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Paleogene paleoclimate reconstruction using oxygen isotopes from land and freshwater organisms: the use of multiple paleoproxies

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Abstract—Understanding past climate change is critical to the interpretation of earth history. Even though relative temperature change has been readily assessed in the marine record, it has been more difficult in the terrestrial record due to restricted taxonomic distribution and isotopic fractionation. This problem could be overcome by the use of multiple paleoproxies. Therefore, the $\delta^{18}O$ isotopic composition of five paleoproxies (rodent tooth enamel, $\delta^{18}O_{Phosphate} = +17.7 \pm 2.0‰$ n = 74 (VSMOW); fish scale ganoine $\delta^{18}O_{Phosphate} = +19.7 \pm 0.7‰$ n = 20 (VSMOW); gastropod shell $\delta^{18}O_{Calcite} = -1.7 \pm 1.3‰$ n = 50 (VPDB); charophyte gyrogonite $\delta^{18}O_{Calcite} = -2.4 \pm 0.5‰$ n = 20 (VPDB); fish otolith $\delta^{18}O_{Aragonite} = \delta^{18}O = -3.6 \pm 0.6‰$ n = 20 (VPDB)) from the Late Eocene (Priabonian) Osborne Member (Headon Hill Formation, Solent Group, Hampshire Basin, UK) were determined. Because diagenetic alteration was shown to be minimal the phosphate oxygen component of rodent tooth enamel (as opposed to enamel carbonate oxygen) was used to calculate an initial $\delta^{18}O_{Local\ water}$ value of $0.0 \pm 3.4‰$. However, a skewed distribution, most likely as a result of the ingestion of evaporating water, necessitated the calculation of a corrected $\delta^{18}O_{Local\ water}$ value of $-1.3 \pm 1.7‰$ (n = 62). This $\delta^{18}O_{Local\ water}$ value corresponds to an approximate mean annual temperature of $18 \pm 1°C$. Four other mean paleotemperatures can also be calculated by combining the $\delta^{18}O_{Local\ water}$ value with four independent freshwater paleoproxies. The calculated paleotemperature using the fish scale thermometry equations most likely represents the mean temperature ($21 \pm 2°C$) of the entire length of the growing season. This should be concordant with the paleotemperature calculated using the *Lymnaea* shell thermometry equation ($23 \pm 2°C$). The lack of concordance is interpreted to be the result of diagenetic alteration of the originally aragonitic *Lymnaea* shell to calcite. The mean paleotemperature calculated using the charophyte gyrogonite thermometry equation ($21 \pm 2°C$), on the other hand, most likely represents the mean temperature of a single month toward the end of the growing season. The fish otolith mean paleotemperature ($28 \pm 2°C$) most likely represents the mean temperature of the warmest months of the growing season. An approximate mean annual temperature of $18 \pm 1°C$, in addition to a mean growing season paleotemperature of $21 \pm 2°C$ (using fish scale only) with a warmest month temperature of $28 \pm 2°C$, and high associated standard deviations suggest that a subtropical to warm temperate seasonal climate existed during the deposition of the Late Eocene Osborne Member. *Copyright © 2003 Elsevier Ltd*

1. INTRODUCTION

The investigation of past global climate change and its impact upon ecosystems, especially with respect to former greenhouse and icehouse worlds, is critical to predicting the future effect of global climate change (e.g., Collinson, 2000; Hooker, 2000). Using isotopic techniques, climate change has been extensively studied in the marine record using the shells of benthic and planktonic foraminifera (for most recent review, see Zachos et al., 2001) and to a lesser extent using other paleoproxies such as the otoliths (ear stones) of marine fish (Ivany et al., 2000). However, the continental response to climate change has been more difficult to track using isotopic techniques. An approach combining the marine and freshwater realms was used by Kolodny and Luz (1991) in an oxygen isotope study of phosphate oxygen in fossil fish from the Devonian to the Recent. However, even though this was a wide ranging study it had low paleoclimatic and temporal resolution. Bryant et al. (1996) and Fricke et al. (1998) both conducted purely continental studies using the oxygen isotope composition of mammalian phosphate in tooth enamel as a proxy for meteoric water. 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 enamel phosphate proxies $\delta^{18}O$ variability of local meteoric water (e.g., Luz et al., 1984). The use of large mammal species (e.g., horse, Bryant et al., 1996, and pantodont, Fricke et al., 1998), whose fossil record before the Neogene is globally relatively sparse, has meant that these studies have generally targeted rapid climatic events occurring over a short time interval when compared to the marine oxygen isotope record. Lindars et al. (2001) reported a new method of phosphate oxygen isotope analysis that allows the measurement of the $\delta^{18}O$ variation in more abundant fossil rodent teeth. Because of the greater abundance of teeth of smaller rather than larger mammals in the fossil record, especially when screen-washing methods are used or when exposure is limited, the use of rodent teeth should allow measurements of higher temporal resolution to be assembled. If rodent teeth are sampled from horizons which indicate a close association with large water bodies the small home ranges and the lack of migratory behavior in most rodents mean that the $\delta^{18}O$ of local water, calculated from the rodent tooth enamel, can be used to calculate a mean annual

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temperature. The $\delta^{18}O$ of local water can also be combined with the $\delta^{18}O$ of other freshwater paleoproxies (i.e., a carbonate or phosphate entity precipitated from that water) to calculate absolute mean paleotemperatures of varying climatic significance.

However, using a single paleoproxy through a long succession of continental deposits is difficult owing to rapid changes in depositional environments that can affect fossilization potential. To overcome this problem we propose using several different paleoproxies that span a variety of depositional environments and preservation types. However, so that their potential can be tested all the paleoproxies must co-occur in at least one single horizon and they must not have experienced significant diagenetic alteration of their primary isotopic composition. The Late Eocene and Early Oligocene Hampshire Basin Solent Group is dominated by sediments which have a pH which is near neutral and show little sign of diagenetic alteration in a depositional setting without volcanogenesis. In addition, the Solent Group presently has minimal overburden and shows no evidence of even having been deeply buried. Intercalated marine stratigraphic markers at known intervals give good correlation to the marine realm and there are tight stratigraphic controls underpinned by mammalian, palynological and charophyte zonations. Moreover, the sediments were deposited across a key interval of global climate change (the Eocene–Oligocene greenhouse–icehouse transition), as documented in the marine proxy isotope record, which is important not only on a European but also a global scale. One horizon in the Late Eocene (Priabonian) Osborne Member of the Headon Hill Formation was chosen for the test as it has the greatest diversity of potential paleoproxies in the sequence.

2. SAMPLE LOCATION AND PALEOPROXIES

Five different paleoproxies, rodent cheek tooth enamel, gastropod shells, charophyte gyrogonites, fish otoliths, and fish scale ganoine, were used in this study (Fig. 1). They were all collected from a single horizon in the Late Eocene (Priabonian) Osborne Member of the Headon Hill Formation, Isle of Wight, UK. The Osborne Member (Table 1) is a sequence of dominantly color-mottled clays with mud-crack horizons and occasional bonebeds, suggestive of the overbank facies of a floodplain.

A summary of the paleoproxies, their composition, and the analytical methods employed on each are outlined in Table 2. *Thalerimys* and *Isoptychus* are two genera of rodents belonging to the extinct European family Theridomyidae. The systematics of the Solent Group theridomyids have been treated in detail by Bosma and Insole (1972) and Bosma (1974). Some changes to the generic assignment have been made by Tobien (1972) and Vianey-Liaud (1979). The teeth are usually found isolated rather than still within the animal's jaw. The following cheek teeth, first/s molars (undifferentiated) (M1/2), third molar (M3), and fourth premolar (P4), were used in this study. Only teeth that showed no obvious sign of corrosion as a result of digestion by predators or scavengers or of abrasion from fluvial transport were used in this study.

The following references provide further information on the gastropods (*Lymnaea*) (Paul, 1989), charophyte gyrogonites (*Nitellopsis*) (Feist-Castel, 1977), fish otoliths (*Umbra*) (Stinton, 1977), and fish scales (*Lepisosteus*) (White, 1931), used in this study. Only specimens with no clear signs of alteration or abrasion were used.

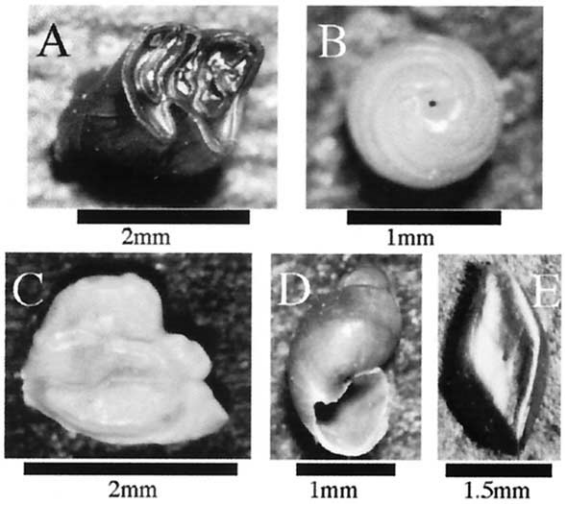

Fig. 1. Representative light microscope images of the five paleoproxies employed in this study. (A) Lower right first or second molar tooth of the rodent *Thalerimys*. (B) Gyrogonite of the charophyte *Nitellopsis*. (C) Otolith (sagitta) of the fish *Umbra valida*. (D) Shell of the pulmonate gastropod *Lymnaea*. (E) Scale of the fish *Lepisosteus*.

