CAN OXYGEN ISOTOPES FROM TURTLE BONE BE USED TO RECONSTRUCT PALEOCLIMATES?

SAMUEL D. MATSON* and DAVID L. FOX
University of Minnesota, Department of Geology and Geophysics, Minneapolis, Minnesota 55455, USA

e-mail: mats0159@umn.edu

ABSTRACT

A substantial complication to using the oxygen isotope composition (δ18O) of vertebrate bioapatite in paleoclimate studies is the need to distinguish variation due to temporal changes in the δ18O of surface waters from that due to temperature-dependent fractionation during biomineralization. One solution is multiple-taxa comparisons using data from coexisting homeothermic and heterothermic animals. Fossil emydid turtles have been suggested to be potentially useful as functional homeotherms because (1) modern emydid employ behaviors, such as basking, to restrict skeletal growth to a narrow temperature range; (2) their aquatic habitat constrains the isotopic variability of dietary inputs; and (3) emydid have a dense fossil record. But because turtles lack teeth and therefore tooth enamel, sampling the bone and enamel is necessary to distinguish variation due to temporal changes in the δ18O of the water they precipitate. Quantification of terrestrial temperature requires delineation of these variables.

INTRODUCTION

Stable isotopes preserved in authigenic or biogenic minerals have been well documented as recorders of paleoclimatic and paleoecological information (Koch, 1998; Lee-Thorp, 2002). Isotopic studies of past terrestrial climates and ecosystems can be complicated, however, in part because of spatial and temporal heterogeneity of terrestrial environments and relatively low preservation potential of minerals appropriate for isotopic analysis. Despite such complications, both the ability and the need to understand continental responses to past global climate change are becoming increasingly important.

A fundamental link between continental climates and the terrestrial isotopic record is the strong positive correlation at mid-to-high latitudes between mean annual temperature (MAT) and the oxygen isotopic composition (δ18O) of meteoric water (Dansgaard, 1964; Rozanski et al., 1993; Fricke and O’Neil, 1999). If MAT-δ18O gradients of the past were similar to those of today, then any terrestrial records that can be used to determine the δ18O of past meteoric water can be used as a proxy for continental MAT. Quantification, however, of meteoric water δ18O from the terrestrial record has been limited by the fact that the variability in the δ18O of inorganic and biogenic minerals (typically carbonates, δ18O_or, or phosphates, δ18OP) measured in isotopic studies is a function of two unknowns. First, δ18O is influenced by temperature-dependent equilibrium fractionation during mineral precipitation. Second, the δ18O of these minerals can vary owing to temporal or spatial changes, or both, in the δ18O of the water from which they precipitate. Quantification of terrestrial temperature requires delineation of these variables.

One approach to this problem is the use of homeothermic animals (such as mammals) that precipitate skeletal hydroxylapatite (HAP) from body water at a constant temperature (Kolodny et al., 1983; Longinelli, 1984; Koch et al., 1989; Bryant et al., 1994; Stuart-Williams and Schwarcz, 1997; Dettman and Lohmann, 2000; Fox and Fisher, 2001; Kohn et al., 2002; Fricke, 2003). Variability in the δ18O of HAP from such animals should be due entirely to variability in the δ18O of body water, which is a function of local meteoric water composition, aridity, and animal physiology. Another approach uses meteoric water δ18O estimates from homeotherms along with δ18O from heterothermic or inorganic minerals (Koch et al., 1995; Fricke et al., 1998; Fricke and Wing, 2004). Since the δ18O of heterothermic and authigenic minerals should vary temporally in terms of both ambient temperature and water δ18O at the time of skeletal or mineral formation, isotopic variability beyond that indicated by homeotherms should be due to changing environmental temperatures. This approach has an additional advantage in that it does not assume modern-day MAT-δ18O gradients for the past.

Previous studies were also complicated by the fact that terrestrial homeotherms often do not closely track the δ18O of meteoric water. The δ18O of skeletal HAP is a reflection of body water δ18O, which can deviate from local meteoric water. Such deviation is generally due to physiological and environmental controls over the δ18O and fluxes of oxygen to and from body water. Several models have demonstrated that differences in body size, physiology, and behavior cause body water δ18O to vary widely across species living in similar climates (Luz et al., 1984; Ayliffe and Chivas, 1990; Bryant and Froelich, 1995; Kohn, 1996). Isotopic variability in aquatic homeotherms should be lower since their body water should closely track the δ18O of the water they inhabit. Interpretations of aquatic habitats for fossil animals, however, are often speculative if the species is extinct or if closely related modern analogs are not available.

Barrick et al. (1999) suggested that δ18O from co-occurring fish and freshwater emydid turtles can be used to estimate terrestrial paleotemperatures. First, the body water δ18O and skeletal δ18O of modern aquatic emydid turtles covary strongly and linearly with their environmental water, as shown in equation 1, which is modified from Barrick et al. (1999) to include only the emydid taxa Pseudemys and Chrysemys:

δ18Ow = (1.03 ± 0.05)(δ18Osw) − (22.57 ± 0.72)

r2 = 0.96, N = 21

(1)

* Corresponding author.

