Bonebeds

BONEBEDS

Genesis, Analysis, and Paleobiological Significance

Edited by
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and Anthony R. Fiorillo

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CHAPTER 8

Stable Isotope Geochemistry of Bonebed Fossils: Reconstructing Paleoenvironments, Paleoecology, and Paleobiology

Henry Fricke

INTRODUCTION

The tissues of living animals contain carbon, oxygen, and nitrogen, which are sourced from the food, water, and air that animals ingest and respire. This linkage holds true for vertebrate hardparts such as bones and teeth, which consist of a matrix of organic molecules (mostly collagen) surrounded by crystals of bioapatite [$Ca_5(PO_4, CO_3)_3(OH, CO_3)$]. For example, carbon found in bioapatite is related to ingested organic material, such as plants in the case of herbivores and flesh in the case of carnivores. Similarly, oxygen in apatite is obtained from the atmosphere and from water ingested from streams, ponds, lakes, and leaves.

These links have proven very important to scientists interested in earth history because the ratio of stable isotopes of carbon, oxygen and nitrogen, in plants and surface waters can vary a great deal. Carbon isotope ratios of plants change in response to environmental conditions and to the type of photosynthetic pathways utilized by the plant; oxygen isotope ratios of waters in streams, lakes, and leaves vary significantly in response to environmental factors such as temperature and aridity, and in relation to the hydrological "history" of air masses that supply precipitation to these surface water reservoirs. As a result, stable isotope analyses of fossils that record these stable isotope variations can be used to address diverse topics, ranging from the nature of ancient environmental conditions, to differences in the behavior, dietary choices, and preferred habitats of extinct

animals. Vertebrate fossils concentrated in bonebeds offer a particularly exciting opportunity to take advantage of these isotopic relations.

The goals of this chapter are to (1) outline the different ways to undertake stable isotope research using fossils, and (2) explore the different types of questions that can be addressed using stable isotope data. The chapter begins with a review that focuses on isotopic variability in plants and surface waters, isotopic relations between these ingested materials and vertebrate remains, and the preservation of primary isotopic information over time. Many excellent reviews of these topics are already available and, thus, only a brief introduction is provided here. The review is followed by a general consideration of study design in relation to bonebed type, and a summary of the critical assumptions and unknowns that may impact study design. The chapter concludes with specific examples of paleoenvironmental and paleoecological reconstructions using stable isotope data, with the hope that they may provide a template and incentive for future research.

This chapter has two important limitations. First, it focuses only on what can be learned from analyzing bioapatite, and does not discuss organic molecules such as protein and collagen, cholesterols, and lipids, which are also an integral part of vertebrate skeletons. The primary reason for limiting the discussion to bioapatite is because organic remains are very susceptible to decomposition via enzymatic, microbial, and geochemical processes, and whereas bioapatite can be preserved for millions of years, organic molecules are rarely found preserved in fossil remains older than ~75 ky. Second, most of the studies described here center on terrestrial mammal remains, which have received the greatest amount of stable isotope research to date. However, much of what is covered in this chapter is theoretically applicable to vertebrates in general, and researchers are encouraged to apply these approaches to other groups.

STABLE ISOTOPES AND ISOTOPIC VARIABILITY OF BONEBED REMAINS

The primary reason that stable isotope ratios of bonebed fossils can be used to investigate environmental conditions or ecological relations of the past is that plants and surface waters distributed across terrestrial landscapes are often characterized by different stable isotope ratios that can be passed on to animal consumers. The underlying causes for isotopic variability are the small differences in atomic mass between isotopes that, in turn, affect rates of reaction and the strength of bonds (O'Neil, 1986). For example, isotopes of oxygen, carbon, and hydrogen are differentiated from each other by the number of neutrons in the atomic nucleus (e.g., ^{18}O and ^{16}O,

^{13}C and ^{12}C, ^{2}H and H) and these differences often result in a separation—or fractionation—of heavy and light isotopes during physical, chemical, and biological processes. Differences in the relative abundance of heavy to light isotopes (the isotope ratio) in various materials can be quite large, particularly when reactions are ongoing (e.g., most biochemical reactions, evaporation of large water bodies). In such cases, products are often enriched in the light isotope.