3. METHODOLOGY

All samples were separated from the host matrix and washed in an ultrasonic bath containing deionized/distilled water before being air-dried and examined under a binocular microscope to ensure no host matrix remained. The rodent tooth samples were crushed and enamel separated from dentine by hand picking. The fish scales were also crushed and ganoine (enameloid) separated by hand picking. The gastropod shells and charo-

Table 1. Sequence of principal lithostratigraphic units showing the context of the Osborne Member of the Hampshire Basin Solent Group, Isle of Wight, UK. Absolute ages are in Ma (Barggren et al., 1995).

Geochronology	Age (Ma)	Formation	Member
Early Oligocene (Rupelian)	33.7	Bouldnor	Cranmore
			Hamstead
Late Eocene			Bembridge Marls
(Priabonian)		Bembridge Limestone	
		Headon Hill	**Osborne**
			Fishbourne
			Lacey's Farm
			Cliff End
			Hatherwood
			Linstone Chine
			Colwell Bay
	37.0		Totland Bay

Table 2. Details of the five different palaeoproxies used in this study, including their group name, their genus, their composition, and the method of analysis employed for each. See text for more details on method of analysis.

Group	Genus	Object studied	Oxygen type	Method of preparation and analysis
Rodent	*Thalerimys* and *Isoptychus*	Separated tooth enamel	Apatite phosphate and carbonate	Direct Laser Fluorination and acid digestion
Fish	*Lepisosteus*	Separated scale ganoine	Apatite phosphate	Direct Laser Fluorination
Gastropod	*Lymnaea*	Whole shell	Calcite	Acid digestion
Charophyte	*Nitellopsis*	Whole gyrogonite	Calcite	Acid digestion
Fish	*Umbra*	Whole otolith	Aragonite	Acid digestion

phyte gyrogonites were first checked to ensure that they did not contain any matrix material in their interiors and then crushed as individual whole samples. Fish otoliths were also crushed as individual whole samples.

X-ray Diffraction (XRD) analysis of a selection of the carbonate paleoproxies (i.e., 7 gastropod shells and 9 fish otoliths) was conducted to check if their primary aragonite mineralogy remained, or if they had been altered by diagenesis to calcite. Analyses were conducted upon powdered samples using a Philips Analytical XRD PW3710 machine with PC-APD diffraction software. A copper tube anode was used and samples scanned between 20° to 50° (2θ).

Two methods of oxygen isotope analysis were used in this study. The analysis of phosphate oxygen (rodent tooth enamel and fish scale ganoine) was conducted using the direct laser fluorination (DLF) technique described by Lindars et al. (2001). Pretreatment of the enamel and ganoine to remove non phosphate bound oxygen involved heating at 400°C for 1 h and fusing under high vacuum in the sample chamber (Lindars et al., 2001). In Lindars et al. (2001) the phosphate oxygen standard NIST 120c was used to validate the technique. Run under the same conditions as the tooth enamel, it gave a value of 21.18 ± 0.30‰ (n = 33) which is in agreement with that quoted by Bryant et al. (1994) (21.4 ± 0.4‰ using $BiPO_4$) and by Lécuyer et al. (1993) (21.7 ± 0.2‰, using $AgPO_4$). However, NIST 120c is not an accredited $\delta^{18}O$ isotopic standard, or a biogenic apatite sample, but rather a phosphate-bearing sediment. As mentioned by Lindars et al. (2001), because of these two points, it may not fully validate the DLF technique. Therefore, results from the analysis of standards and samples donated by Vennemann (Université de Lausanne) and Tütken (Universität Tübingen) are presented in the Appendix. The results of these tests show that reported $\delta^{18}O_{PO4}$ values for both silver phosphate samples and tooth enamel samples can be successfully reproduced using the DLF technique. However, the technique does not appear to be valid for dentine or, by association, bone samples. Fourier Transform Infrared (FT-IR) spectral analysis, using a Buker IFS 113v FT-IR spectrometer at Cambridge University, indicates that a possible reason for this is the retention of hydroxyl oxygen in fused dentine-rich samples, which appears to have been removed from enamel samples during fusing. FT-IR analysis also indicates that carbonate oxygen, if present, is only a very minor component of fused enamel and dentine. The DLF $\delta^{18}O_{PO4}$ values obtained on the enamel samples from other laboratories are slightly lower, but usually within error, of those reported elsewhere using silver phosphate techniques (see Appendix). This indicates that, during the fusing of our enamel samples in the pretreatment step before analysis, there is no mixing between PO_4^{3-} and CO_3^{2-} oxygen.

Owing to the small sample mass of hand picked enamel (~2 mg) and the requirement to conduct where possible both phosphate and carbonate oxygen analysis, replicate analyses could not be obtained on individual teeth. As a consequence, the error on the individual tooth phosphate oxygen isotope composition was taken to be the same as that on the enamel sample FZ-MANS8, ± 0.2‰ **(**see Appendix).

Analysis of the CO_3^{2-} component of the rodent tooth enamel, plus carbonate paleoproxies (individual whole samples of gastropod shells, fish otoliths and charophyte gyrogonites), was conducted using an acid digestion technique with a continuous helium flow Micromass Isoprime multiflow Mass-spec. For the analysis of tooth enamel, approximately 1 mg of sample (compared with ~300 μg for carbonate samples) was weighed into individual, septum sealed, vials and placed in a hot plate maintained at 90°C. Each vial was automatically flushed with helium before excess H_3PO_4 was added. After approximately an hour of equilibration time the CO_2 was analyzed by continuous flow Mass Spectrometry.

For the analysis of the CO_3^{2-} component of enamel an internal lab apatite standard gave a $\delta^{18}O$ value of +18.55 ± 0.20‰ (n = 7) and a $\delta^{13}C$ value of −3.68 ± 0.30‰ (VPDB) (n = 7). Owing to the small sample masses of the hand picked enamel (~2 mg on average), replicate carbonate oxygen analyses could not be conducted. As a consequence, the error on individual tooth carbonate analyses is taken to be that of the internal lab apatite standard ($\delta^{18}O$ = ± 0.20‰, $\delta^{13}C$ = ± 0.30‰).

For the analysis of the oxygen component of the carbonate paleoproxies the in run NBS-19 standard gave a $\delta^{18}O$ value of −2.28 ± 0.05‰ (VPDB) (n = 19) and a $\delta^{13}C$ value of +2.1 ± 0.04‰ (VPDB) (n = 19). The internal lab carbonate standard gave a $\delta^{18}O$ value of −9.80 ± 0.06‰ (VPDB) (n = 53) and a $\delta^{13}C$ value of +3.32 ± 0.10‰ (VPDB) (n = 53). All the carbonate paleoproxy samples were calibrated to the NBS-19 standard. The error on the individual carbonate analyses was taken to be the same as that on the NBS-19 standard (± 0.05‰) as replicate analyses on all the carbonate paleoproxies could not be obtained because of small sample sizes. Preparation of the carbonate paleoproxy samples before acid digestion followed that outlined in White et al. (1999) (gastropods), Jones et al. (1996) (charophyte gyrogonites) and Patterson et al. (1993) (otoliths).

Electron microprobe Wave Dispersive Spectral (EM-WDS) analyses, using the techniques of Denys et al. (1996), were specifically employed to determine Ca, P and F weight percents

in selected *Thalerimys* and *Isoptychus* tooth samples. The WDS analyses were carried out at the Natural History Museum, London, UK, using a Cameca SX50 EM-WDS (Lindars, 1998). Spot analyses were standardized using natural and synthetic oxides, silicates and phosphates, and were collected at an accelerating voltage of 15 keV, with a beam current of 20 nA and a spot size of 15 μm. The detection limit on all elements was in the order of 0.01 wt%. The samples were embedded in epoxy resin, sectioned and then polished to reveal fresh internal cross sections of the teeth. Spot analyses were conducted upon both the tooth enamel and dentine along transects through the cross section of the teeth.

4. CALCULATION OF A MEAN δ^{18}O LOCAL WATER VALUE AND ABSOLUTE PALEOTEMPERATURES

The methodology for calculating the δ^{18}O local water value using tooth enamel and all the thermometry equations used in the calculation of mean paleotemperatures is outlined below.

4.1. Calculation of a Mean δ^{18}O Local Water Value and a Mean Annual Temperature Using Mammal Tooth Enamel

The method is described in detail in Lindars et al. (2001) and summarized below.

Longinelli and Nuti (1973) reported a phosphate thermometer which was recalibrated by Kolodny et al. (1983) such that

$$\text{Temp. (°C)} = 113.3 - 4.38(\delta^{18}O_{\text{Tooth phosphate (VSMOW)}} - \delta^{18}O_{\text{Local water}}) \quad (1)$$

If local water is assumed to be body water maintained at a constant body temperature of 37°C, then the equation can be simplified to

$$\delta^{18}O_{\text{Body water}} = \delta^{18}O_{\text{Phosphate (VSMOW)}} - 17.42 \quad (2)$$

Furthermore, Luz et al. (1984) reported the following relationship for lab rodents:

$$\delta^{18}O_{\text{Local water}} = \{\delta^{18}O_{\text{Body water}} - 0.24\} / 0.59 \quad (3)$$

Therefore, combining Eqns. 2 and 3 gives

$$\delta^{18}O_{\text{Local water}} = \{[\delta^{18}O_{\text{Tooth phosphate (VSMOW)}} - 17.42] - 0.24\}/0.59. \quad (4)$$

It should be noted that the relationship reported by Luz et al. (1984) for lab rodents is based upon the analysis of bone material and as a consequence may not be fully applicable to the analysis of teeth. However, it is from the same group of small mammals (rodents) that we are analyzing. There is a known taxon-dependent fractionation with respect to local water and body water, therefore, we have chosen this equation over that of other published equations for other groups of mammals (see, among others, Longinelli, 1984; D'Angela and Longinelli, 1990; Ayliffe et al., 1994; Huertas et al., 1995).

