The palaeoecology of the non-mammalian cynodonts *Diademodon* and *Cynognathus* from the Karoo Basin of South Africa, using stable light isotope analysis

Jennifer Botha[a,*], Julia Lee-Thorp[b], Anusuya Chinsamy[a]

[a]*Zoology Department, University of Cape Town, Rondebosch, Cape Town, 7700, South Africa*
[b]*Archaeology Department, University of Cape Town, Rondebosch, Cape Town, 7700, South Africa*

Abstract

The palaeoecology of the coeval Middle Triassic non-mammalian cynodonts, *Diademodon* and *Cynognathus* (Therapsida) remains poorly understood although their gross morphology has been studied intensively. Significant differences in their growth patterns suggest inherent biological differences, despite having inhabited similar environments. In this study, the palaeoecology of *Cynognathus* and *Diademodon* specimens were examined using intra-tooth stable carbon and oxygen isotope analyses of enamel carbonate. The resulting stable isotope patterns of *Cynognathus* and *Diademodon* were compared with that of *Crocodylus niloticus* and published mammalian tooth enamel data. Predictably, the non-mammalian cynodont $\delta^{13}C$ values fall within the expected range for C_3 plant diets. Both $\delta^{18}O$ and $\delta^{13}C$ values of *Diademodon* are markedly more depleted than those of *Cynognathus*, suggesting that the former fed in shadier, damper areas, was nocturnal and/or depended more directly on environmental water. The seasonal amplitude reflected in the *Cynognathus* teeth is relatively low. However, high amplitude, directional $\delta^{18}O$ intra-tooth variations in the *Diademodon* teeth are comparable to, or higher than, those observed for extant mammalian and *C. niloticus* teeth from semi-arid, seasonal regions. This suggests that marked seasonality prevailed in the Karoo Basin during the Middle Triassic, and that *Diademodon* was sensitive to these variations. These isotopic differences between *Diademodon* and *Cynognathus* indicate differing responses to climatic fluctuations and reveal new insights into the palaeoecology of non-mammalian cynodonts.
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1. Introduction

* Corresponding author. Current address: Karoo Palaeontology Department, National Museum, P. O. Box 266, Bloemfontein, 9300, South Africa. Tel.: +27 51 447 9609; fax: +27 51 447 6273.
 E-mail address: jbotha@nasmus.co.za (J. Botha).

Diademodon and *Cynognathus* are Middle Triassic, derived non-mammalian cynodonts, whose remains are found in deposits from the *Cynognathus*

Assemblage Zone, Burgersdorp Formation, Beaufort Group, Karoo Super Group of South Africa (Rubidge, 1995). The non-mammalian cynodonts (Therapsida; Cynodontia) are particularly significant as their long fossil record provides information on the evolutionary sequence leading to the first true mammals. The cranium of *Diademodon* is characterized by a narrow snout, wide orbital region and antero-dorsally placed eyes (Seeley, 1895; Watson, 1911; Kemp, 1982), and the temporal fenestrae are large and separated from one another by a narrow, high sagittal crest (Kemp, 1982). The postorbital region of the zygomatic arch is also unusually deep (Brink, 1955). *Diademodon* is one of the first non-mammalian cynodonts to exhibit a combination of a mammal-like jaw adductor musculature and precise postcanine occlusion (Grine, 1976, 1977; Grine et al., 1979; Kemp, 1982). The development of tooth occlusion facilitated the modification of different tooth types to encompass a varied diet (Hopson, 1991) and, therefore, it has been argued that *Diademodon* was omnivorous (Grine, 1978). The cranial features that distinguish *Cynognathus* are long, narrow snout, enlarged dentary bone, robust orbital bar and zygomatic arch and an unusually large squamosal bone. The sectorial teeth have wear-facets indicating that the teeth occluded and studies have inferred carnivory for this genus (Seeley, 1908; Broom, 1911, 1913; Gregory and Camp, 1918; Kemp, 1982).

Although they have distinctive cranial and dental morphologies, the postcranial skeletons of these genera are, with the exception of slight differences in the neural spines and centra of the vertebral column (Brink, 1955), relatively indistinguishable from one another (Brink, 1955; Jenkins, 1971). As their remains are frequently found in the same fossil assemblages, it is difficult to distinguish their postcrania from one another when no associated cranial material is preserved. Recently, however, Botha and Chinsamy (2000) found distinct histological patterns in the limb bones of *Diademodon* and *Cynognathus*, respectively. The bone tissue of *Diademodon* is zonal, indicating cyclical patterns of growth, which *may* have been seasonally influenced. In contrast, the bone tissue of *Cynognathus* consists of uninterrupted, rapidly forming, fibro-lamellar bone, which indicates a rapid, sustained growth strategy (Botha and Chinsamy, 2000).

Thus, although these genera were contemporary, experienced similar climate and broad environmental conditions, and are similar in size (up to 2 m in length) and postcranial morphology, their diets and their growth patterns differed. The latter might be due to inherent physiological differences, or to differing sensitivity to seasonal fluctuations (Botha and Chinsamy, 2000). Since there are significant differences in the growth patterns of these genera, their habitat or microhabitat preferences may also have differed.

Stable light isotope analysis is a tool frequently used to examine the ecology of extant (e.g. Vogel, 1978; Koch et al., 1995; Cerling et al., 1999; Sponheimer and Lee-Thorp, 2001; Sponheimer et al., 2003), and extinct animals (Koch et al., 1989; Lee-Thorp et al., 1989; Thackeray et al., 1990; Quade et al., 1992; Bocherens et al., 1996; MacFadden, 1998; Sharp and Cerling, 1998; Franz-Odendaal et al., 2002; Zazzo et al., 2002; Stanton Thomas and Carlson, 2004; Tütken et al., 2004). Stable light isotope ratios in the tissues of living organisms track or record the isotopic composition of features of the environment in which an animal lives. Certain aspects of an extinct animal's behaviour, dietary preferences and the nature and amplitude of the seasonal variation in its environment can be determined by examining the stable oxygen and carbon isotope signatures in fossil bones and teeth (Koch, 1998). The proviso is that the stable isotope composition has not been substantially altered by diagenetic processes over time. Since tooth enamel is more crystalline, has minimal organic content, and is compact with little pore space (LeGeros, 1991), it is more resistant to physical, chemical and isotopic alteration as compared to dentine or bone. Furthermore, since enamel is not subjected to remodelling as is the case for bone, the isotope composition of enamel increments reflects relatively discrete intervals of time.