1 Stable carbon and oxygen isotopic ratios reported in this paper follow the conventional δ notation:

δ value = [(Rsample/Rstandard) − 1] × 1000,

where R = 13C/12C or 18O/16O, and standard is Vienna Pee Dee Belemnite (VPDB) for carbon and Vienna Standard Mean Ocean Water (VSMOW) for oxygen. The δ values are given in parts per thousand (‰).
Second, although emydid turtles are heterothermic, they employ behavioral mechanisms, such as basking, to restrict their body temperature to a narrow range (Cagle, 1954; Boyer, 1965), and the growth rate of the shell is biased toward this temperature (Gibbons, 1967; Costanzo et al., 1995; Koper and Brooks, 2000). Thus, oxygen isotopes from emydid shell HAP should reflect a roughly constant temperature of skeletal precipitation, and the data of Barrick et al. (1999) seem to support this suggestion. Finally, the family Emydidae exhibits a temporally and geographically extensive fossil record (Hutchinson, 1998). Observation of the life history and behavior of modern emydid turtles can support inferences about the physiology of extinct, closely related taxa. In their study, Barrick et al. (1999) reconstruct Cretaceous terrestrial climate for Alberta using δ18O from fossil soft-shelled turtles from the family Trionychidae, since a modern representative of this taxon seems to have a similar temperature of bone deposition to emydid. The approach has yet to be demonstrated using fossil emydid turtles.

Diagenetic alteration is a concern in any isotopic study using fossil skeletons. Furthermore, bone should be especially susceptible to diagenesis since the plate-shaped crystallites of primary bone HAP are smaller and less densely intergrown than those of enamel (Weiner et al., 1989; Chillon et al., 1994; Kolodny et al., 1996; Person et al., 1996). Several studies have shown that fossil bones often have lower carbonate content [(CO3)2−] and higher fluoride content ([F]−) and are more perfectly crystalline than modern bones (Land et al., 1980; Shemesh, 1990; Bryant et al., 1994; Person et al., 1995; Wright and Schwarz, 1996; Zazzo et al., 2004). Furthermore, Zazzo et al. (2004) demonstrate that 18O can be exchanged through inorganic (for 18O) or microbially mediated (for 18O2) recrystallization of primary HAP or addition of diagenetic apatite. Either of these processes should cause greater crystallographic perfection of bone. While decreased [CO3][2−] increased [F], and increased crystallinity cannot definitively determine whether 18O exchange has occurred, they can indicate primary HAP recrystallization (Shemesh, 1990; Person et al., 1995; Wright and Schwarz, 1996).

The isotopic integrity of fossil turtle bone must be verified prior to extending interpretations of paleoclimate. The results of Barrick et al. (1999) are in general agreement with a global-scale climate simulation for the mid-Cretaceous (Barron et al., 1995). No independent isotopic records for the terrestrial section studied by Barrick et al. (1999) were available for comparison, however, and a subsequent study of these sediments has suggested alteration of fossil bone (Trueman et al., 2003). More detailed studies of the isotopic fidelity of fossil bone are warranted before this material can be used to reconstruct paleotemperatures.

This study focuses on fossils from the Paleoene-Eocene (P-E) boundary section of the Clarks Fork Basin, Wyoming, and has three goals. First, this study provides a robust test of the approach suggested by Barrick et al. (1999) and offers several advantages for doing so. The P-E boundary was a period of rapid global warming known as the Paleocene-Eocene Thermal Maximum (Shackleton, 1986; Kennett and Stott, 1991; Zachos et al., 1994; Bains et al., 1999). Previous studies have examined records of this climate change in the Clarks Fork Basin (Wing et al., 1991; Koch et al., 1995; Fricke et al., 1998; Gunnell, 1998; Wing, 1998; Bowen et al., 2001; Gingerich, 2001; Koch et al., 2003). Thus, a well-documented record of climatic change from the same section is available for comparison with the results obtained here. Second, this study explores mechanisms of bone diagenesis and its implications for interpretation of the isotopic record. Since uncertainty remains regarding the limitations of isotopic studies, further investigation of diagenesis is necessary. Finally, this study contributes to the record of terrestrial climate change across the P-E boundary.

Clarks Fork Basin, Wyoming

The Bighorn Basin in northwest Wyoming (Fig. 1) preserves one of the world’s most complete records of Paleocene and Eocene continental sediments, and its rich fossil record has been the focus of more than a century of paleontological studies (Gingerich, 1980). This study concentrates on P-E sediments of the Clarks Fork Basin, a northwestern extension of the Bighorn Basin. Exposures of these sediments have been studied extensively along the margins of Polecat Bench, a Pleistocene fluvial terrace located approximately 10 km northwest of the town of Powell, Wyoming. The fossils used in this study come from a series of badland exposures to the south and west of Polecat Bench known as the Sand Coulee area, where more than 400 fossil localities have been identified by workers associated with the University of Michigan. The P-E stratigraphy of interest to this study is composed of the Fort Union and Willwood Formations. The P-E boundary (55.5 Ma) is stratigraphically above the contact of the late Paleocene Fort Union Formation and the latest Paleocene–early Eocene Willwood Formation in the Clarks Fork region. The Fort Union Formation consists primarily of grayish-totan lignitic shales, carbonateous mudstones, and lenticular sandstones (Hickey, 1980). The Willwood Formation is characterized by sheet sandstones and brightly variegated mudstones that are generally more pedogenically mature than those of the Fort Union (Kraus, 1980, 1997, 2001). Fort Union and Willwood sediments are interpreted to represent more or less continuous deposition along meandering river systems draining the Beartooth and Absaroka Mountains to the west and southwest and the Bighorn Mountains to the east.