Assuming that the calculated $\delta^{18}O_{\text{Local water}}$ value is comparable to that of $\delta^{18}O_{\text{Meteoric water}}$ then a mean annual temperature (MAT) for mid to high latitudes can be calculated using Dansgaard's (1964) equation.

$$\text{MAT (°C)} = (\delta^{18}O_{\text{Local water}} + 13.6)/0.695 \quad (5)$$

4.2. Calculation of Paleotemperature Using Fish Scale Ganoine

This is achieved using Longinelli and Nuti's (1973) phosphate thermometer which has been recalibrated by Kolodny et al. (1983).

$$\text{Temp. (°C)} = 113.3 - 4.38(\delta^{18}O_{\text{Phosphate (VSMOW)}} - \delta^{18}O_{\text{Local water}}) \quad (6)$$

Where $\delta^{18}O_{\text{Local water}}$ is that calculated using mammal tooth enamel and $\delta^{18}O_{\text{Phosphate}}$ is that measured on the fish scale ganoine. This method has been used by Fricke et al. (1998) to estimate changes in temperature across the Paleocene–Eocene boundary.

4.3. Calculation of Paleotemperature Using the Gastropod Lymnaea

This is achieved using White et al.'s (1999) Lymnaea genus-specific carbonate thermometer.

$$\text{Temp. (°C)} = 21.36 - 4.83(\delta^{18}O_{\text{Carbonate (VPDB)}} - \delta^{18}O_{\text{Local water}}) \quad (7)$$

Where $\delta^{18}O_{\text{Local water}}$ is that calculated using mammal tooth enamel and $\delta^{18}O_{\text{Carbonate}}$ is that measured on the *Lymnaea* shells.

4.4. Calculation of Paleotemperature Using Charophyte Gyrogonites

Jones et al. (1996) reported that this could be achieved by using Hays and Grossman's (1991) freshwater carbonate thermometer.

$$\text{Temp. (°C)} = 15.7 - 4.36(\delta^{18}O_{\text{Carbonate (VPDB)}} - \delta^{18}O_{\text{Local water}}) + 0.12\,(\delta^{18}O_{\text{Carbonate (VPDB)}} - \delta^{18}O_{\text{Local water}})^2 \quad (8)$$

Where $\delta^{18}O_{\text{Local water}}$ is that calculated using mammal tooth enamel and $\delta^{18}O_{\text{Carbonate}}$ is that measured on the charophyte gyrogonites.

4.5. Calculation of Paleotemperature Using Fish Otoliths

This is achieved using Patterson et al.'s (1993) freshwater otolith specific thermometer.

$$\text{Temp (°C)} = (18564 / ((\text{Ln}\,(((10^3 + (\delta^{18}O_{\text{Carbonate (VPDB)}} * 1.03091 + 30.91)) / (10^3 + \delta^{18}O_{\text{Local water}}))) * 10^3) + 33.491)) - 273.15 \quad (9)$$

where $\delta^{18}O_{\text{Local water}}$ is that calculated using mammal tooth enamel and $\delta^{18}O_{\text{Carbonate}}$ is that measured on freshwater fish otoliths.

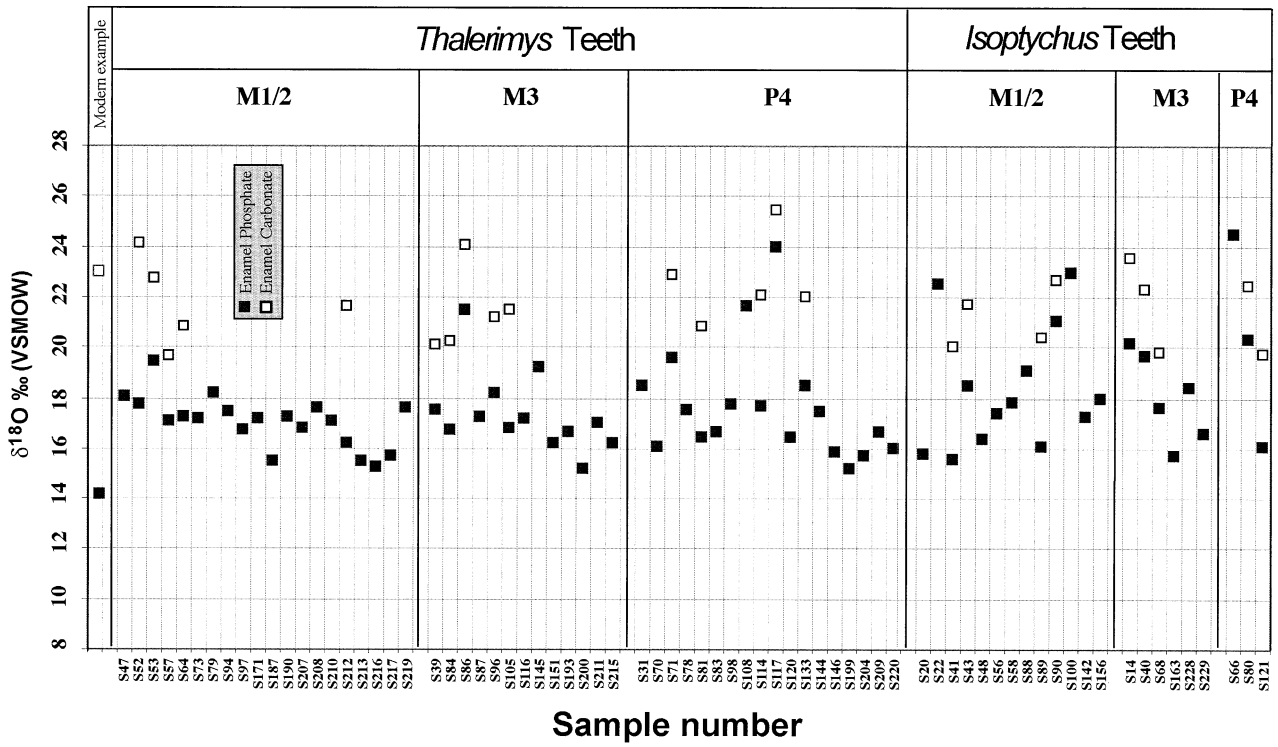

Fig. 2. Phosphate and carbonate $\delta^{18}O$ isotope results from enamel from *Thalerimys* and *Isoptychus* teeth. The modern tooth is a hypothetical example, based upon an expected 8.6–9.1‰ fractionation between phosphate and carbonate $\delta^{18}O$ isotope results. M1/2 (first or second molar), M3 (third molar) and P4 (fourth premolar) refer to tooth types. All $\delta^{18}O$ results are reported with respect to VSMOW.

4.6. Calculation of Paleotemperature Errors

The standard error and standard deviation on each paleotemperature are propagated from the standard errors and standard deviations on the paleoproxies using a recognized error propagation equation. For example, for White et al. (1999) the *Lymnaea* genus-specific carbonate thermometer (Eqn. 7) can be written as

$$Y = K_1 - K_2(A - B), \tag{10}$$

from which the 1σ error (∇) propagates as

$$\nabla^2 Y = \nabla^2 A\,(dY/dA)^2 + \nabla^2 B\,(dY/dB)^2 \tag{11}$$

Similar error propagations were applied to all the other thermometry equations.

5. RESULTS

5.1. Tooth Diagenesis Results

The results of both the DLF analysis of phosphate oxygen and the acid digestion analysis of carbonate oxygen, from the enamel of *Thalerimys* and *Isoptychus* teeth, are shown in Figure 2 (data for this figure can be found in Table E1 of an electronic supplement to this paper on the Elsevier Website).

In total, seventy four $\delta^{18}O_{PO4}$ results and twenty four $\delta^{18}O_{CO3}$ results are reported. As shown at the top of Figure 2, in modern unaltered samples, owing to the fractionation of oxygen between CO_3^{2-} and PO_4^{3-}, the $\delta^{18}O_{CO3}$ values should be 8.6–9.1‰ higher than the $\delta^{18}O_{PO4}$ values (Iacumin et al., 1996). It is clear from the results in Figure 2 that enamel in fossil *Thalerimys* and *Isoptychus* teeth do not conform to this expectation. The enamel $\delta^{18}O_{CO3}$ values are not in equilibrium with their corresponding $\delta^{18}O_{PO4}$ values. They are on average only 4.5‰ higher, rather than the expected 8.6–9.1‰.

Nonequilibrium isotopic separations between $\delta^{18}O_{CO3}$ and $\delta^{18}O_{PO4}$ isotope ratios in fossil tooth enamel is not uncommon (Fricke et al., 1998). However, this is the first time it has been observed using the DLF technique for the analysis of phosphate oxygen. As a consequence, it must first be shown that the less than 8.6–9.1‰ difference between $\delta^{18}O_{PO4}$ and $\delta^{18}O_{CO3}$ in the enamel of our teeth is not the result of isotopic mixing between CO_3 and PO_4 oxygen isotopes during DLF analysis of phosphate oxygen. We have analyzed tooth enamel samples (ZEQ56 and FZMAN-S8), and a whole shark tooth sample (GW1) and compared our DLF results with those reported by Vennemann et al. (2002) and Tütken (Universität Tübingen, personal communication). These results show that there is no evidence for mixing between CO_3 and PO_4 oxygen isotopes during the fusing stage before the DLF analysis of phosphate oxygen (see Appendix).