The stable light isotopes of oxygen ($^{18}O/^{16}O$) obtained from skeletal tissues such as bones and teeth provide information relating to environmental fluctuations in meteoric water, with a series of biological filters related to the drinking behaviour (Luz et al., 1984; Luz and Kolodny, 1985; Fricke et al., 1998; Sponheimer and Lee-Thorp, 2001) or thermophysiology of a species (Kohn, 1996; Stuart-Williams and Schwarcz, 1997). Meteoric water (either surface, flu-

vial, or lacustrine) is usually the main source of ingested water, although many species rely heavily on leafwater. The temperature of biomineral formation and the $\delta^{18}O$ (expressed as parts per thousand (‰) using the δ (delta) notation) of body water determine the oxygen isotope composition of skeletal tissues. As mammals maintain relatively constant body temperatures, their skeletal tissue $\delta^{18}O$ values are controlled by body water composition (Luz and Kolodny, 1985). This is not the case for reptiles, although reptiles frequently control their body temperatures by basking or shuttling between land and water in the case of semi-aquatic species (Seebacher and Grigg, 1997; Stoskopf et al., 2001). Body water $\delta^{18}O$ is controlled by the mass balance of oxygen entering and exiting the body. Oxygen enters the body principally as inspired atmospheric oxygen, drinking water and water in food and as oxygen bound in food. Oxygen is lost as liquid water in urine, sweat and faeces, and as water vapour and CO_2 in respiratory gases (Bryant and Froelich, 1995). Atmospheric oxygen is well mixed so that isotopic composition is relatively constant with the result that $\delta^{18}O$ of ingested water is the major variable influencing $\delta^{18}O$ variability in bones and teeth (Luz and Kolodny, 1985; Kohn, 1996; Kohn et al., 1996).

The isotopic composition of meteoric water is sensitive to a series of climatic factors including oceanic source, latitude and mean annual temperature (Dansgaard, 1964). As the isotopic composition of skeletal tissues is mainly controlled by the $\delta^{18}O$ value of body water, these tissues can be used to obtain information relating to environmental temperature and precipitation (Luz and Kolodny, 1985; Bocherens et al., 1996; Stuart-Williams and Schwarcz, 1997). In addition, $\delta^{18}O$ variations amongst different animals in one area can provide indirect information about habitat, foraging preferences, and thermophysiological adaptations (Bocherens et al., 1996; Kohn, 1996; Sponheimer and Lee-Thorp, 2001). For example, herbivorous animals feeding at night are expected to be depleted in ^{18}O compared to diurnal animals (Bocherens et al., 1996), as plant water is not enriched at night (Yakir, 1992). Animals that obtain their liquid water from ^{18}O-enriched plant sources, such as leaves, are frequently more enriched in ^{18}O compared to animals that obtain their liquid water more directly, by drinking regularly (Sponheimer and Lee-Thorp, 2001).

Oxygen is present in both phosphate (PO_4^{3-}) and carbonate (CO_3^{2-}) ions in tooth enamel apatite. $\delta^{18}O$ phosphate ($\delta^{18}O_p$) and $\delta^{18}O$ carbonate ($\delta^{18}O_c$) are offset, but positively correlated (Bryant et al., 1996a; Iacumin et al., 1996) showing that they reflect the same phenomena. Many studies have concentrated on $\delta^{18}O_p$, as the P–O bond is exceptionally strong, rendering phosphate oxygen less exchangeable compared to carbonate oxygen (Shemesh et al., 1983; Longinelli, 1984; Luz and Kolodny, 1989; Iacumin et al., 1996). Recent studies, however, have shown that reliable biogenic oxygen signals can be obtained from tooth enamel carbonate ranging in age from Pleistocene (Bocherens et al., 1996; Sponheimer and Lee-Thorp, 1999) to Miocene (Cerling et al., 1997; MacFadden, 1998). The reliability of $\delta^{18}O$ from both phosphate and carbonate is dependent on the lack of recrystallization resulting in replacement of the original ions (Lee-Thorp, 2002; Botha et al., 2003). The advantage of apatite carbonate analysis is that it yields $\delta^{18}O$ and $\delta^{13}C$ simultaneously, from small samples and without requiring intricate chemical preparation.

Stable carbon isotopes ($^{13}C/^{12}C$) provide information relating to plants at the base of the food web. All plants discriminate against ^{13}C in favour of ^{12}C during photosynthesis, but the direct Rubisco process in plants following C_3 photosynthesis leads to much higher fractionation (Berry, 1988). Plants using the C_3 photosynthetic pathway, including most trees, shrubs, herbs and temperate grasses, exhibit values between $-30‰$ and $-23‰$ (Smith and Epstein, 1971; Vogel, 1978). Amplitude of fractionation–and hence $\delta^{13}C$ value of the plant–is influenced by a number of climatic and environmental factors, which explains the wide range of carbon isotope values for C_3 plants. Shade, moist conditions and lower temperatures are associated with lower $\delta^{13}C$, while conversely, high light levels, aridity and warm temperatures are associated with higher $\delta^{13}C$ values (Tieszen and Boutton, 1989). Plants using the C_4 photosynthetic pathway (including tropical grasses, some shrubs), or the Crassulacean Acid Metabolism photosynthetic pathway (including mostly succulents) are more recent evolutionary developments (Smith and Epstein, 1971; Ehleringer et al., 1997; Keeley and Rundel, 2003) and do not apply to this study.