MATERIALS AND METHODS

Sampling Protocol

Fossils sampled in this study are from the collections of the University of Michigan Museum of Paleontology (Ann Arbor). Some of the specimens used are represented by single individuals of a given taxon. These individual skeletons are variably complete, and taxonomic identifications range from family to species. Other isolated skeletal fragments were obtained from cataloged miscellaneous boxes that contain material from several individuals and taxa. For these specimens, turtle-shell fragments displaying smooth exterior carapacial and plastral surfaces and a relatively flat shell (characteristics of aquatic emydid turtles) were used.

Powder samples were drilled from the fossils using a diamond bur bit mounted into a variable-speed, handheld rotary drill. All samples were drilled at low speed to minimize heating and potential isotopic fractionation. Powder from the outer ~1 mm was discarded to avoid surface contaminants. For the turtle samples, ~50 mg of powder was drilled from.
a clean cross-sectional surface of the shell, avoiding the interior and exterior margins.

Crocodilian specimens used in this study include teeth from the genera *Leidyosuchus* and *Allognathosuchus* (Crocodylia, Alligatoridae), as well as isolated, unidentified crocodilian teeth. Enamel powder was drilled from the entire external surface of each tooth. The crocodilian teeth were relatively small (~<1.5 cm length) with thin enamel, so only 10–14 mg of powder was obtained without incorporating underlying dentine. Seven specimens possessed teeth in situ within jaw bone fragments. For these specimens, an additional 40–50 mg of powder was drilled from a cross-sectional surface of the bone for comparison with associated teeth.

All but two of the fish specimens used in this study are body scales from *Lepisosteus*, or gar (Osteichthyes, Lepisosteiformes). The outer surfaces of gar scales are covered with an enamel-like apatitic tissue (ganoin) that is densely crystalline and is thus more resistant to diagenesis than bone. Gar scales from these localities have been studied previously (Fricke et al., 1998; Fricke and Wing, 2004), allowing comparison with the data presented here. Since most of the fish material consists of isolated scales and it is difficult to establish the number of individuals represented, each scale sampled was treated as an individual specimen. While this approach eliminated the possibility of interindividual mixing, it also limited sample sizes to approximately 10–20 mg per scale. In addition to the gar scales, samples were obtained from two isolated, unidentified fish vertebrae.

Isotopic Analysis of Carbonate in Bioapatite

For oxygen isotope analysis of the structural carbonate component of turtle bone HAP, 25 mg aliquots from each powder sample were weighed into 15-ml centrifuge tubes. For the crocodilian and fish samples, 5 mg aliquots were weighed into 2-ml centrifuge tubes. All samples were pre-treated following the method of Koch et al. (1997). To oxidize any organic matter, the samples were soaked in 2%–3% NaOCl (0.04 ml NaOCl per 1 mg sample) for 24 h and then rinsed five times with an excess of distilled, deionized (DDI) water. Each sample was then soaked in a solution of 1 M acetic acid buffered with an equal volume of 1 M calcium acetate (0.08 ml solution per 1 mg sample) for 24 h to remove diagenetic carbonate minerals. The samples were then rinsed another five times with an excess of DDI water and lyophilized overnight. Each sample was then reacted with 100% phosphoric acid at 71.5°C in a Finnigan Kiel II automatic carbonate preparation device, and the isotopic composition of the resulting CO₂ was measured using a Finnigan MAT 252 isotope ratio mass spectrometer at the University of Minnesota Stable Isotope Laboratory. Analytical precision was maintained at better than 0.1‰ through repeated measurements of National Bureau of Standards (NBS)-18 and NBS-19 and values were normalized to NBS-19.

Isotopic Analysis of Phosphate in Bioapatite

For oxygen isotope analysis of the phosphate component of turtle, crocodilian, and fish skeletal hydroxyapatite, 5–10 mg aliquots from each sample were prepared in 15-ml centrifuge tubes following the procedure outlined by O’Neill et al. (1994) and modified by Dettman et al. (2001) and Kohn et al. (2002). One ml of 2 M HF was added to each sample, and the samples were then sonicated for 60 min and left in the HF to dissolve for 24 h. In contrast to the methods of Dettman et al. (2001) and Kohn et al. (2002), in which the HF was neutralized with 500 μl of 20% NH₄OH, only 430 μl of 10% NH₄OH were used to prevent high pH and poor Ag₃PO₄ precipitation. Following neutralization, 300 μl of DDI water was added to each tube. The samples were then centrifuged to separate out any CaF₂ and other insoluble material, and the solution from each tube was decanted into a clean 15-ml centrifuge tube. The precipitate from the first tube was rinsed with 5 ml of DDI water, and the rinse was centrifuged and decanted into the solution in the second tube. At this point in the method of Kohn et al. (2002) an additional 300 μl of NH₄OH would be added to the solution in the second tube, but this step was not followed to avoid raising the pH even further. One ml of 2 M AgNO₃ was then added to the approximately 10 ml of decanted solution, causing rapid precipitation of finely crystalline, yellow Ag₃PO₄. The best yields were obtained by letting each tube stand undisturbed in a dark area for approximately 5 h to let the precipitate settle to the bottom of the tube. The samples were then centrifuged and rinsed five times with an excess of DDI water and dried overnight at 60°C. The initially yellow-brown Ag₃PO₄ took on a dark brown color during the process of rinsing and drying. This dark color is unusual for Ag₃PO₄ and may reflect silver nitrate contamination. Dettman et al. (2001), however, also report samples that turned dark brown in the final preparation stages, and they provide several lines of evidence suggesting that the dark samples were not contaminated.