It is generally accepted that when there is nonequilibrium isotopic separations between the enamel $\delta^{18}O_{CO3}$ and $\delta^{18}O_{PO4}$ isotope ratios, this is as a result of alteration of the weakly bonded carbonate oxygen rather than the strongly bonded phosphate oxygen. For example, Kolodny et al. (1983) and Fricke et al. (1998) both argued that phosphate oxygen is less prone to

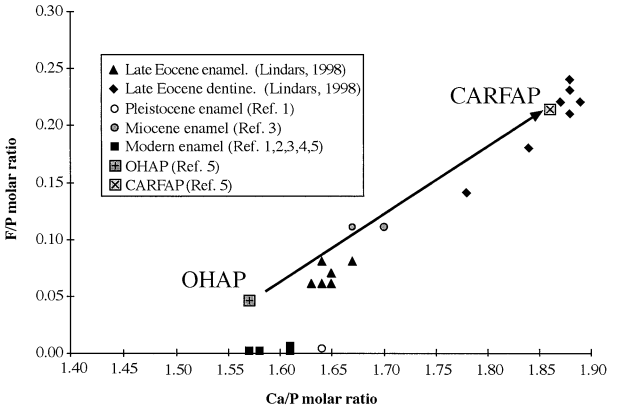

Fig. 3. Plot of F/P against Ca/P molar ratios for modern and fossil mammal teeth, including those from Lindars (1998), plus mineral phosphates. References are as follows: 1, Michel et al. (1995); 2, Williams and Elliott (1979); 3, Bryant (1995); 4, LeGeros (1981); 5, Nriagu and Moore (1984). The arrow indicates the postdepositional diagenetic alteration from OHAP to CARFAP.

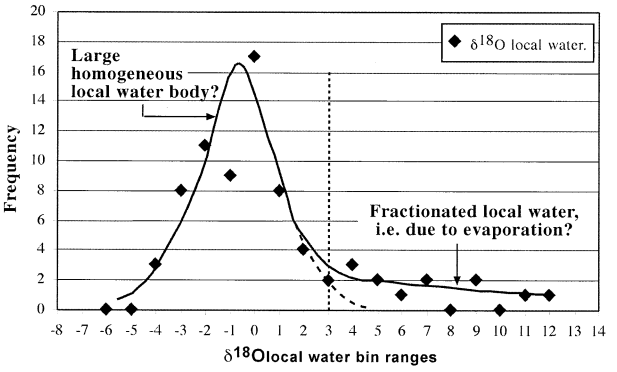

Fig. 4. Plot of the frequency of $\delta^{18}O_{Local\ water}$ values calculated from *Thalerimys* and *Isoptychus* teeth. The curve plotted on the graph is just for qualitative interpretation and not a modeled distribution.

diagenetic alteration than carbonate oxygen because of an observed larger range in enamel $\delta^{18}O_{CO3}$ values when compared to their corresponding $\delta^{18}O_{PO4}$ values. In our study, however, the opposite is the case, with enamel $\delta^{18}O_{PO4}$ values having a larger range (8.37‰) than $\delta^{18}O_{CO3}$ values (5.85‰). This however, does not necessarily indicate that in our *Thalerimys* and *Isoptychus* teeth phosphate oxygen is more digenetically altered than carbonate oxygen as diagenesis might also lead to a convergence of $\delta^{18}O$ values toward an equilibrium with the diagenetic fluid.

To support further our notion that the enamel phosphate oxygen has not been diagenetically altered, we also investigated the elemental composition of the *Thalerimys* and *Isoptychus* enamel and dentine using EM-WDS analysis and related these results to apatite mineralogy. For example, Bryant (1995) showed that there was a gradual trend over time in fossil biogenic calcium phosphates Ca/P and F/P molar ratios from primary hydroxy- apatite (OHAP) to a more stable carbonate fluoro-apatite (CARFAP) as a result of diagenesis. The fossil enamel F/P and Ca/P molar ratios from this study are shown in Figure 3 (data for this figure can be found in Table E2 of an Electronic Annex to this paper on the Elsevier Website**)**. These results indicate that, even though our enamel have Ca/P molar ratios (1.63–1.67) slightly greater than OHAP (1.57), the ratios are similar to those previously reported for both modern and fossil enamel (modern enamel 1.58–1.78, fossil enamel 1.64–1.70; Williams and Elliott, 1979; LeGeros, 1981; Nriagu and Moore, 1984; Bryant, 1995; Michel et al., 1995). Our fossil dentine, on the other hand, has Ca/P molar ratios (1.78–1.89) that are very close to CARFAP (1.86). A similar distribution is evident with the F/P molar ratios. Therefore, as indicated in Figure 3, the results from this study suggest that *Thalerimys* and *Isoptychus* enamel is still principally an OHAP and has experienced, at most, only minor diagenetic alteration. The degree of alteration is less than that recorded on Miocene *Pseudhipparion retrusum* and *Merychippus* enamel (Bryant, 1995), but greater than that experienced by Pleistocene red deer enamel (Michel et al., 1995). The *Thalerimys* and *Isoptychus* dentine, on the other hand, has experienced extensive diagenetic alteration from OHAP to CARFAP. Even though this does not prove that the phosphate oxygen isotopic component of enamel is unaltered is does suggest that it has at most only experienced minor alteration.

5.2. Tooth Enamel Results and Local Water Calculations

Based upon the above indications of differential alteration of phosphate and carbonate oxygen during diagenesis, only enamel $\delta^{18}O_{PO4}$ results and not enamel $\delta^{18}O_{CO3}$ results, were considered for the paleoclimate part of this study.

As two genera of fossil rodents (*Thalerimys* and *Isoptychus*) and four tooth types (M1/2, M3, P4) were used in this study, it is important to show that there are no significant differences in the enamel $\delta^{18}O_{PO4}$ values between tooth types or genera. As can be seen in Figure 2 this appears to be the case. Therefore, an overall mean for all enamel $\delta^{18}O_{PO4}$ results is $+17.7 \pm 2.0$‰ (VSMOW)**,** which equates to a $\delta^{18}O_{Local\ water}$ value of 0.0 ± 3.4‰ (Eqn. 4). However, in Figure 4 the frequency of the measured $\delta^{18}O_{Local\ water}$ values clearly shows a skewed distribution toward higher values. This skewed distribution is not a result of either taxon type or tooth type, as all contribute at least one result to it. It is also highly unlikely to be the result of diagenetic alteration, as analysis of the carbonate oxygen component of enamel indicates that diagenesis is resulting in a reduction (lowering) of oxygen isotopic values. This is in the opposite direction to the skewed distribution. There is also no evidence, in the form of transport abrasion associated with the depositional setting, to indicate that the few teeth with the highest $\delta^{18}O_{PO4}$ values could have been reworked from older horizons with different $\delta^{18}O_{PO4}$ values. Fluvial transport from other more distinct water bodies cannot be fully discounted, but the diversity of rodent bone types in the Osborne Member seems comparable with assemblages from lake margin deposits in other Solent Group units where long distance transport has been shown to be unlikely (Hooker, 1994). There is evidence, however, in the overbank deposits of the Osborne Member, in the form of mud cracks and paleosols, which suggests that short lived periodic evaporation is likely to have affected water bodies on the floodplain. This would have led to isotopic fractionation of oxygen. Thus ingestion of this fractionated water source during a drier interval could account for the

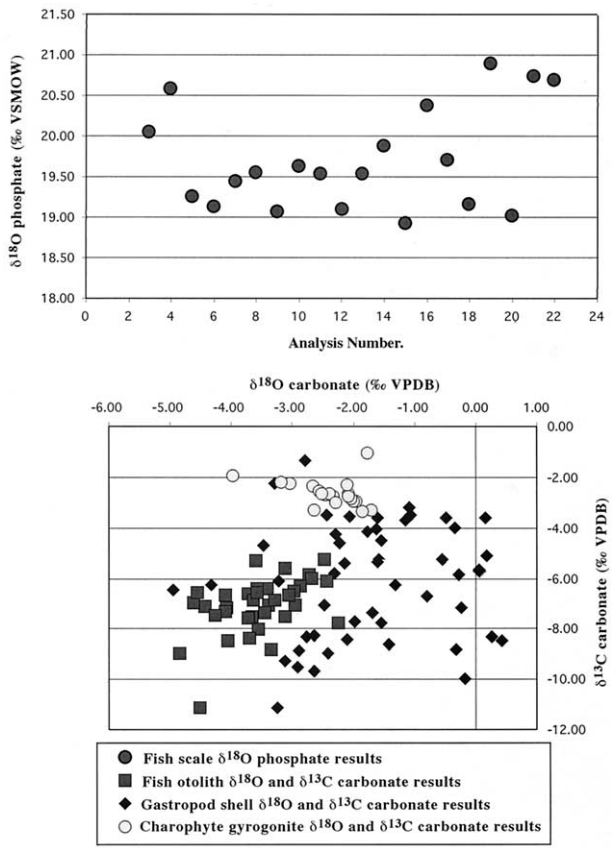

Fig. 5. $\delta^{18}O$ results of the phosphate analysis of fish scale ganoine (top), and the carbonate analysis of *Lymnaea* shells, charophyte gyrogonites, and fish otoliths (bottom).

skewed distribution in the $\delta^{18}O_{Local\ water}$ values. Alternatively, ingestion of fractionated water from isolated water sources, like ponded water on leaves, by a small proportion of the rodents could produce a similar skewed distribution. The latter explanation seems less likely in view of the availability of an apparently nearby water body for drinking. The first hypothesis could potentially be tested by analyzing other sites with different depositional settings. As a consequence of the skewed distribution the mean $\delta^{18}O_{Local\ water}$ value and the standard deviation are considered to be unrepresentative of the water ingested by the majority of the rodents. Therefore, all $\delta_{18}O_{Local\ water}$ values $> +3‰$ (n = 12) were removed from the data set and a corrected $\delta^{18}O_{Local\ water}$ value of $-1.3 \pm 1.7‰$ (n = 62) calculated (Fig. 4).