Herbivores incorporate dietary plant $\delta^{13}C$ signatures into their tissues with some further fractionation

occurring during biochemical processes and precipitation of carbonate, that result in apatite $\delta^{13}C$ values that are about 12–14‰ more positive than the plants (Lee-Thorp et al., 1989; Cerling and Harris, 1999). Thus, extant herbivores feeding exclusively on C_3 plants have $\delta^{13}C$ values for mineral carbonate of approximately $-17‰$ to $-10‰$. These typical values for plants and animals are ultimately dependent on the $\delta^{13}C$ value of atmospheric CO_2 of the time.

As tooth enamel is laid down more or less incrementally from the top to the base of the crown, short-term seasonal changes that occur during enamel maturation are recorded in individual teeth, providing information about seasonality. For example, Fricke and O'Neil (1996) found a 5.6‰ intra-tooth oxygen isotopic variability in 500-year-old Bison teeth, which reflected the extent of $\delta^{18}O$ environmental seasonality reasonably well. Thus, a time series of changes in ingested water $\delta^{18}O$ values, which in turn reflect variability in the local climate, can be obtained by the serial sampling of teeth (Fricke et al., 1998). Similarly, Balasse et al. (2002) found that $\delta^{13}C$ and $\delta^{18}O$ values in the tooth enamel of archaeological sheep in the Western Cape, South Africa, corresponded to seasonal variations of 3.5‰ in both cases. However, enamel matures for some time after initial mineralisation, so that $\delta^{13}C$ and $\delta^{18}O$ variations obtained by serial sampling cannot represent entirely discrete time intervals, but rather a dampened isotopic sequence (Passey and Cerling, 2002; Ballasse, 2003).

Most isotope-based studies of seasonality on incremental vertebrate tissues have focused on mammals, since mammals retain constant body temperatures. Therefore, any variability in the isotope signature can be ascribed to environmental variation and not to variability in the temperature of crystallization. Crocodilians and lizards do not maintain constant body temperatures and might, therefore, have larger intra-bone and inter-bone isotopic variability, which may be enhanced or reduced depending on the effect of body temperature (Barrick and Showers, 1995) and their thermoregulatory behaviours, such as heat seeking or heat avoidance (Kohn, 1996; Seebacher and Grigg, 1997).

As Diademodon and Cynognathus utilized significantly different growth strategies (Botha and Chinsamy, 2000), it is likely that their ecological niches differed as well. This study examines the palaeoecology of these derived non-mammalian cynodonts using stable carbon and oxygen isotope analyses, to deduce whether ecological differences, such as water-related and foraging behaviours, occurred between the two taxa. Sequential analysis of an intra-tooth series provides a tool to examine the nature and amplitude of the seasonal variability experienced by these animals, and to be compared with that of extant mammals and reptiles.

2. Materials and methods

The fossil material used in this study consists of two Cynognathus and eight Diademodon teeth, collected from Karoo deposits in Burgersdorp and Lady Frere, respectively. As Cynognathus specimens are relatively rare, only a small sample set of their teeth was available for analysis.

As Diademodon and Cynognathus are derived non-mammalian cynodonts with several mammal-like morphological traits (Kemp, 1982; Hopson, 1991), and because of their transitional status between reptiles and mammals, the results were compared with both Crocodylus niloticus (Table 1) and published extant mammalian data. C. niloticus was chosen as the representative extant reptile because they have large teeth with reasonably thicker enamel compared to other reptiles. These teeth were obtained from Stellenbosch, in the Western Cape, South Africa, which is today a relatively arid area that experiences marked summer warmth and aridity, and cool winter precipitation. Therefore, the $\delta^{13}C$ and $\delta^{18}O$ in these teeth are expected to reflect these marked seasonal changes. The teeth of the monitor lizard, Varanus, were also tested, but proved too small and the enamel too thin for isotopic analysis.

The time reflected in the tooth enamel depends on the duration of enamel formation but, enamel mineralisation, eruption and tooth replacement rates have not been quantified for many extant animals. Line (2000) found that enamel in an extant crocodilian took approximately 2.5 months to form. In contrast, human enamel in later erupting teeth (e.g., the M3's) may take 4 to 6 years to form (Brown et al., 1960; Reid and Dean, 2000). Tooth replacement rates in reptiles provide clues about enamel formation periods. The rate of tooth replacement in young Alligator mississippiensis

Table 1
The specimens and their localities examined in this study

Genus	Specimen number	Assemblage zone	Locality
Crocodylus niloticus	CROC 1	–	Le Bonheur Crocodile Farm, Stellenbosch
	CROC 2	–	Le Bonheur Crocodile Farm, Stellenbosch
	CROC 3	–	Le Bonheur Crocodile Farm, Stellenbosch
Cynognathus	SAM-PK-K3029	*Cynognathus*	Burgersdorp
	BP/1/1675d	*Cynognathus*	Burgersdorp
Diademodon	UMCZ T504a, 443	*Cynognathus*	Lady Frere
	470, 471, 472	*Cynognathus*	Lady Frere
	473, 474, 487	*Cynognathus*	Lady Frere

Institutional abbreviations: SAM-PK-K, Iziko South African Museum of Cape Town; BP/1/, Bernard Price Institute for Palaeontological Research, University of the Witwatersrand, Johannesburg; UMCZ T, Cambridge University, England.

is between 8 and 16 months (Edmund, 1969) and the replacement rate decreases with age. Although non-mammalian cynodonts also experienced multiple tooth replacement, it is believed that *Cynognathus* and *Diademodon* had a slower rate of tooth replacement compared to the early non-mammalian cynodonts, such as *Procynosuchus* (Hopson, 1971). If the tooth replacement rates of the more derived non-mammalian cynodonts were intermediate between that of extant reptiles and mammals, it is likely that their rates of enamel formation were also intermediate. Therefore, the length of time reflected in their teeth is likely to be in the order of several months at least.