The dried Ag₃PO₄ was crushed with a spatula, and 0.5–1.5 mg triplicates (for turtle bone) or 0.5–1.0 mg duplicates (for crocodilians and fish) were weighed into 3.5 × 5 mm Ag capsules. The prepared samples were then reduced to CO through high-temperature pyrolysis in a Finnigan MAT high temperature conversion–elemental analyzer (TC–EA) at the University of Missouri Stable Isotope Laboratory. The CO was then introduced via a continuous He flow into a Finnigan Delta-Plus isotope ratio mass spectrometer, and its isotopic composition was measured. The phosphate oxygen isotope compositions are reported in conventional δ notation relative to Vienna Standard Mean Ocean Water (VSMOW).

Fifteen samples from two separately prepared aliquots of the standard NBS-120c, a porphorite rock from Florida, were analyzed separately from the turtle, crocodilian, and fish samples. The δ¹⁸O values for the NBS-120c samples ranged from 19.7‰±21.5‰ (mean = 21.1% ± 0.48‰). Crowson et al. (1991) and Lécuyer et al. (1996) report mean δ¹⁸O values for NBS-120c of 21.33‰±0.05‰ (N = 15) and 21.7%±0.14‰ (N = 10), respectively, based on fluorination of Ag₃PO₄, while O’Neil et al. (1994) report a value of 21.80‰±0.34‰ (N = 5) based on reaction with graphite in sealed silica tubes. Fox and Fisher (2001) report a value of 21.85‰±0.36‰ (N = 6) based on reaction with graphite. Vennemann et al. (2002) report mean values for NBS-120c of 22.1%±0.51‰ (N = 18) based on TC–EA analysis, 22.6%±0.09‰ (N = 3) based on fluorination with BrF₅, and 21.27‰±0.02‰ (N = 2) based on reaction with graphite.

Carbonate Coulometry and X-Ray Diffraction

Carbonate content has been suggested as a proxy for the extent of recrystallization of bone HAP (Wright and Schwarz, 1996; Zasso et al., 2004). Measurements of [CO₃⁻] from turtle bone samples in this study were made through total inorganic carbon coulometric titration (Engleman et al., 1985) using a UIC 5030 carbonate carbon apparatus at the Limnological Research Center Core Laboratory at the University of Minnesota. The coulometry procedure used here assumes that no original organic carbon is present in the fossils. Ten milligram aliquots of each sample were used since experimental results obtained from pure CaCO₃ standards and samples of pristine modern African elephant (*Loxodonta africana*) molar enamel suggest that a minimum of 8–10 mg of powder is required to obtain precise results. The smaller sizes of crocodilian and fish samples precluded coulometry analyses of these materials. Two coulometry analyses were performed for each turtle bone sample. The first analysis was done on untreated sample powder, while the second analysis was done on powders that were pretreated with NaOCl and acetic acid following the procedure outlined by Koch et al. (1997).

Crystallinity has been suggested as another proxy for diagenetic HAP recrystallization (Shemesh, 1990; Person et al., 1995). In this study, crystallinity was measured through X-ray diffraction (XRD) analyses on turtle bone samples using cobalt radiation on a Siemens D-500 wide-angle diffractometer at the Characterization Facility at the University of Minnesota. The diffractometer was equipped with a rotating sample holder, which gives a good statistical distribution of crystalline orientation. The Crystallinity Index (CI) was determined following the method of Person
et al. (1995), in which the sum of the heights of the [112], [300], and [202] intensity peaks on the X-ray diffractogram (defined as the difference between the maximum value at the top of each peak and the minimum value of the valley preceding the peak) is divided by the height of the [211] peak (defined as the difference between the maximum value at the top of the peak and the baseline between 27.9° and 44.4° 2θ with Co Kα radiation).

RESULTS
Carbonate δ18Oc

All data with temporal associations shown in this study have been recalibrated to follow the age model outlined by Koch et al. (2003). Turtle bone δ18Oc values (see Supplementary Data 12; Fig. 2A) exhibit a relatively large range, from 16.3‰ to 28.0‰ (mean = 21.9‰, SD = 3.72, N = 25). This range is slightly wider than previous studies of paleosol carbonates (Koch et al., 1995; Bowen et al., 2001), the large and mid-sized mammals Coryphodon and Phenacodus (Koch et al., 1995; Fricke et al., 1998), and the smaller mammals Ectocion and Hyracotherium (Koch et al., 1995). Many of the turtle values are higher than paleosol carbonates, Coryphodon, and Phenacodus but are similar to those observed in the smaller mammals. Analysis of different samples from single specimens indicates that intraindividual variability (0.3‰–2.0‰, mean = 1.2‰, N = 7) is lower than intralocality variability (1.2‰–8.5‰, mean = 3.8‰, N = 5), which is in turn lower than interlocality variability (mean = 4.7‰, N = 6).

The δ18Oc values of crocodilian tooth enamel and bone (see Supplementary Data 12; Fig. 2B) display a high range and mean value relative to previous studies, from 20.6‰ to 31.9‰ (mean = 27.2‰, SD = 3.05, N = 23) for enamel and 21.7‰ to 25.7‰ (mean = 23.1‰, SD = 1.47, N = 8) for bone. The majority of these values are more positive than all previously measured values from various taxa. Also, the δ18Oc of tooth enamel is consistently higher than that of bone δ18Oc from the same individual, though the degree of offset is variable.