5.3. Carbonate and Phosphate Paleoproxy Results

Results of the DLF analysis of fish scale ganoine and acid digestion analysis of gastropod shells and charophyte gyrogonites, plus fish otoliths, are summarized in Figure 5 (data for this figure can be found in Table E3 of an electronic supplement to this paper on the Elsevier Website). The mean $\delta^{18}O$ values for each are: fish scale ganoine $\delta^{18}O = +19.7 \pm 0.7‰$ n = 20 (VSMOW); gastropod shell $\delta^{18}O = -1.7 \pm 1.3‰$ n = 50 (VPDB); charophyte gyrogonite $\delta^{18}O = -2.4 \pm 0.5‰$ n = 20 (VPDB); and fish otolith $\delta^{18}O = -3.6 \pm 0.6‰$ n = 20 (VPDB).

XRD analyses of seven *Lymnaea* shells and nine *Umbra* otoliths were conducted (results can be found in Table E4 of an electronic supplement to this paper on the Elsevier Website). Unfortunately, owing to small sample masses not all the gastropods or otoliths used in the isotope study could be analyzed by XRD. However, a selection was available which give an indication of the degree of diagenetic alteration experienced by each group. The results show that all seven of the *Lymnaea* analyzed have experienced diagenetic alteration from their primary aragonite mineralogy to that of calcite. This in turn would indicate that the majority, if not all, of the *Lymnaea* used in the isotope study had experienced some degree of diagenetic alteration, which could have affected their primary $\delta^{18}O$ values. In contrast, six of the nine otoliths appear to have retained their primary aragonite mineralogy, whilst the other three have an approximate 28–42% calcite component. As a consequence, it can be argued that the majority of the otoliths have probably only experienced minor diagenetic alteration of their primary $\delta^{18}O$ values.

With the exception of the *Lymnaea* shells all the other paleoproxies which lived in the water body show reasonably tight distributions (<0.7‰). The larger scatter in the distribution for the *Lymnaea* shells (1.3‰) is most likely linked to the diagenetic alteration of aragonite to calcite. However, it should also be noted that the distribution could also result from their habit of moving to the water surface and climbing onto emergent plants or into marshy ground (Paul, 1989). In contrast, the other nonmammal paleoproxies are likely to have been fully aquatic. Unfortunately, un-like the results from the rodent teeth, the large scatter in the distribution in the *Lymnaea* $\delta^{18}O$ results cannot be corrected for, as there is no obvious skewness in the data set.

With respect to the fish scale ganoine (phosphate enameloid) results, it should be noted that there is no skewed distribution like that found in the rodent tooth enamel. This indicates that the skewed distribution in the rodent enamel cannot be a result of the DLF analytical method as both were analyzed using the same technique. Furthermore, it suggests that the skewed distribution in the rodent tooth enamel is unlikely to be caused by diagenesis as there is no reason why it would effect only the tooth enamel and not the fish scale ganoine (phosphate enameloid). Therefore, this further supports the notion that the skewed distribution in the rodent tooth enamel most likely results from their individual drinking habits, i.e., ingestion of fractionated water from evaporating small water bodies. In contrast drying out of the water bodies inhabited by the fish would be likely to inhibit growth and therefore deposition of ganoine, leaving no evidence of the fractionation of the oxygen isotopes.

5.4. Calculations of Mean Paleotemperatures

The mean paleotemperatures calculated from the thermometry Eqns. 5 to 9 are shown in Table 3. Using the $\delta^{18}O_{Local\ water}$ value alone gives a mean annual temperature of $18 \pm 1°C$ (Eqn. 5). Using the $\delta^{18}O_{Local\ water}$ value in combination with the *Lymnaea* Eqn. 7 gives a mean paleotemperature of $23 \pm 2°C$; whilst the charophyte gyrogonite and fish scale equations (6

Table 3. Paleotemperature results using the palaeoproxies shown in Table 2 and the paleothermometry equations given in the text. Phosphate $\delta^{18}O$ results (rodent tooth enamel and fish scale ganoine) are quoted relative to VSMOW. Calcite $\delta^{18}O$ results (charophyte gyrogonites and *Lymnaea* shells) and aragonite $\delta^{18}O$ results (fish otoliths) are quoted relative to VPDB.

Local water proxy	$\delta^{18}O$ local water ± stdev	Precipitate	$\delta^{18}O$ precipitate ± stdev	Mean annual Temp. (°C)	Water Temp. (°C)	2 σ std Error	2 σ stdev	Equation number
Thalerimys and *Isoptychus* rodent tooth enamel	−1.3 ± 1.7‰			18		1	5	5
		Fish scale ganoine	+19.7 ± 0.7‰		21	2	15	6
		Lymnaea shell	−1.7 ± 1.3‰		23	2	14	7
		Charophyte gyrogonite	−2.4 ± 0.5‰		21	2	16	8
		Fish otolith	−3.6 ± 0.6‰		28	2	18	9

Std = standard error, Stdev = standard deviation. See text for information on the calculation of errors on the palaeotemperatures.

and 8) both give a mean paleotemperature of 21 ± 2°C, and the fish otolith Eqn. 9 gives a mean paleotemperature of 28 ± 2°C.

6. DISCUSSION

Absolute paleotemperatures, as opposed to trends in the $\delta^{18}O$ of water over time, have been reported previously by, among others, Patterson (1999) and Wurster and Patterson (2001) using fish otoliths alone; as well as by Lécuyer et al. (1996) and Fricke et al. (1998), both using mammal teeth and fish scales. However, there has never been an attempt to compare or interpret paleotemperatures calculated using multiple paleoproxies at the same horizon. In addition, rodent teeth have never before been used to calculated paleo-$\delta^{18}O_{Local\ water}$ values.

6.1. Suitability of Using Rodent Tooth Enamel to Calculate the $\delta^{18}O$ Value of Local Water

Two factors are critical to the use of mammal teeth in the determination of paleo-$\delta^{18}O_{Local\ water}$ values. First it is necessary to demonstrate that the majority of their water intake came from homogeneous water sources, rather than from their consumption of food. Previously, large mammals have been preferred to small mammals because drinking water forms a larger proportion of their oxygen intake (Bryant and Froelich, 1995). However, with large mammals the potential of long distance migrations means that the $\delta^{18}O_{PO4}$ values in their cheek tooth enamel is representative of their site of weaning which may be quite different from that of their death, the one whose reconstruction is being attempted. Rodents, on the other hand, typically have small home ranges and do not migrate, removing this problem. However, the question of the source of their water intake must be addressed.

The evidence that the rodents *Thalerimys* and *Isoptychus* most likely obtained their water intake principally from ground water rather than food is as follows. Firstly, the ready availability of water is indicated by the biota and sedimentology of the Osborne Member. The source of the fossils is a large bone rich lens in a sequence of dominantly color-mottled clays with mud-crack horizons and occasional bonebeds, indicative of a pond in the overbank facies of a floodplain. Ecological diversity analysis of the Osborne Member mammal fauna independently suggests that the habitat was an open one with woodland patches (Hooker, 1992). This was thus a mesic environment, the kind where modern rodents typically obtain their water by drinking rather than from their food (Schröpfer, 1974; Wright, 1976). Moreover, *Thalerimys* and *Isoptychus*, from the anatomy of attributed ankle bones (J.J.H., personal observation) and articulated skeletons of near relatives (Schmidt-Kittler and Storch, 1985) were essentially ground dwelling animals. Their combined dominance through almost the entire Solent Group regardless of lithofacies strongly suggests that they lived and died close to the edges of the water bodies in which they became entombed. Supporting evidence is that their co-dominants in the faunas, members of the extinct dormouse genus *Glamys*, had a close spatial relationship to the water bodies concerned, as fossil gnaw marks show that they fed on the seeds of the water plant *Stratiotes* (Collinson and Hooker, 2000). Therefore, all this evidence indicates that *Thalerimys* and *Isoptychus* probably obtained the majority of their water intake from ground water rather than food. However, the skewed distribution in their $\delta^{18}O_{Local\ water}$ results does indicate the occasional consumption of fractionated water by a few animals, most likely from evaporating small ponded water sources. This can be observed in the data set and corrected for.

The second requirement to demonstrate the suitability of rodent tooth enamel in the determination of paleo-$\delta^{18}O_{Local\ water}$ values is evidence that enamel phosphate oxygen used in the calculations, has not been diagenetically altered. In previous studies of mammal teeth the enamel that was used was thick simply because the animals body size was large (among others, horse, Bryant et al., 1996, and pantodont, Fricke et al., 1998). The enamel of the small rodents studied here 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. This has been shown to be the case in section 5.1.