Enamel was drilled in bands along each tooth (Fig. 1), from the top of the crown to the base of the crown, parallel to the occlusal surface, using a low-speed mini-rotary drill with a 1.5 mm diamond-tipped bit. The layer of tooth enamel in the non-mammalian cynodont teeth, and *C. niloticus* teeth, is very thin, and great care was taken to avoid drilling into the dentine layer. Thus, the samples obtained at each point were very small—each sample per band weighed approximately 0.7 mg, which was just enough powder for one mass spectrometric determination. We avoided using any of the pretreatment procedures that have been developed to eliminate carbonate contamination in Pliocene and Pleistocene mammalian material, because of high sample losses (>50% of sample is routinely dissolved during acetic acid pretreatments).

This course of action is justified as follows. The purpose of applying weak (or buffered) acetic acid to fossil powders is to remove post-depositional carbonates as well as more soluble apatite from enamel samples, with the understanding that they are, or likely to be, diagenetic. However, in this case the

fossils were not buried in carbonate-rich matrices and no carbonate was visible, or detectable in Fourier Transform Infrared (FTIR) spectra (Botha et al., 2003). FTIR analyses of three *Diademodon* enamel samples showed spectra typical of fossilized biological apatites, with some degree of recrystallization and ionic reorganization indicated. Given the tendency of calcium phosphate apatites to grow and attain higher crystallinity over time, such changes in fossils of this great age are not unexpected. However, the likelihood of preferential removal of apatites formed after deposition, using the standard, simple acetic acid pretreat-
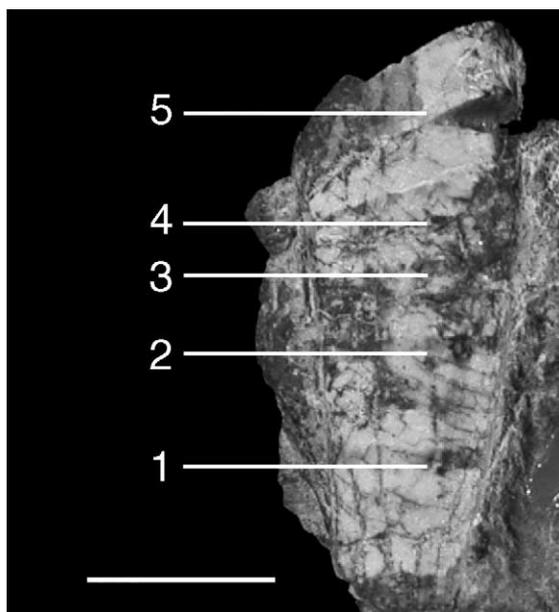

Fig. 1. Photograph of a *Diademodon* canine tooth showing the sampling trajectory. The bands drilled along the tooth (from the top of the crown to the bottom of the crown) are numbered 1–5.

ments, is remote. The spectra also showed a higher content of substituted carbonate (~6%) compared to that of extant mammalian enamel (typically 3–4%), and very similar to that of extant reptilian enamel (Botha et al., 2003). Higher carbonate contents are more susceptible to acid dissolution, providing a further argument against the use of acid pretreatment.

CO_2 was obtained by acid hydrolysis using 100% pure phosphoric acid (H_3PO_4) at 70 °C and collected by cryogenic distillation in a Kiel II automated carbonate device. Isotopic ratios were determined in a Finnigan Mat 252 mass spectrometer. Samples were calibrated against a regression obtained from two international standards (NBS18 and NBS19) and two inter-laboratory standards. Ratios are reported relative to the PDB standard. Precision, determined from replicate analyses of the standards, is <0.1‰ for $\delta^{13}C$, and <0.2‰ for $\delta^{18}O$, respectively.

3. Results

3.1. Non-mammalian cynodont stable light isotope patterns

Mean $\delta^{13}C$ and $\delta^{18}O$ values were calculated from the serial measurements across each tooth (Fig. 2). The results show that *Diademodon* $\delta^{18}O$ and $\delta^{13}C$ values are clustered and are significantly more depleted than the *Cynognathus* values (independent 1-tailed *t*-test: $\delta^{18}O$: $t_s = -3.42$; $df = 51$; $p < 0.05$ and $\delta^{13}C$: $t_s = -6.002$; $df = 51$; $p < 0.05$). The $\delta^{13}C$ values reflect diets that are somewhat similar in carbon isotope composition to today's C_3 plants, implying that $\delta^{13}C_{CO_2}$ was perhaps similar. In contrast, $\delta^{18}O$ values reflect hydrological values markedly more depleted than those currently observed in southern Africa (Harris et al., 1999).