The δ18Oc values of gar ganoine (see Supplementary Data 12; Fig. 2A) range from 16.8‰ to 31.4‰ (mean = 24.1‰, SD = 5.1, N = 9), and the two values of fish bone δ18Oc are 17.7‰ and 22.9‰. The majority of the gar ganoine and fish bone δ18Oc values fall within the range of 17.8‰–23.3‰ for gar ganoine reported by Fricke et al. (1998). Several of the gar ganoine values, however, are much higher than expected from previous studies, similar to that reported for crocodilian enamel.

Phosphate δ18Op

Turtle bone δ18Op values (see Supplementary Data 12; Fig. 3A) exhibit a more restricted range than the corresponding δ18Oc values, from 9.8‰ to 15.3‰ (mean = 11.6‰, SD = 1.07, N = 27) VSMOW. Triplicate analyses from each prepared sample display a range in variability from 0.1‰ to 1.6‰, with a mean value of 0.8‰. This triplicate variability is slightly higher than the maximum standard deviation of triplicate NBS-120c TC-EA analyses (0.51‰) reported by Vennemann et al. (2002) and observed from our own repeat analyses of NBS-120c splits from individual aliquots (0.48‰). As with the turtle δ18Oc data, mean intralocality variability (1.1‰) is lower than mean interlocality variability (1.9‰). Turtle δ18Op has a similar range to that of measured and estimated δ18Oc values for Coryphodon and Phenacodus (Koch et al., 1995; Fricke et al., 1998) but is slightly more negative and more restricted than δ18Oc estimates for Ectocion and Hyracotherium (Koch et al., 1995).

Crocodilian bone δ18Oc values (see Supplementary Data 12; Fig. 3B) range from 9.3‰ to 12.6‰ (mean = 11.0‰, SD = 1.1, N = 8). Variability between duplicate analyses of single samples ranges from 0.0‰ to 1.0‰ (mean = 0.4‰). Enamel values are slightly higher, ranging from 10.1‰ to 14.3‰ VSMOW (mean = 11.6‰, SD = 1.0, N = 26). Duplicate analyses of single enamel samples exhibit a higher intrasample
variability than that of bone, ranging from 0.0% to 1.9% (mean = 0.5%). The range of 3.3% for crocodilian bone $\delta^{18}O_c$ throughout the entire stratigraphic interval is comparable to that of $\delta^{18}O_c$ (4.0%), but the large range (11.3%) and anomalously high values (ca. 32%) of enamel $\delta^{18}O_c$ values are not seen in enamel $\delta^{18}O_p$.

Gar ganoine $\delta^{18}O_c$ values (see Supplementary Data 1; Fig. 3A) range from 9.6‰ to 14.1‰ (mean = 12.3‰, SD = 1.7, N = 12), while the two fish bone samples display values of 11.2‰ and 11.7‰. Variability between duplicate analyses of single ganoine samples ranges from 0.0‰ to 1.0‰ (mean = 0.4%). Similar to crocodilian enamel, the high variability (18%) and very heavy values (~31%) seen in gar ganoine $\delta^{18}O_c$ are not observed in ganoine $\delta^{18}O_p$.

**Carbonate Content and Crystallinity**

Coulometric titration of untreated samples reveals that several specimens have extremely high [CO$_3^-$], sometimes in the range of 50%–60%. The pretreated samples have lower values, ranging from ~3.5% to 26%. While previous measurements of modern bone [CO$_3^-$] indicate taxonomic variation, the values are typically no higher than ~5% (Rey et al., 1991; Rink and Schwarz, 1995; Bryant et al., 1996; Wright and Schwarz, 1996; MacFadden et al., 2004; Zazzo et al., 2004). The extra carbonate observed in the turtle bone samples is likely diagenetic calcite spar within the pore spaces of the bone. Indeed, preliminary electron microprobe analyses of two turtle bone samples suggest that the Haversian canals of the bone are lined with iron oxides and filled completely with calcite. The coulometry results suggest that the 24 h acetic acid pretreatment does not appear to be long enough to remove all diagenetic carbonate from some of the samples (see Supplementary Data 2). Subsequent experiments and coulometry analyses of the five samples with the highest initial [CO$_3^-$] indicate that the samples can be brought down to ~11% carbonate if they are treated with 1 M acetic acid with a 1 M calcium acetate buffer (2 ml solution per 25 mg sample) for 72 h, with the acetic acid being replaced once during that time. Treatment for longer periods and additional acid replacement did not reduce [CO$_3^-$] to below ~11% (higher than previous estimates of bone [CO$_3^-$]), and the 72 h pretreatment was employed for samples used for XRD analyses. Koch et al. (1997) describe potential isotopic effects if bone HAP samples are treated for longer than 3 days with 1 M acetic acid, but the isotopic effects of the 72 h treatment (replacing the acid once) used here are unknown. The crystallinity indices determined from XRD range from 0.28 to 0.55 (mean = 0.40, N = 20).