The relative abundance of heavy to light isotopes in most materials is on the order of parts per thousand, and stable isotope ratios in any material are reported relative to a standard using the δ notation, where $\delta = (R_{sample}/R_{standard} - 1)1000‰$ (rather than %). Isotope standards are often derived from common materials and include standard mean ocean water (VSMOW) for oxygen, and marine carbonate (VPDB) for carbon. Hydrogen isotope ratios of bioapatite are not commonly analyzed, do not appear to be preserved well over time (Kohn et al., 1999), and are not discussed further.

Carbon Isotope Variability of Plants

Plants living in terrestrial environments may exhibit a large range in $\delta^{13}C$, from approximately –36‰ to –10‰, even across a relatively small geographic area. As illustrated schematically in Figure 8.1, plants growing in open, dry, saline, or nutrient-poor settings generally have higher $\delta^{13}C$ than those living in closed, wet, nutrient-rich environments. Fully aquatic plants, however, often have higher carbon isotope ratios than fully terrestrial plants. Such carbon isotope variability can be key in helping to differentiate between fossil herbivores that routinely ate different plants, or ingested water from different parts of a large paleoecosystem.

Causes of carbon isotope variability in plants have been the focus of several excellent reviews (O'Leary, 1988; Farquhar et al., 1989; O'Leary et al., 1992; Kohn and Cerling, 2002) and are briefly summarized here. One very important factor is the photosynthetic pathway utilized by the plant. Most carbon in plant organic material is derived from the reduction of atmospheric CO_2 during photosynthesis. Stable isotopes of carbon are fractionated from one another during this process, and the extent of this fractionation (i.e., carbon isotope discrimination between organic material and the atmosphere) varies depending on which specific photosynthetic pathway is utilized by a given plant. For example, plants using the C3 (Calvin) pathway are characterized by a large isotopic discrimination and have $\delta^{13}C$ values that range from approximately –36‰ to –21‰

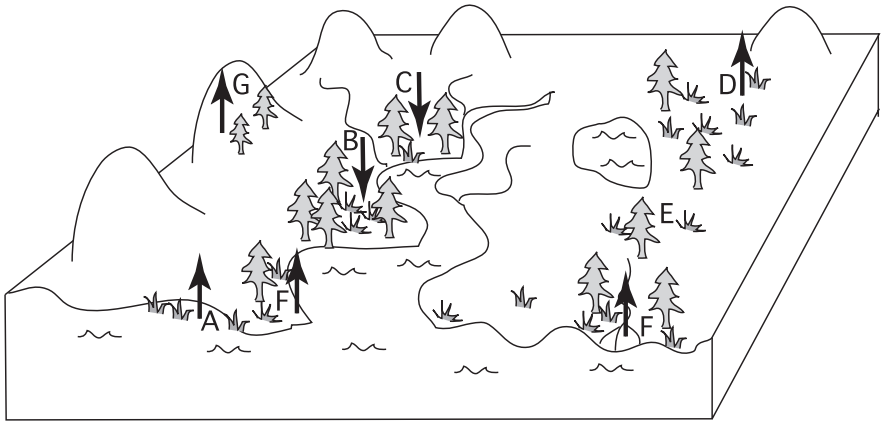


Figure 8.1. Schematic illustration of relative $\delta^{13}C$ values that may be found for C3 plants occupying different parts of a coastal/terrestrial ecosystem. The latter include: A, fully marine plants; B, closed-canopy forest; C, open forest, water available; D, open grassland; E, mixed forest/grassland; F, coastal marsh/mangrove forest; G, high-elevation areas. Using area E as a baseline, plants living in areas A, D, F, and G are likely to have higher $\delta^{13}C$, while those living in areas B and C are likely to have lower $\delta^{13}C$. Reasons for relatively higher $\delta^{13}C$ values include slow rates of diffusion of CO_2 through water (A); enhanced aridity and thus less moisture availability in open environments (D); osmotic stress in brackish waters along with the possibility of evaporative tidal flat settings (F); lower concentrations of CO_2 in the atmosphere at higher elevations (G). Reasons for relatively lower $\delta^{13}C$ values include recycling of CO_2 in the understory of dense, closed-canopy forests (B), and the abundant availability of fresh water (C). C4 plants living in any of these areas can greatly increase the average $\delta^{13}C$ of plants living in them. Many C4 plants are grasses common to open, dry environments (such as D) or warm tropical regions. Because combinations of different factors such as regional climate, hydrology, and plant type may all act to modify the general pattern presented here, this figure alone should not be used to interpret isotope data from bonebed remains.