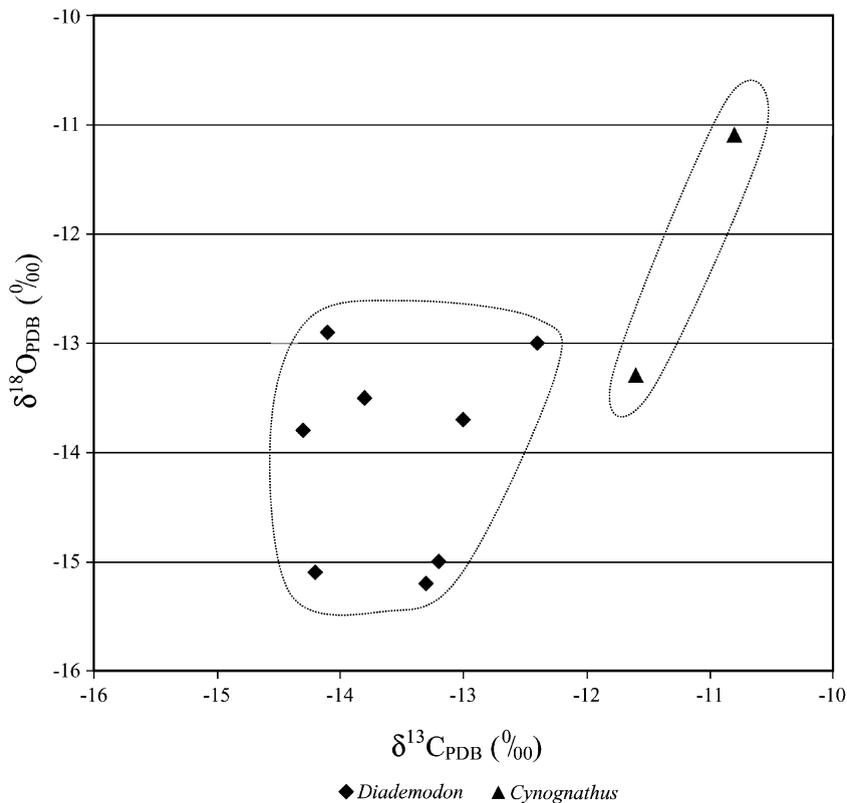Fig. 2. Mean $\delta^{13}C$ versus mean $\delta^{18}O$ of the non-mammalian cynodonts, *Cynognathus* and *Diademodon*, calculated from the intra-tooth serial analyses for each specimen. Note that the $\delta^{13}C$ and $\delta^{18}O$ ratios of *Diademodon* are more depleted than those of *Cynognathus*. Both $\delta^{13}C$ and $\delta^{18}O$ are expressed relative to PDB.

Serial analysis of two *Cynognathus* teeth (SAM-PK-K3029; BP/1/1675d) revealed an overall range of $\delta^{18}O$ values from $-10.7‰$ to $-13.7‰$, and $\delta^{13}C$ values from $-10.5‰$ to $-12‰$ (Fig. 3). SAM-PK-K3029 shows slightly more variability in both $\delta^{18}O$ and $\delta^{13}C$ than BP/1/1675d, although it is slightly less than 1‰.

Eight *Diademodon* teeth provided a reasonable sample size for the examination of intra-tooth variation. For clarity, the $\delta^{18}O$ and $\delta^{13}C$ results are shown separately. Fig. 4A shows that $\delta^{18}O$ values of individual *Diademodon* teeth range from $-10.3‰$ to $-16.5‰$. The average variability within each tooth was 3.8‰ (range from 1.5‰ to 6‰). $\delta^{13}C$ values range from $-12.2‰$ to $-15‰$ (Fig. 4B) and the variability is lower ($\sim1.3‰$) than that of $\delta^{18}O$. All eight *Diademodon* teeth show directional intra-tooth trends in $\delta^{18}O$ and $\delta^{13}C$, that is, positive or negative values are maintained through several bands.

3.2. Extant C. niloticus stable light isotope patterns

The *C. niloticus* $\delta^{18}O$ values, ranging from $-2‰$ to $-5.3‰$, are consistent with precipitation values in this region today. The $\delta^{13}C$ values range from $-3.9‰$ to $-8.8‰$. Both $\delta^{18}O$ and $\delta^{13}C$ show an average intra-tooth variability of 2‰ (Fig. 5). Of the three teeth that were analyzed, CROC 1 shows the highest intra-tooth variability of 3.3‰ for $\delta^{18}O$ and 4.9‰ for $\delta^{13}C$. In all three of these specimens, $\delta^{18}O$ and $\delta^{13}C$ co-vary, a feature that is not present in the *Diademodon* teeth.

4. Discussion

Most palaeo-isotope studies using fossil tooth enamel have analyzed Cenozoic material (e.g. Bocherens et al., 1996; Bryant et al., 1996b; Cerling et al., 1997). Apart from those of Thackeray et al. (1990) and MacLeod et al. (2000), none have attempted to extract ecological information from stable light isotope composition in non-mammalian therapsid biological apatite of Permian and Triassic age. Both these studies used as sample material dicynodont tusks that consist entirely of dentine (King, 1981; Hotton, 1986), a tissue highly susceptible to diagenesis (Lee-Thorp and van der Merwe, 1991; Wang et al., 1994). In spite of this problem, the scale and direction of $\delta^{13}C$ trends in Thackeray et al.'s study matched, reasonably well,
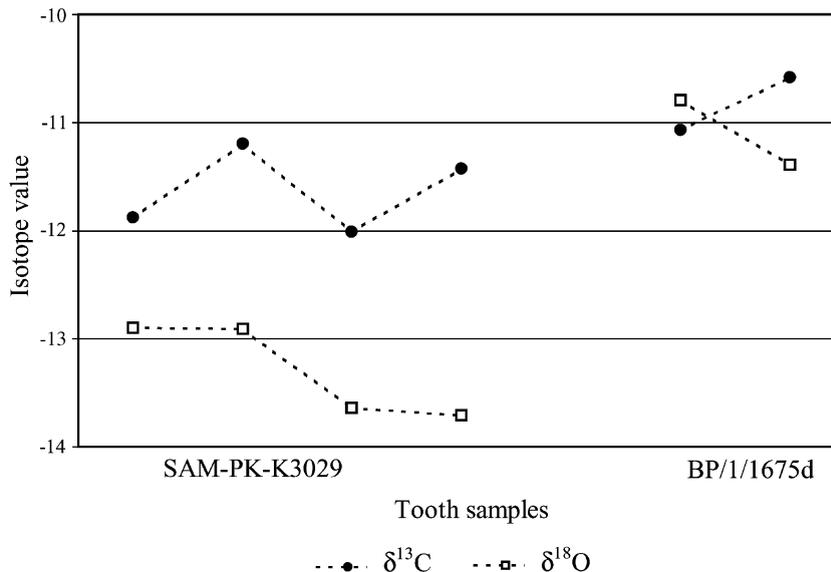