**DISCUSSION**

Estimates of Paleocene-Eocene Paleotemperature

The use of co-occurring emydids and heterotherms to estimate paleotemperature (T) relies on two equations. Equation 1 above is used to estimate surface-water isotopic composition ($\delta^{18}O_w$) from measured bone $\delta^{18}O_p$. This method requires the assumption that P-E emydids turtles controlled their body temperature behaviorally and also that they had body water $\delta^{18}O$ that was in isotopic equilibrium with $\delta^{18}O_w$. Similar to modern emydids, Estimates of $\delta^{18}O_w$ are then used in the phosphate-water temperature equation of Longinelli and Nuti (1973) along with heterotherm $\delta^{18}O_p$ values to calculate $T$:

$$T({}^\circ C) = (111.4 \pm 4.78) - (4.3 \pm 0.21)(\delta^{18}O_p - \delta^{18}O_w)$$

$$r^2 = 0.94, \quad N = 27$$

Modern surface water temperatures exhibit a strong correlation with overlying air temperatures, though the former are generally ~1°C warmer, presumably because of differences between water and air in terms of heat capacity and cooling rates (Fricke and Wing, 2004). Estimates of Paleocene-Eocene MAT constructed using emydid $\delta^{18}O_p$ with that of co-occurring crocodilians (Fig. 4A) and gars (Fig. 4B) can be compared with independent MAT estimates from previous studies using leaf-margin analysis (LMA; Wing et al., 1999) and $\delta^{18}O_p$ from co-

**FIGURE 3**—Comparisons across the Paleocene-Eocene boundary of phosphate $\delta^{18}O_p$ from this and previous studies. A) Turtle bone, gar ganoine, and fish bone. B) Crocodilian enamel and bone. C) Coryphodon enamel. D) Phenacodus, Ectocion, and Hyracotherium enamel. For the Coryphodon data, black circles = measured values; gray circles = values estimated from carbonate using a combined linear regression. $\delta^{18}O_p = 0.97 \times \delta^{18}O_w - 7.93$, $r^2 = 0.98$ (Bryant et al., 1996; Iacumin et al., 1996) for modern mammalian tooth and bone. Data sources and abbreviations as indicated in Fig. 2. Measured values from Fricke et al. (1998) corrected using a linear regression (outlined by Vennemann et al., 2002) to account for discrepancies between fluorination and temperature conversion–elemental analyses.
occurring Coryphodon and gars (Fig. 4; Fricke et al., 1998; Fricke and Wing, 2004). Based on the previous estimates of MAT from the Clarks Fork Basin, there are two primary patterns that should be reconstructed from the emydid, crocodilian, and gar data if they are reliable as paleo-temperature proxies. First, a rapid warming event should be observed across the Wasatchian-0 (Wa-0) biozone, from ca. 55.0 Ma to 54.9 Ma, since this period is during the Paleocene-Eocene Thermal Maximum observed in the marine record (Zachos et al., 2001). Second, an early Eocene cooling trend on the order of 6–8°C should be observed from ca. 54.7 Ma to 53.6 Ma, since both LMA and the δ¹⁸O of authigenic hematite independently record this phenomenon (Bao et al., 1999; Wing et al., 1999).

Reconstructions of MAT from emydid turtles and heterotherms appear to be in agreement with the patterns described above. During the Wa-0 interval, emydids and gars record a temperature increase of ~4°C, while emydids and crocodilians suggest a temperature increase of >12°C. Based on the age model used here (which follows Koch et al., 2003), this warming event took place over a period of approximately 30 kyr, which is in agreement with expectations of rapid climate change recorded in marine and terrestrial records (Fricke et al., 1998; Wing et al., 1999;
Although a lack of co-occurring emydids and gars after ca. 54.4 Ma precluded their use for estimating temperatures during the early Eocene cooling trend reported by Wing et al. (1999) and Bao et al. (1999), the trend is reproduced by the emydid and crocodilian data, as seen in Figure 4A. During the interval from ca. 54.4 Ma to 53.8 Ma, reconstructions based on emyids and crocodilians exhibit a temperature decrease of approximately 8°C. Furthermore, while MAT estimates from Coryphodon and gar (−20−26°C) are generally higher than the approximately 13−18°C range suggested by LMA, the estimates from emyids and crocodilians (−11−23°C) and emyids and gars (−13−24°C) are in much better agreement with each other and with the LMA results. This result testifies to the utility of both oxygen isotope and LMA approaches to reconstructing paleotemperatures, since each method provides an independent estimate through entirely different sample materials, assumptions, and taphonomic biases.

It is important to note that equations 1 and 2 both have error associated with their slopes and intercepts, and propagation of this error results in relatively large uncertainty for paleotemperature estimates. Even without considering intralevel variability or analytical error, uncertainty for the temperature estimates ranges from ±7.4 to 7.8 °C. The relatively large error is inherent to this method of paleotemperature reconstruction, and any future applications or interpretations based on the method must be made in light of this fact. Additional uncertainty involves assumptions of emydid homeothermy and crocodilian heterothermy. Although emyids constrain their body temperatures to a narrow range relative to other heterotherms, the range is broader than that of endotherms. Also, previous studies show that crocodilian heterothermy is equivocal and varies across taxa (Diefenbach, 1975; Smith, 1979; Grigg and Seebacher, 2000). Our study used these assumptions to determine if the emyrid-crocodilian approach results in reasonable temperature estimates. The fact that our temperature estimates agree with independent records suggests the assumptions are perhaps justified, though further exploration of emydid homeothermy and crocodilian heterothermy could certainly be justified.