compared to $\delta^{13}C$ of about $-8‰$ for modern atmospheric CO_2. In contrast, C4 (Hatch-Slack pathway) plants are characterized by less isotopic discrimination and variability, with $\delta^{13}C$ values generally ranging from –14 to –10‰. Less common CAM (Crassulacean acid metabolism pathway) plants utilize a combination of C3 and C4 photosynthetic processes and have $\delta^{13}C$ values intermediate to those of C3 and C4 plants.

Local environmental conditions also play an important role in influencing carbon isotope ratios of plants, particularly in the case of plants utilizing the C3 photosynthetic pathway. These plants are very sensitive to the amount of CO_2 in a leaf cell, and when there is an excess of CO_2, $\delta^{13}C$ values in synthesized tissues will be lower, reflecting greater selectivity for ^{12}C. In contrast, when CO_2 concentrations are limited, a greater proportion of all C atoms will be utilized for tissue generation, and the associated isotopic discrimination between ^{12}C and ^{13}C will be reduced (Farqhar et al., 1989). In turn, concentrations of CO_2 in a leaf cell are influenced a great deal by the opening and closing of leaf stomata, which controls the flux of CO_2 into the plant. Stomata are more likely to remain closed when

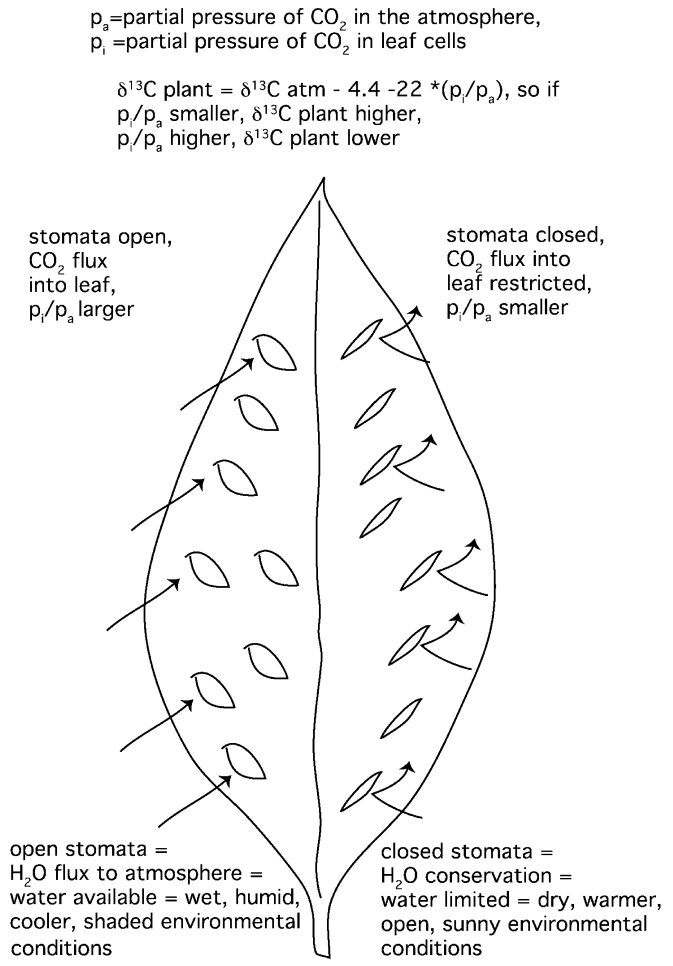


Figure 8.2. Influences on the $\delta^{13}C$ of C3 plants can be represented by the following equation: $\delta^{13}C_{plant} = \delta^{13}C_{atmosphere} - a - (b - a)*(p_i/p_a)$, where "a" represents the isotopic fractionation associated with the diffusion of CO_2 into a leaf and is typically about 4.4‰, "b" is the isotopic fractionation associated with carbon fixation into organic matter and is typically about 29‰, p_i is the partial pressure of CO_2 inside a leaf cell, and p_a is the partial pressure of CO_2 in the atmosphere (after Farquhar et al., 1989).