Fig. 3. Intra-tooth $\delta^{13}C$ and $\delta^{18}O$ analyses of the enamel carbonate of the non-mammalian cynodont, *Cynognathus*. Two teeth, each from different individuals (SAM-PK-K3029 and BP/1/1675d), were serially sampled. Each point represents a sample taken from one band of crown enamel. Each group of points, connected by a line, indicates all the points taken in bands, in one tooth, from the top of the crown (left) towards the base of the crown (right).
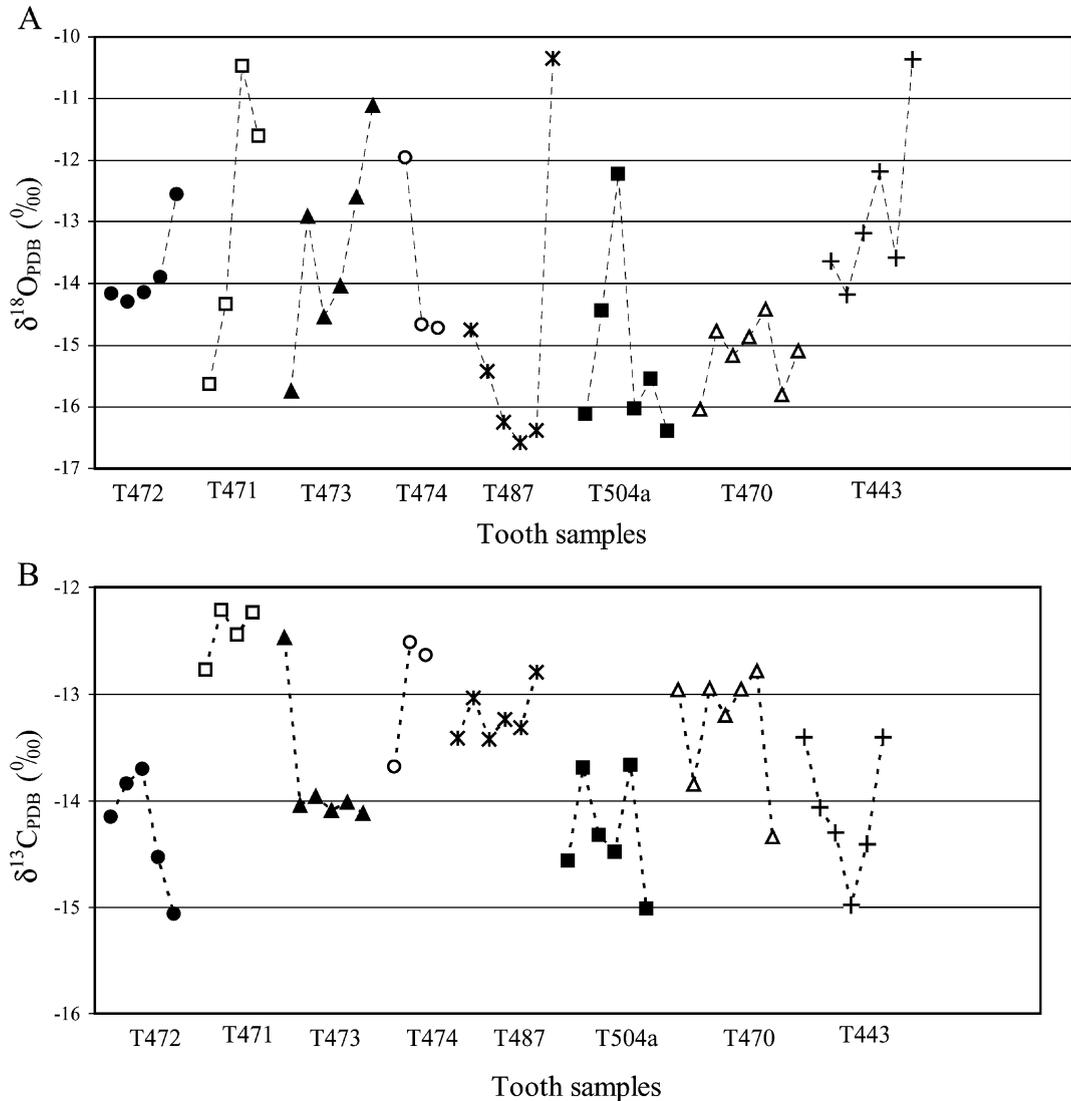
Fig. 4. Intra-tooth $\delta^{18}O$ ratios (A) and $\delta^{13}C$ ratios (B) of the enamel carbonate of the non-mammalian cynodont, *Diademodon*. Each group of points represents samples taken along each of eight teeth from several individuals. Note that the variation is directional for both the $\delta^{18}O$ and $\delta^{13}C$ values. Each tooth is a UMCZ T specimen.

those measured from contemporary northern hemisphere sedimentary sequences, and suggested long-term climate change (Thackeray et al., 1990). As the non-mammalian cynodont enamel used in this study is of Triassic age, we attempted to assess the integrity of the material, and of the stable light isotope data, using two approaches. The FTIR spectra showed that the material was indeed fossil biological apatite, and that the ~6% carbonate content was typical of reptilian