The use of dentine phosphate oxygen in paleoclimate studies has already been ruled out for larger mammals, regardless of the thickness of their enamel, by previous workers owing to its proven susceptibility to diagenetic alteration (among others, Wang and Cerling, 1994; Kohn et al., 1999). As a consequence, we have also excluded dentine for the same reasons. However, the use of the carbonate oxygen isotopic composition of thick enamel from large mammals in paleoclimate studies has been justified (Cerling and Sharp, 1995; Bocherens et al., 1996; Cerling et al., 1997) on the basis of no diagenetic alteration. The results from our study clearly indicate that diagenetic alteration has affected the CO_3^{2-} oxygen isotopic

component of both *Thalerimys* and *Isoptychus* enamel. As a result, we do not recommend the use of carbonate oxygen extracted from the enamel of small mammals in paleoclimate studies, because of the increased chance of diagenetic alteration of the CO_3^{2-} oxygen. On the other hand, our evidence indicates minimal alteration of the PO_4^{2-} oxygen isotopic component of *Thalerimys* and *Isoptychus* enamel. Therefore, like previous authors (Longinelli, 1984; Luz et al., 1984, 1990; Ayliffe and Chivas, 1990; Fricke et al., 1996, 1998; Stuart-Williams and Schwarz, 1997; Kohn et al., 1998), we argue that the enamel PO_4^{2-} oxygen isotopic composition of *Thalerimys* and *Isoptychus* teeth can also be used reliably in paleoclimate reconstruction.

6.2. Climatic Significance of the Calculated Paleotemperatures

With the exception of the MAT, the calculated paleotemperatures in Table 3 represent those of shallow water. However, because of the close relationship between water and air temperature (most recently, Livingstone and Lotter, 1998) these temperatures have paleoclimatic significance. To understand further the meaning of these temperatures we first need to establish the time interval in which the *Thalerimys* and *Isoptychus* tooth enamel mineralized, itself dependent upon the breeding season of the rodents, which is unknown. However, from modern studies, the smaller the mammal, the less seasonal the breeding, probably because of a positively correlated short gestation period (Kiltie, 1988). As a working hypothesis we are treating *Thalerimys* and *Isoptychus* as low seasonality breeders and assume that the mineralization of their cheek teeth, and therefore the calculated $\delta^{18}O_{Local\ water}$ value, would encompass many, but not all, of the temperature fluctuations throughout a year. As a consequence of this, the MAT calculated from the $\delta_{18}O_{Local\ water}$ value (18 $\pm$ 1°C, Eqn. 5) is likely be a slight over-estimate, as it may not incorporate the coldest months of the year.

The fish scales and *Lymnaea* shells, on the other hand, mineralize throughout the growing season. The charophyte gyrogonites are known to mineralize over a 1 month period toward the end of the growing season (Jones et al., 1996). 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 like *Aplodinotus grunniens*, growth is most rapid during the warmest part of the year (Patterson et al., 1993).

Therefore, in theory, the mean paleotemperature calculated by combining the $^{18}O_{Local\ water}$ value with the ^{18}Ovalue from the fish scales and *Lymnaea* shells thermometry equations (number 5 and 6) should be concordant and most likely represent the mean temperatures of the growing seasons. However, our results are not concordant (Fish scales = 21 $\pm$ 2°C, *Lymnaea* = 23 $\pm$ 2°C). This may be due to diagenetic alteration of the *Lymnaea*. The original aragonite shell has been altered to calcite which may have resulted in an increased scatter in, and potential alteration of, the mean $\delta^{18}O$ value.

The mean paleotemperature calculated using the charophyte gyrogonite thermometry equation (number 7, 21 $\pm$ 2°C) on the other hand, most likely represents the mean temperature of a single month toward the end of the growing season and the fish otoliths mean paleotemperature (Eqn. 8, 28 $\pm$ 2°C) most likely represents the mean temperature of the warmest months of the growing season. However, in interpreting the fish otolith data one should also consider whether the paleotemperature might be related to the original determination of the freshwater otolith thermometry equation (Patterson et al., 1993, Eqn. 8). The freshwater otolith species used herein, *Umbra valida*, is extinct. However, the genus *Umbra* in the family Umbridae is not, but neither it, nor its closest family relative Esocidae (pikes), were represented in the Patterson et al. (1993) study. 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 may need to be made at least family/genus specific like the *Micropogonias undulatus* marine otolith thermometer of Thorrold et al. (1997) and the *Lymnaea* gastropod thermometry equation of White et al. (1999) used here. However, it should be noted that the thermometry equation used for the fish scales was not species, genus or family specific but composition specific, i.e., phosphate. It produced a similar mean temperature to that of the genus specific *Lymnaea* equation, which was to be expected as they both should represent the mean temperatures of the growing seasons. Therefore, the fact that the fish otolith genus used in this study was not one used by Patterson et al. (1993) in their determination of a freshwater fish otolith thermometry equation should be noted, but at the same time it probably does not affect the result.

Based upon the mean paleotemperatures calculated in this study, i.e., 1) a mean annual temperature of 18 $\pm$ 1°C (possibly slightly overestimated); 2) a mean temperature of the growing season of 21 $\pm$ 2 °C (based upon the fish scales only and ignoring the diagenetically altered *Lymnaea* shells); 3) a warmest month mean temperature of 28 $\pm$ 2°C; 4) a month toward the end of the growing season having a mean temperature of 21 $\pm$ 2°C; it can be argued that the climate during deposition of the Osborne Member was likely to have been subtropical to warm temperate.

6.3. Errors, Standard Deviations, and Seasonality

The five mean paleotemperatures reported here are each accompanied by a 2σ standard error and a 2σ standard deviation which have been propagated from the standard errors and standard deviations on the mean $\delta^{18}O$ values of the individual paleoproxies (see Eqns. 9 and 10). The standard error represents the accuracy to which the mean of a data set, in our case temperature, has been calculated. Therefore, when comparing the level of agreement between the four calculated mean paleotemperatures the standard error is used. Even though this indicates that three out of the five mean paleotemperatures are concordant it does not necessarily mean that they record the same paleoinformation, i.e., two represent the mean paleotemperature of the growing season and one the mean paleotemperature of a single month toward the end of the growing season. The standard deviation, on the other hand, represents the fluctuation of data around its mean value. In the case of our data these potentially contain four principle components: 1. analytical error ($\delta^{18}O_{Phosphate}$ = $\pm$ 0.2‰ and $\delta^{18}O_{Carbonate}$ = $\pm$ 0.05‰); 2. variable nonequilibrium metabolic fractionation

in both enamel and carbonate precipitation; 3. variable water source intake, such as water sourced from food; 4. variable water temperature due to seasonal fluctuations in temperature. Of these four components only that contributed by analytical error can be directly determined and this is relatively small with an average contribution of 1°C to the standard deviation. In addition, it has already been argued that the deviation contributed by *Thalerimys* and *Isoptychus* sourcing their water intake from food is also likely to be minor. The contribution from variable nonequilibrium metabolic fractionation in enamel precipitation may be more significant and could be related to the physiology of the small mammal species used in this study. Models derived by Bryant and Froelich (1995) indicate that the larger the mammal body size, the greater the proportion of the oxygen intake from drinking water and the lower the body-mass fractionation and potential error. However, Lindars et al. (2001) showed that in a study of modern rodents there was a good correlation between the calculated $\delta^{18}O$ of local water using *Apodemus sylvaticus* teeth and a local water source from which they drank. This would indicate that the potential errors associated with the use of small mammals may not be as significant as indicated by the models of Bryant and Froelich (1995). This, therefore, leaves a significant proportion of the standard deviation in the paleotemperatures resulting from real variations in water temperature attributable to seasonal fluctuations. To determine the extent of seasonal temperature fluctuation in the Osborne Member individual growth bands in either the fish otoliths (see, e.g., Wurster and Patterson, 2001), or the gastropods (e.g., Andreasson and Schmitz, 1996) could be analyzed in the future. Here we suggest that the large 2σ standard deviations on all the paleotemperatures (14–18°C) are probably indicative of a seasonal climate during deposition of the Osborne Member.

7. CONCLUSIONS

The enamel of fossil rodent teeth (Eocene Headon Hill Formation, UK) is shown to have largely resisted diagenetic alteration. Therefore, the phosphate oxygen isotopic composition of the enamel of *Thalerimys* and *Isoptychus* cheek teeth (and by extrapolation that of other rodents, or other small plant-eating mammals) can be used to determine the paleo-$\delta^{18}O$ value of ingested local water. In addition, ingestion of fractionated local water (possibly as a result of evaporation) can also be observed and corrected for. The $\delta^{18}O$ local water value can then be used to calculate a mean annual temperature and can also be combined with the $\delta^{18}O$ value of four other paleoproxies (i.e., fish otoliths, charophyte gyrogonites, fish scales and *Lymnaea* shells) to determine four other mean paleotemperatures. However, the annual breeding period of the rodents and the timing of carbonate and phosphate precipitation of the other paleoproxies determine the exact meaning of these different mean paleotemperatures. As a result of our interpretation of the most likely annual breeding period and the timing of precipitation of each paleoproxy it is argued that, firstly, the mean annual temperature of 18 ± 1°C is likely to be a slight overestimate. Secondly, of the other four calculated mean paleotemperatures, that determined using the fish scale thermometry equations (21 ± 2°C) is most likely to represent the mean temperature of the growing season. In theory this should be concordant with the mean temperature calculated using the *Lymnaea* shells (23 ± 2°C), however, that this is not the case and may be due to the diagenetic alteration of their original aragonite shells. The mean paleotemperature calculated using the charophyte gyrogonite thermometry equation (21 ± 2°C), on the other hand, most likely represents the mean temperature of a single month toward the end of the growing season. The mean paleotemperature (28 ± 2°C) calculated from fish otoliths most likely represents that of the warmest months within the growing season. Our estimates of mean annual temperature (18 ± 1°C), mean growing season temperature (21 ± 2°C), and mean temperature of the warmest months (28 ± 2°C), the latter two with associated large standard deviations, suggest a seasonal subtropical to warm temperate climate during deposition of the Late Eocene Osborne Member.