environmental factors such as temperature, water availability, and light intensity are such that water needs to be conserved (Fig. 8.2) (O'Leary, 1988; Farqhar et al., 1989; Tieszen, 1991; O'Leary et al., 1992). For example, under arid, high-temperature, or high-light conditions, stomata remain closed to minimize water loss. As a result, CO_2 concentrations in leaf cells decrease, and $\delta^{13}C$ increases. Conversely, plants growing in wetter, shaded, cooler areas keep their stomata open for a longer period, CO_2 concentrations in the leaf are higher, and $\delta^{13}C$ values are lower.

Summaries of these environmental effects are also provided by Heaton (1999) and Arens et al. (2000).

$\delta^{13}C$ of plant material may also be affected by the "canopy effect." In this situation, CO_2 under the canopy of a closed forest exhibits lower carbon isotope ratios than the open atmosphere due to plant respiration and decomposition on or near the forest floor. When incorporated into understory plants during photosynthesis, the lower $\delta^{13}C$ values stand out in comparison to the same plants living in open canopy settings (van der Merve and Medina, 1991; Cerling et al., 2004). Because the canopy effect is due to local changes in $\delta^{13}C$ of CO_2, it is independent of photosynthetic pathways or specific environmental conditions.

Lastly, there may be taxon-specific differences in $\delta^{13}C$ of C3 plants living in any one place (Tieszen, 1991; Heaton, 1999; Arens et al., 2000, Codron et al., 2005). However, they have not been studied systematically for many modern and ancient environments and are not discussed further. $\delta^{13}C$ of atmospheric CO_2 can also change over time in response to perturbations in the global carbon cycle, and absolute $\delta^{13}C$ of all plant types will change in a similar direction (Koch et al., 1992), although not necessarily to the same degree (Bowen et al., 2004). It should also be noted that most of the paleobotanical fossil record prior to the late Cenozoic is dominated by C3 plants. There is evidence that plants utilizing both C4 and CAM photosynthetic pathways may have evolved several times during the Mesozoic; their occurrence however, appears to have been limited in number and restricted to only certain kinds of terrestrial settings (Wright and Vanstone, 1991; Kuypers, et al., 1999; Krull and Retallack, 2000; Edwards et al., 2004). It is not until the Miocene, ~8 Ma, that the global expansion of C4 grasslands occurred, resulting in modern terrestrial ecosystems (Cerling et al., 1997a). The C4 pathway is well adapted to high-light, high-temperature, and arid conditions, thus the recognition of these kinds of plants in the fossil record can be used to indicate plant biology as well as broad paleoenvironmental conditions.

Oxygen Isotope Variability of Surface Waters

Oxygen isotope systematics of surface waters are more complicated than those of carbon in plants because large regional and global scale patterns in oxygen isotope ratios exist (Fig. 8.3), and any local variations in oxygen isotope ratios of surface waters that may occur over a small area (e.g., Fig. 8.4) are then superimposed on the larger-scale patterns. Despite this additional complexity, oxygen isotope ratios in the hardparts of vertebrates