enamel (reported in Botha et al., 2003). FTIR spectra, however, provide no information about the integrity of the isotope values other than to show that no simple carbonate was present. The conventional test for reliability of $\delta^{13}C$, using the distinction between C_3 and C_4 feeders was obviously not available since no C_4 plants existed at this time. Therefore we relied on observations of predicted seasonal trends and patterned variability in both the $\delta^{18}O$ and $\delta^{13}C$
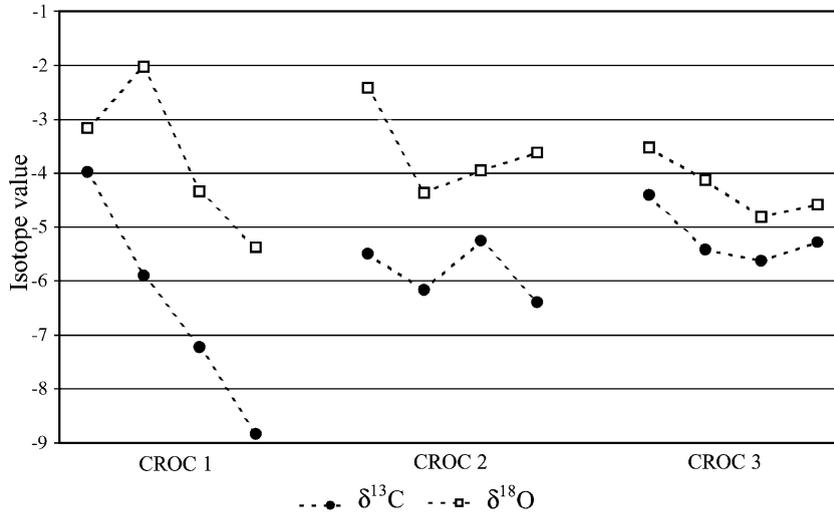
Fig. 5. δ^{13}C and δ^{18}O ratios of the enamel carbonate of extant *C. niloticus*. Three teeth (CROC 1, CROC 2, CROC 3) from three different individuals were analyzed.

sequences to indicate that the isotope data provided a reasonable reflection of ambient conditions. The serial isotope analyses, therefore, provided both a check on the data quality, and information about the palaeoecology of the two fossil genera.

4.1. Palaeoecology

The mean δ^{13}C value for all the non-mammalian cynodont specimens analyzed in this study is $-13.5‰ \pm 1.34$. If a $\Delta_{\text{diet-enamel}}$ offset of 12‰ (Lee-Thorp et al., 1989) or 14‰ (Cerling and Harris, 1999) is used, and assuming no diagenetic shifts, this value would reflect a δ^{13}C plant range of $-25.5‰$ to $-27.5‰$. The δ^{13}C atmospheric CO_2 in the Triassic ($\sim$220 Ma) is believed to have been approximately $-7.4‰$ (based on palaeosol carbonates; Ekart et al., 1999), which is close to today's value of almost $-8‰$. All Triassic plants would have used the C_3 photosynthetic pathway. Therefore, the δ^{13}C range of plants in the Triassic should indeed be similar to the range of modern values ($-30‰$ to $-23‰$). Our results are consistent with this prediction.

The *Diademodon* δ^{13}C values are markedly lower than the *Cynognathus* values (Fig. 2). Admittedly, there are few *Cynognathus* values, but the pattern also holds for all the serial isotope analyses, so that there is little overlap. The lower *Diademodon* δ^{13}C values could be indicative of its particular dietary

habits or preferred habitat. For instance, this animal may have lived under closed canopy conditions where it was cool or damp, and may, therefore, have eaten ^{13}C-depleted plants because of the "canopy effect" causing low light and a recycling of respired CO_2 (van der Merwe and Medina, 1991).

With regard to the δ^{18}O results, reasonable interpretations may be made about the trends and differences between the genera. The mean *Diademodon* δ^{18}O value is depleted compared to the mean δ^{18}O value of *Cynognathus* (Fig. 2). This suggests a fundamental difference in their ecology. Bocherens et al. (1996) found that δ^{18}O values in *Hippopotamus amphibius* were depleted compared to the rest of the terrestrial herbivores in their study, largely because of their habits of terrestrial foraging at night and remaining in water during the day where they might also feed on aquatic vegetation. Lower δ^{18}O in aquatic plant water is associated with reduced evapotranspiration, while body water loss due to transcutaneous evaporation is substantially reduced in aquatic or semi-aquatic animals (Bocherens et al., 1996). These factors combine to produce low δ^{18}O values in hippopotamus compared to terrestrial mammals with large evaporative water loss fluxes. The semi-aquatic *C. niloticus* also exhibits relatively low δ^{18}O values ($-3.95‰$; Fig. 5) compared to that of published data for terrestrial extant mammals in the Western Cape (e.g. bontebok $-2.65‰$, giraffe 1.17‰, gnu $-0.96‰$; Franz-

Odendaal, 2002), suggesting that aquatic or semi-aquatic mammals have low $\delta^{18}O$ values (Clementz et al., 2003).

Thus, the depleted *Diademodon* $\delta^{18}O$ values may reflect nocturnal and/or semi-aquatic habits. The morphology of *Diademodon* does not indicate adaptations for a semi-aquatic or aquatic lifestyle (Kemp, 1982), but it is possible that *Diademodon* ate aquatic vegetation located in shallow water. Alternatively, the lower $\delta^{18}O$ values may indicate that *Diademodon* obtained its drinking water more directly or more frequently than did *Cynognathus* (Sponheimer and Lee-Thorp, 1999). Taken together, the lower $\delta^{18}O$ and $\delta^{13}C$ values for *Diademodon* suggest preferences for shady, closed, and/or near aquatic habitats and their associated foods. *Cynognathus*, as a carnivore, might be expected to have lower $\delta^{18}O$ than associated herbivores as shown in two studies of extant and Pleistocene mammals (Sponheimer and Lee-Thorp, 1999, 2001), but this is not the case here. A possible explanation is that the factor causing the lower $\delta^{18}O$ in *Diademodon* is more influential.

Although diagenetic effects cannot be excluded, the consistency of the results–including the serial analyses discussed below–suggest that these isotopic differences between the genera are real and due to ecological differences. Although the material was collected from different localities, all sites are from a similar latitude and longitude (30°40′ to 31°45′S and 26°20′ to 27°15′E), equating to a Triassic latitude of somewhere between 35°S and 40°S (Visser, 1991). The similarity of *place*, if not site, excludes the effects of large-scale differences in rainfall.