One result from the approach used here that has not been observed in any previous study is the apparent −7°C cooling recorded by emyids and crocodilians immediately preceding Wa-0. Intuitively, this pattern is not in agreement with predictions of a rapid temperature increase at the P-E boundary. Furthermore, crocodilians would not be expected to survive in areas with MAT of <14 °C (Markwick, 1998). For a temperature increase to be calculated from equation 2, estimates of δ13Οm made from emyids must increase relative to δ13Οm measured from heterotherm, since temperature is inversely proportional to the former subtracted from the latter in this equation. As seen in Figs. 3A and 3B, the increase in crocodilian δ13Οm across Wa-0 is greater than that of emyids. Thus, calculations from equation 2 indicate a temperature decrease since estimates of δ18Οm do not increase enough to indicate warming. Despite intensive sampling, however, neither Bowen et al. (2001) or Koch et al. (2003) report a significant departure in the δ18Ο of paleosol carbonates at the P-E boundary in the Clarks Fork Basin, even though a positive excursion was observed in similar samples from the southern Bighorn Basin. In this case, the lack of a large increase in δ18Οm estimates from emyids may actually be in agreement with expectations from other studies. While the sharp cooling immediately prior to the carbon isotopic excursion suggested by the emyid and crocodilian data does not agree with expectations, this result could easily be tested by using the emydid-heterotherm approach to reconstruct paleotemperatures for other areas of the Bighorn Basin.

**Isotopic Integrity of Bone δ18Ο and δ18Οp**

While turtle bone, crocodilian enamel and bone, and gar ganoine seem to reconstruct meaningful paleotemperatures, the possibility of their diagenetic alteration warrants further discussion. The mean δ18Ο value of turtle bone is not statistically distinguishable from that of paleosol carbonate or mammals (Mann–Whitney U-test, 2-tailed p > 0.124) for the P-E interval as a whole. Many of the individual crocodilian enamel and gar ganoine values, however, deviate strongly from coeval paleosol carbonates, mammals, and emydis in a positive direction (Fig. 2). The most positive anomalous values observed in this study (30‰−32‰) cannot be explained by equilibrium isotopic exchange at low or high temperature with pore waters, unless unreasonably high pore-water compositions (e.g., >10‰) are considered. If crocodilians and gars inhabited water evaporatively enriched in 18Ο, a consistent offset between taxonomic groups should be expected for each locality. Crocodilian enamel and gar ganoine, however, are not consistently enriched relative to emydis, with differences ranging from −1.0‰ to +14.9‰. A final explanation for the high δ18Ο values is that our pretreatment methods did not completely remove all organic material, permitting analysis of SO2 contaminants (Cerling and Sharp, 1996; MacFadden, 1998).

The negative carbon isotopic excursion coincident with the P-E boundary in both marine and terrestrial records (Kennett and Stott, 1991; Koch et al., 1992; Bowen et al., 2001) provides another means to assess the isotopic fidelity of structural carbonate in bone. Only turtle bone δ13Ο exhibits a significant negative shift (t-test, p = 0.017) at Wa-0. Following the Wa-0 interval, the mean turtle bone δ13Ο does not record a significant shift back to pre-Wa-0 values (Mann–Whitney U-test, p = 0.896). The fact that the carbon isotopic excursion is not recorded by crocodilian or gar δ13Ο (and is only partially recorded by turtle bone δ13Ο) provides further evidence that structural carbonate has been altered.

Phosphate δ18Ο is less relative variance to carbonate. The mean values for turtle and crocodilian δ18Ο, are statistically indistinguishable from the combined mean value predicted from Coryphodon and Pheneodus δ18Ο (turtles: Mann–Whitney U-test, 2-tailed p = 0.665; crocodilians: t-test, 2-tailed p = 0.767). The mean δ18Ο values for turtle bone (11.6‰), crocodilian enamel (11.6‰) and bone (11.0‰), and gar ganoine (12.7‰) are all slightly more negative than the combined data set predicted from δ18Ο of the terrestrial taxa Ectocion and Hyracotherium (13.3‰). This observation was also made for Coryphodon by Frick and Wing (2004), who argue that the lower Coryphodon values reflect an aquatic habitat. If it is assumed that Coryphodon δ18Ο is pristine, then in order for turtle and crocodilian bone δ18Ο to be altered, the diagenetic end member must lie within the range of 10‰−14‰ predicted from Coryphodon, suggesting equilibrium diagenetic water δ18Ο up to −4‰. While not unreasonable, this is higher than previous estimates of −12‰ to −6‰ for P-E δ18Ο in the Bighorn Basin.

In unaltered fossil mammalian HAP δ18Ο and δ18Οp values should covary as for modern mammals (Bryant et al., 1996; lacumin et al., 1996). Phosphate and carbonate δ18Ο values from this study do not show a strong linear relationship (Fig. 5), which is not uncommon in fossil material (Fricke et al., 1998; Trueman et al., 2003; Zazzo et al., 2004). Without knowing end-member diagenetic values, however, we cannot determine whether δ18Ο or δ18Οp, or both, have been altered.