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APPENDIX

Vennemann et al. (2002) reported interlab $\delta^{18}O$ values for a range of new phosphate oxygen isotope samples. Vennemann and Tütken (Universität Tübingen) supplied us with a range of these published phosphate samples, in addition to other laboratory samples, to further validate the pretreatment and direct laser fluorination (DLF) techniques of Lindars et al. (2001). A list of all the samples supplied to us is given in Table A1. The $\delta^{18}O$ values we measured using the DLF technique, in addition to the values quoted in Vennemann et al. (2002), and those obtained by independent analyses, are shown in Table A2.

A.1. $\delta^{18}O_{PO4}$ Values from the DLF Analysis of Silver Phosphate Precipitates

Four silver phosphate samples were analyzed using the DLF technique without any prior pretreatment (i.e., they were not heated to 400°C and not fused in the sample chamber before analysis). As can be seen in Table A2 and Figure A1 our results compare well with those reported in Vennemann et al. (2002) over a $\delta^{18}O$ range of 5–22‰. When comparing our DLF results with the three different techniques (BrF_5 fluorination, high temperature reduction, and silica tube) reported in Vennemann et al. (2002), all our values, with the exception of that for the synthetic apatite lab standard (HAP), plot within error. HAP is slightly lower, but there is only a silica tube technique value with which to compare it. Considering the range between values reported for GW1 and TU2 using the 3 different techniques, we are confident that, if HAP had also been measured using all available techniques rather than just one, our result would plot within error of them.

A.2. $\delta^{18}O_{PO4}$ Values from the DLF Analysis of Pretreated NIST 120c and Various Other Tooth Samples

Five original unprecipitated phosphate samples (whole tooth, tooth enamel and phosphate sediment) were pretreated by heating to 400°C and fused under vacuum in the sample chamber before DLF analysis (Lindars et al., 2001). In Table A2 and Figure A2 our pretreated DLF $\delta^{18}O_{PO4}$ values are compared with both published and reported values obtained using different techniques to measure silver or bismuth phosphate precipitates of the same samples.

A.2.1. NIST 120c

As can be seen in Figure A2 our pretreated DLF mean $\delta^{18}O_{PO4}$ value for NIST 120c (21.2 ± 0.3‰) compares well with values reported by Fricke et al. (1998) (using $AgPO_4$ 21.8 ± 0.3‰), Bryant et al. (1994) (using $BiPO_4$ 21.4 ± 0.4‰) and Lécuyer et al. (1993) (using $AgPO_4$ 21.7 ± 0.2‰). Vennemann et al. (2002) record slightly higher values for an $AgPO_4$ precipitate of NIST 120c than has been previously reported (Table A2 and Fig. A2). However, even though our pretreated DLF $\delta^{18}O_{PO4}$ value is lower than that reported by Vennemann et al. (2002) on a silver phosphate precipitate, it does plot within error of the

Table A1. A list of the phosphate samples, their references, and method of analysis, which were used to test the validity of our direct laser fluorination technique.

Name	Type of sample	Reference	Method of analysis
TU1	Synthetic KH_2PO_4 precipitated as Ag_3PO_4	Vennemann et al. (2002)	DLF
TU2	Synthetic KH_2PO_4 precipitated as Ag_3PO_4	Vennemann et al. (2002)	DLF
ZEQ56	Separated enamel from one recent horse tooth (M3)	Thomas Tütken (Personal Communication)	Heat 400°C + fused before DLF
FZMAN-S8	Separated tooth enamel from a fossil mammoth collected from the bottom of the North Sea off the coast of the Netherlands	Thomas Tütken (Personal Communication)	Heat 400°C + fused before DLF
NIST120c	Phosphate-bearing sediment	Internal lab standard	Heat 400°C + fused before DLF
GW1	Mixture of tooth dentine and enamel from one Great White Shark tooth (*Carcharodon carcharias*) Original sample + a sample precipitated as Ag_3PO_4	Vennemann et al. (2002)	Original sample Heat 400°C+ fused before DLF Ag_3PO_4 sample DLF
HAP	Synthetic apatite precipitated as Ag_3PO_4 Universität Tübingen internal lab standard	Thomas Tütken (Personal Communication)	DLF

Table A2. The $\delta^{18}O_{PO4}$ results from the direct laser fluorination of the phosphate samples listed in Table 1. In addition, the $\delta^{18}O_{PO4}$ results quoted in Vennemann et al. (2002) and those quoted to us as unpublished results are also listed.

	TU1	TU2	HAP	GW1 Ag3PO4	GW1 raw	ZEQ56	FZMANS8	NIST 120c
Analytical method and reference	Synthetic apatite samples			Whole tooth sample		Enamel samples		Sediment sample
	Silver phosphate samples					Unprecipitated, original samples		
DLF results	20.21 ± 0.4‰ n = 7	4.74 ± 0.17‰ n = 4	16.20 ± 0.13‰ n = 4	21.54 ± 0.3‰ n = 5	16.59 ± 0.39‰ n = 12	13.68 ± 0.15‰ n = 8	15.04 ± 0.17‰ n = 8	21.13 ± 0.40‰ n = 40
BrF5 results, Vennemann et al. (2002)	21.11 ± 0.19‰ n = 7	5.45 ± 0.10‰ n = 7		22.65 ± 0.18‰ n = 13				22.58 ± 0.09‰ n = 3
HTR normalized results Vennemann et al. (2002)	21.11 ± 0.57‰ n = 22	5.35 ± 0.62‰ n = 14		22.66 ± 0.14‰ n = 18				22.09 ± 0.51‰ n = 18
Silica tube results Vennemann et al. (2002)	21.38‰ n = 1	6.23 ± 0.17‰ n = 2		21.3 ± 0.12‰ n = 5				21.70 ± 0.38‰ n = 4
Silica tube results Thomas Tüiken (Personal Communication)			17.03 ± 0.3‰			14.50‰ n = 1	15.50 ± 0.4‰ n = 2	
Some other referenced results								
Geophysical Laboratory HTR result on $AgPO_4$ in Vennemann et al. (2002)								23.3 ± 0.5‰
On $BiPO_4$, Bryant et al. (1994)								21.4 ± 0.4‰
On $AgPO_4$ Lécuyer et al. (1993)								21.7 ± 0.2‰
On $AgPO_4$, Fricke et al. (1998)								21.8 ± 0.3‰

silica tube and high temperature reduction technique values. Regardless of this, NIST 120c is not a phosphate oxygen isotope standard, but rather a phosphate bearing sediment. Therefore, even though it was used routinely by previous authors as justification for the validity of their biogenic apatite results (see, among others, Lécuyer et al., 1993; Bryant et al., 1994; Fricke et al., 1998), our ability, using the DLF technique, to produce results similar to those already published may not be sufficient justification for the DLF technique.

A.2.2. ZEQ56 and FZMAN-S8 Tooth Enamel Samples

The pretreated FZMAN-S8 enamel sample, even though it has a slightly lower DLF value than that reported by Thomas Tütken (personal communication) using the silica tube technique on a silver phosphate precipitate, does plot within error of the value he reports. This is not the case with the ZEQ56 enamel sample. However, there is only one silica tube result with which to compare it. Until more results become available, if we assume that the same error is present on the ZEQ56 sample as on the FZMAN-S8 sample (±0.4‰, see dashed white error bars on Fig. A2) then our DLF result plots within error of the silica tube result. In addition, it should also be noted that with respect to NIST 120c, that there is an approximate 2‰ variation in its value depending upon which technique and which laboratory was used to analyze it (Table A2). If a similar distribution were repeated on these two enamel samples then it is clear that our DLF values would be likely to be indistinguishable from them.

Slightly lower $\delta^{18}O_{PO4}$ values obtained by DLF of ZEQ56 and FZMAN-S8 enamel samples indicates that during fusing before DLF analysis there is no evidence for isotopic mixing between PO_4^{3-} and CO_3^{2-} oxygen. If exchange had occurred then, because CO_3^{2-} oxygen is between 8.6–9.1‰ higher than the PO_4^{3-} oxygen (Iacumin et al., 1996), it would be expected that mixing would have resulted in our DLF $\delta^{18}O_{PO4}$ values being higher than those reported using a silver phosphate precipitate.

A.2.3. GW-1 Whole-Tooth Sample

GW-1 is a crushed and homogenized sample of numerous whole teeth from a single Great White Shark, and as their teeth have relatively thin enamel, it can be assumed that the sample is dominated by dentine. As can been seen in Figure A2 and Table A2, the pretreated DLF $\delta^{18}O$ result from this dentine-rich sample is approximately 6‰ lower than that reported by Vennemann et al. (2002) using a silver phosphate precipitate of it. Vennemann (personal communication) obtained a similar value to ours (16.5‰) on the same original GW1 sample when he had heated it to 650°C and then analyzed it using a laser fluorination system similar to the DLF technique (note that he did not fuse the sample before analysis). However, when he used the same laser fluorination technique on enamel separated from the same Great White Shark he obtained a $\delta^{18}O$ value of ~22‰ (Vennemann, personal communication). Unfortunately, there was no remaining enamel sample for us to compare with that result.