4.2. Seasonality

The patterns of directional intra-tooth isotope variability in the fossil *Diademodon* teeth suggest that the isotopic data are real and reflect changing conditions; one can reasonably expect that diagenetic effects would induce either complete homogenization, or random shifts. This is clearly not the case. $\delta^{18}O$ variability is high, varying from 1‰ to 6‰ (Fig. 4A), and $\delta^{18}O$ does not co-vary with $\delta^{13}C$. Since $\delta^{18}O$ variability in tooth enamel provides only a dampened measure and not an absolute reflection of the full amplitude, true seasonal amplitude would have been even higher than the results indicate. The

large amplitude in *Diademodon* $\delta^{18}O$ values is noteworthy. Extant aquatic mammals tend to show less variability in $\delta^{18}O$ because terrestrial mammals usually experience greater physiological and environmental variability than aquatic mammals (Clementz et al., 2003). Thus, the high $\delta^{18}O$ variation in *Diademodon* teeth argues against a purely aquatic lifestyle.

Variability in *Cynognathus* tooth $\delta^{18}O$ is just less than 1‰ (Fig. 3). A 1‰ intra-tooth variation is large enough to suggest that the data points reflect seasonal variation (Kohn et al., 1999), and the directional sequence of the data further points to seasonal patterns.

The *C. niloticus* $\delta^{18}O$ and $\delta^{13}C$ results also exhibit directional intra-tooth variability (Fig. 5). These trends most likely reflect external seasonal shifts, although variations in body temperature may also influence fractionation during formation of enamel, and hence $\delta^{18}O$ values. These teeth were collected from a region with a strongly seasonal climate with warm, dry summers and cool, wet winters. High evaporation during the summer leads to higher water $\delta^{18}O$, while environmental water $\delta^{18}O$ values in winter are lower by ~3–4‰ (Harris et al., 1999). In this case, higher $\delta^{18}O$ values in *C. niloticus* teeth represent warm seasons and lower values reflect the cool (winter) season.

The intra-tooth variation in the *C. niloticus* teeth (up to 3‰) is similar to the findings in previous studies conducted on extant and historic mammals (Balasse et al., 2002) in the Western Cape. Elsewhere, several studies of mammalian teeth, including a study of continuously growing beaver teeth (Stuart-Williams and Schwarcz, 1997), extant sheep and 500-year-old bison (Fricke and O'Neil, 1996), and Holocene horse enamel phosphate (Sharp and Cerling, 1998) have shown intra-tooth variability on the order of 3–3.6‰. Notwithstanding that amplitude of variability would be expected to be greater at higher latitudes, in combination, it suggests that reptiles may in effect exhibit a similar variability compared to mammals in a region.

The *mean* intra-tooth $\delta^{18}O$ variability of 3.8‰ in *Diademodon* is similar to both the extant *C. niloticus* and published mammalian data. However, it varied in some cases by as much as 6‰ (Fig. 4A). If the biological dampening effect is considered, the true amplitude would have been even higher. High $\delta^{18}O$

variability might be due to extreme variations in body temperature related to environmental fluctuations, but this explanation seems biologically improbable. Moreover, *Cynognathus* in similar environments did not experience the same stresses. Indeed, variability of at least 6‰ is too high to be accredited to variations in body temperature alone (Barrick, pers. comm., 2001). The high variability is more plausibly and more simply ascribed to seasonal shifts in δ^{18}O since isotope effects in meteoric water are known to be large and highly variable even on short time-scales (Dansgaard, 1964).

Earlier studies have suggested that the Karoo environment of the time was semi-arid with a seasonal climate (Tucker and Benton, 1982; Anderson and Anderson, 1983, 1985; Smith et al., 1993), in which warm, dry summers were followed by cool, wet winters. This seasonal cycle is similar to that found in the Western Cape today, although the Triassic was more arid. Thus, we may deduce from the high intra-tooth δ^{18}O variability in *Diademodon*, that it was particularly sensitive to environmental fluctuations. The cyclical nature of its bone tissues in which alternating fast and slow growth rates, as reflected by the zonal bone tissue, were independently suggested to have been due to sensitivity to environmental fluctuations (Botha and Chinsamy, 2000). Using the modern data for the Western Cape as a model, high δ^{18}O values in the *Diademodon* teeth would reflect a summer season, and the lower δ^{18}O values reflect an autumn or winter season, similar to that seen in *C. niloticus*.

4.3. Conclusions

δ^{13}C values of the *Diademodon* and *Cynognathus* teeth reflect a δ^{13}C plant range of -25.5‰ to -27.5‰, indicating the presence of C_3 plants. This result is to be expected as earlier studies have found that Triassic terrestrial plants used only the C_3 photosynthetic pathway. The δ^{18}O and δ^{13}C values of *Diademodon* teeth are more depleted than the *Cynognathus* values, suggesting different microhabitats or differences in water-related behaviour. Distinct cycles in δ^{18}O are observed in the *Diademodon* teeth, possibly indicative of seasonal fluctuations. This phenomenon is also reflected in the bone histology of this animal where periodic interruptions in growth in the form of annuli and lines of arrested growth have

been observed (Botha and Chinsamy, 2000). In contrast, the bone histology of *Cynognathus* is continuous and uninterrupted, and the intra-tooth δ^{18}O isotopic variability is much less than that observed in the *Diademodon* teeth. This suggests that these contemporary genera responded very differently to a seasonal environment. The differences must be located in differential use of microhabitats, and behaviour (including dietary ecology), and possibly differences in physiology.

The findings in this study reveal the potential for isotope tools to be used for investigating the biology and ecology of fossil taxa, even as old as 220 million years old and shows how stable oxygen and carbon isotopes can unlock key information about Triassic vertebrates and their environment.

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