An additional test for bone diagenesis is to compare enamel and bone from the seven crocodilian specimens with teeth preserved in situ within the jaw. Tooth enamel of crocodilians grows daily, teeth are replaced throughout the life of the animal (Erickson, 1996), and bone is constantly remodeled, so enamel and bone δ18Ο from crocodilians should be relatively comparable temporally. The amount of variation in crocodilian bone that can be explained by variation in enamel is low for δ18Ο (Fig. 6A). Furthermore, δ18Ο values for bone are consistently lower than enamel values (Fig. 3B), and the amount of offset is variable, as is expected for bone that is more extensively altered than enamel, but by variable amounts depending on the locality. Although the dataset is small, the linear regression of bone δ18Ο onto that of enamel (Fig. 6B) shows a much stronger correlation than for δ18Ο (r2 = 0.70). In addition, the regression has a positive slope (1.04) near unity that is statistically significant (p = 0.02), with an intercept (−0.91) near zero and a 95% confidence interval on the intercept that includes zero. These observations suggest that bone and enamel δ18Ο, precipitated in equilibrium from the same body-water reservoir. Unlike the δ18Ο values, bone δ18Ο is not
FIGURE 5—Comparisons of δ¹⁸O and δ¹³C values. A) Turtle bone and gar ganoin. B) Crocodilian enamel and bone. Solid line = linear regression from the combined data set of Iacumin et al. (1996) and Bryant et al. (1996). VSMOW = Vienna Standard Mean Ocean Water.

FIGURE 6—Comparisons of intraindividual samples of crocodilian enamel and bone. A) δ¹³C values. B) δ¹⁸O values. VSMOW = Vienna Standard Mean Ocean Water.

Consistently more negative than that of enamel. In summary, these observations suggest that while δ¹⁸O may be altered, primary δ¹⁸O seems to be preserved in bone.

Crystallinity and [CO₃⁻] as Proxies for ¹⁸O Exchange

The CI values of turtle bone from this study (0.28–0.55) are of the same general magnitude as presumably altered archaeological and fossil bone samples measured by Person et al. (1995), suggesting similar recrystallization of the samples used here. If the supplementary or recrystallized apatite implied by the high CI values was precipitated from relatively ¹⁸O-depleted waters, however, a strong negative relationship should exist between CI and δ¹³C. A negative relationship does exist (Fig. 7A), but it is weak (r² = 0.04). Similarly, recrystallization evinced from decreasing [CO₃⁻] (Zazzo et al., 2004) should be correlated to δ¹⁸O. This is also not the case for bone samples both before (r² = 0.03) and after (r² = 0.01) acetic acid pretreatment (Fig. 7B). One explanation for these results is that the diagenetic end member may lie within the range of primary δ¹⁸O values. Both Zazzo et al. (2004) and Sharp et al. (2000), however, observe that altered HAP samples are generally depleted in ¹⁸O relative to unaltered samples. Furthermore, previous estimates of −12‰ to −6‰ δ¹⁸O, for P-E of the Bighorn Basin suggest that samples exchanging oxygen with these waters should similarly be ¹⁸O depleted. A second explanation is that CI and [CO₃⁻] are poor indicators of δ¹⁸O alteration. A lack of correlation between crystallinity and extent of diagenetic ¹⁸O exchange was reported independently by Pucéat et al. (2004), suggesting that crystallinity may not be a reliable proxy for isotopic fidelity.

Turtle bone [CO₃⁻] measured in this study was relatively high for some samples. Since carbonate δ¹⁸O was measured on samples receiving a single 24 h pretreatment, incorporation of exogenous carbonate into our δ¹³C values is a possibility, and our interpretations should take this into account. If calcite spar that formed in equilibrium with depleted pore fluids influences our measured isotopic values, we might expect a pattern in which high [CO₃⁻] is associated with low δ¹³C. In contrast, the weak relationship between δ¹³C and [CO₃⁻] is positive (Fig. 8A). Furthermore, while preliminary data show that turtle bone structural carbonate might be as high as 10%–11% (Fig. 8B), even samples with biologically reasonable [CO₃⁻] of −5%–6% have a δ¹⁸O range similar to that of samples having presumably high amounts of exogenous carbonate. This
suggests that although some of our isotopic data might include diagenetic calcite spar, even structural carbonate content appears to have decreased in some specimens and exchanged with pore fluids in others.

CONCLUSIONS

Several observations in this study generally support the hypothesis of Barrick et al. (1999), which suggests that phosphate $\delta^{18}$O of fossil emydid turtle bone can be used in conjunction with $\delta^{18}$O from co-occurring heterothermic taxa to quantify paleotemperatures. First, the oxygen isotopic values of bone phosphate measured in this study fall within the range of $\delta^{18}$O values measured from tooth enamel phosphate and estimated from tooth enamel carbonate from the same localities. Since altered bone should generally be $^{18}$O-depleted relative to unaltered bone, a similar range for enamel and bone $\delta^{18}$O implies that the bone is relatively unaltered. Second, in contrast to carbonate $\delta^{18}$O, phosphate $\delta^{18}$O from intraindividual crocodilian samples exhibits a fairly strong positive correlation between bone and enamel. If bone phosphate were extensively altered relative to enamel, these values would not covary, and bone should generally be $^{18}$O-depleted relative to enamel. Finally, paleotemperature estimates based on $\delta^{18}$O$_p$ from co-occurring emydid turtle bone, crocodilian enamel, and gar ganoine are in agreement with independent temperature estimates based on oxygen isotope data from mammals and paleosols, as well as leaf-margin analysis. The turtle, crocodilian, and gar data successfully reconstruct patterns of well-documented climate change observed from these other approaches. The results of this study are significant in that they suggest the reliability of a potentially powerful approach to estimating paleotemperature that does not assume modern gradients between MAT and meteoric water $\delta^{18}$O. Combined with independent estimates from other proxies, this approach may allow a better understanding of past climate change in terrestrial environments. The interpretations of this study, however, cannot necessarily be extended to all fossil bone, and each locality must be evaluated on a case-by-case basis using methods similar to those applied here.

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