A.2.4. Fourier-Transform Infrared (FT-IR) Spectral Results

The measured $\delta^{18}O_{CO3}$ value for GW-1 is ~31‰ (Vennemann, personal communication). If there was isotopic mixing between PO_4^{3-} and CO_3^{2-} oxygen during fusing of the original unprecipitated GW-1 it would be expected that the DLF technique would give a $\delta^{18}O$ value greater than that reported on the silver phosphate precipitate. This is

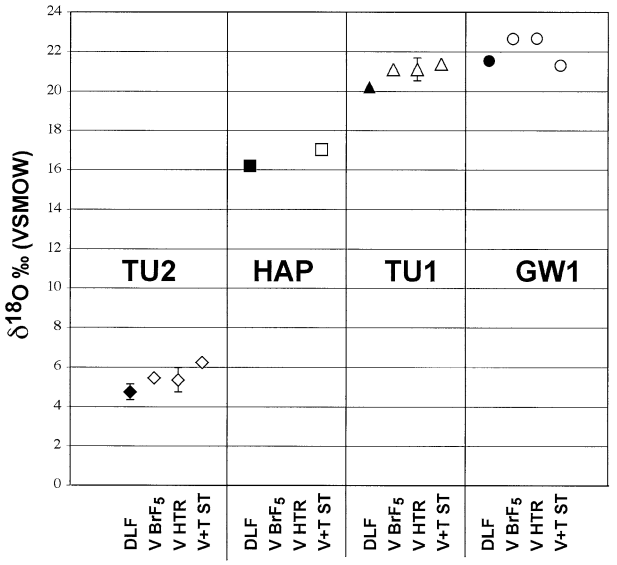

Fig. A1. Plot of our $\delta^{18}O$ results (black symbols) from DLF of four silver phosphate samples against those reported using other techniques (white symbols). See Table A1 for a full description of the samples. V BrF_5 = fluorination technique reported in Vennemann et al. (2002). V HTR = high-temperature reduction technique reported in technique reported in Vennemann et al. (2002). V+T ST = silica tube technique reported in Vennemann et al. (2002) and that used by Thomas Tütken (personal communication). Unless shown, error bars are contained within individual plotted points.

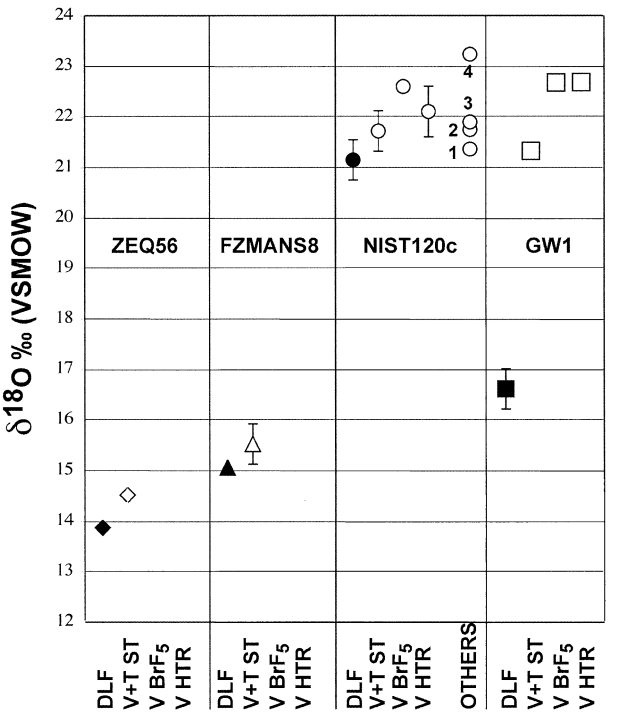

Fig. A2. Plot of our $\delta^{18}O$ results (black symbols) from the DLF of four original unprecipitated phosphate samples against those reported using other techniques on silver phosphate precipitates (white symbols). See Table A1 for a full description of the samples. V BrF_5 = fluorination technique reported in Vennemann et al. (2002). V HTR = high-temperature reduction technique reported in Vennemann et al. (2002). V+T ST = silica tube technique reported in Vennemann et al. (2002) and that used by Thomas Tütken (personal communication). Others = other referenced values, as follows: 1, Bryant et al. (1994); 2, Lécuyer et al. (1993); 3, Fricke et al. (1998); 4, Geophysical Laboratory HTR result in Vennemann et al. (2002). The white dashed error bar on the ZEQ56 sample measured by the silica tube technique (V+T ST) is ±0.3‰. As there is only one result for this sample, no error bar could be assigned. Therefore, until more analyses are conducted it is assumed that it will have the same error as is present on the FZMANS8 enamel sample, which was measured using the same silica tube technique. Unless shown, error bars are contained within individual plotted points.

clearly not the case as our DLF value is lower than that obtained by Vennemann et al. (2002) on a silver phosphate precipitate.

To investigate what may be causing the low $\delta^{18}O$ value Fourier Transform Infrared (FT-IR) analysis of a fused bead of original unprecipitated GW-1, prepared as for DLF analysis, was conducted on the Buker IFS 113v FT-IR spectrometer at Cambridge University. As can be seen in Figure A3, the spectrum for this GW-1 sample shows large absorption peaks at around 680–1280 cm^{-1} and around 1970–2094 cm^{-1}, which are representative of the first and second overtones of PO_4^{3-}, and a broad peak between 2750–3623 cm^{-1}, which is representative of O-H stretching. FT-IR spectra of fused beads of the enamel samples ZEQ56 and FZMAN-S8, prepared under the same conditions as GW-1, show the same two PO_4^{3-} overtone peaks, but a significantly reduced, O-H stretching peak (Fig. A3). In all spectra there is also evidence for minor peaks around 1388 cm^{-1} and 2321 cm^{-1}. The peak around 1388 cm^{-1} is difficult to resolve because it is located upon the shoulder of the second PO_4^{3-} overtone. However, if it represents residual CO_3^{2-} it would only indicate trace amounts. The peak around 2321 cm^{-1}, which fluctuates in intensity between samples is representative of CO_2 and is part of the blank background. GW-1 is the only sample that shows a significant O-H stretching peak. Therefore, it can be argued that the reason why the DLF of this dentine-rich sample gave a $\delta^{18}O$ value ~6‰ lower than that reported by Vennemann et al. (2002) on a silver phosphate precipitate, is probably due to the retention of residual hydroxyl oxygen after fusing. Unfortunately, as no biogenic apatite standard of known composition was available for our FT-IR analysis the percentage of residual hydroxyl oxygen in the GW-1 fused bead could not be determined. Furthermore, un-like with carbonate oxygen, the $\delta^{18}O$ value of hydroxyl oxygen compared to phosphate oxygen in biogenic apatite is unknown. However, the fact that GW-1 was the only sample with residual hydroxyl oxygen detected after fusing, and assuming that the contribution of this residual oxygen is the reason for the low $\delta^{18}O$ value obtained by DLF, would indicate that the $\delta^{18}O$ value of hydroxyl oxygen must be < 16‰. Finally, an FT-IR spectrum was also run upon a fused bead of sample S79 (*Thalerimys* M1/2 enamel, $\delta^{18}O = 18.16$‰). As can be seen in Fig. A3, the *Thalerimys* enamel sample has a similar spectrum to that of the enamel samples ZEQ56 and FZMAN-S8. Our DLF analyses on enamel samples ZEQ56 and FZMAN-S8 gave results within error of those obtained using the silica tube technique. This validates the use of our DLF technique for analysis of *Thalerimys* and *Isoptychus* enamel samples.

A.3. Conclusions

The DLF and its associated pretreatment technique was designed to analyze small mammal teeth with a separated enamel sample mass of approximately 1 mg. This sample mass is at the lowest limit of any available technique that required prior precipitation of silver phosphate (see Vennemann et al., 2002, for review). Even though it was first reported in Lindars et al. (2001) validated by the reproduction of NIST 120c values similar to those already reported, this was considered to be insufficient justification following the publication of Vennemann et al.'s (2002) paper. Therefore, Vennemann and Tütken supplied a selection of samples to enable further testing of our DLF technique. The results of these tests show that reported $\delta^{18}O$ values for both silver phosphate samples and tooth enamel samples can be successfully reproduced using the DLF technique. However, the technique does not appear to be valid for dentine-rich samples and, by association, bone samples. FT-IR analyses indicate that the reason for this may be the retention of hydroxyl oxygen in fused dentine-rich samples. In addi-

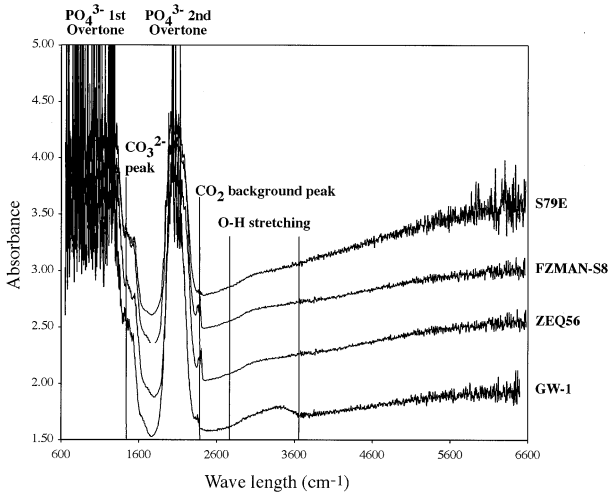

Fig. A3. FT-IR spectra of fused beads of original unprecipitated GW1, ZEQ56, FZMANS8, and *Thalerimys* enamel sample S79. All fused beads were prepared using the same technique as for DLF analysis. See Table A1 for a description of each sample.

tion, the fact that our DLF $\delta^{18}O$ results on the enamel samples from other laboratories are slightly lower than, but usually within error of, those reported using silver phosphate techniques indicates that there is no mixing between PO_4^{3-} and CO_3^{2-} oxygen during fusing of our enamel samples.