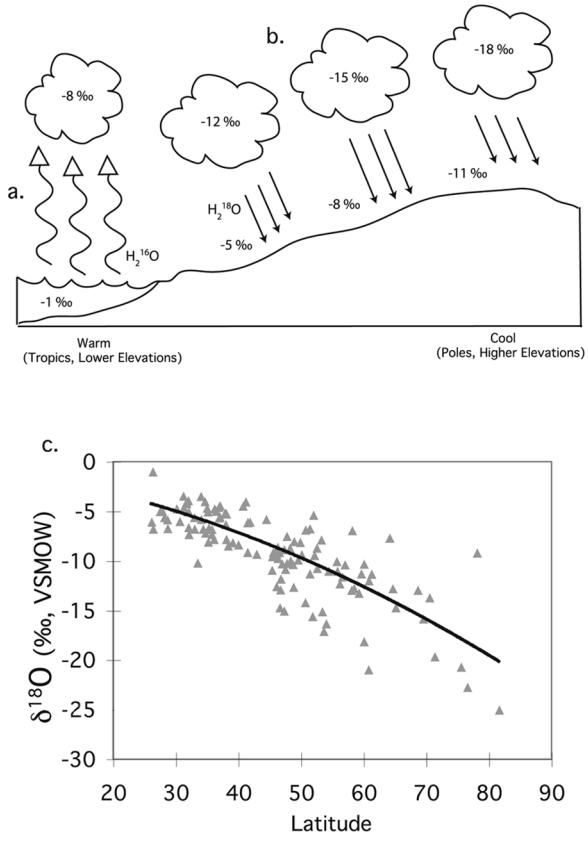


Figure 8.3. Schematic illustration of the hydrologic processes which effect oxygen isotope ratios of precipitation (and surface waters) at global and regional scales. A. Because the lighter isotope is preferentially incorporated into the vapor phase during evaporation, water in air masses over the ocean have lower $\delta^{18}O$ values than ocean water. B. Condensation takes place as these air masses move over land or up elevation. The heavier isotope is preferentially incorporated into the liquid or solid phase so that precipitation has a higher $\delta^{18}O$ than the remaining water vapor. Ongoing cooling and, thus, distillation of air masses results in progressively lower $\delta^{18}O$ values of both vapor and precipitation. C. As latitude increases, air-mass distillation generally results in precipitation with a decreased $\delta^{18}O$ (after Rozanski et al., 1993).

that drink surface water can record oxygen isotope variability and, in turn, can be used to infer both paleoenvironmental and paleoecological conditions. The following is a brief summary of causes of oxygen isotopic variability in surface waters on global and local scales.

At present, $\delta^{18}O$ of global precipitation ranges from approximately 0‰ to –30‰ (Dansgaard, 1964; Rozanski et al., 1993). The primary cause of isotopic variability in precipitation is the preferential incorporation of ^{18}O into condensate as water is precipitated and removed from cooling air masses (Fig. 8.3). As more precipitation is removed from an air mass,

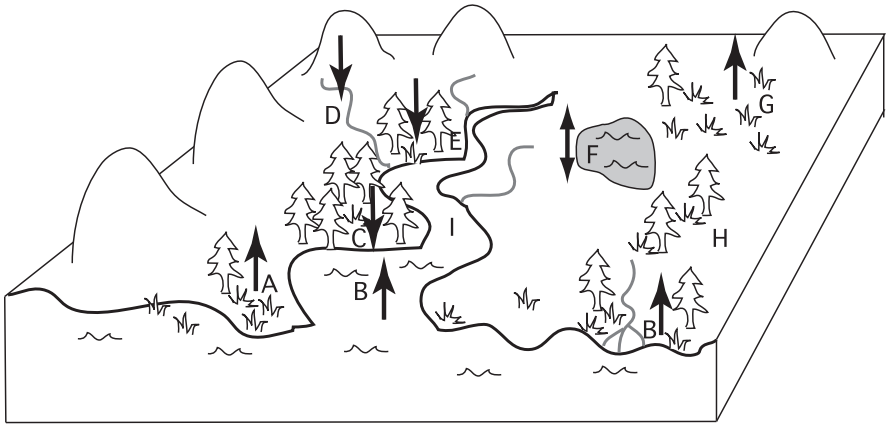


Figure 8.4. Schematic illustration of relative $\delta^{18}O$ values that may be found for surface water (both standing and leaf water) located in different parts of a coastal to terrestrial ecosystem. A. Freshwater swamps. B. Zone of marine and fresh water mixing. C. Forest canopy. D. Streams with precipitation from high elevation. E. River and river margin. F. Ponds and lakes. G. Open grassland. H. Mixed forest/grassland. I. Trunk river. Using area H as a baseline, water from A, B, and G are likely to have higher $\delta^{18}O$, while that from C, D, and E are likely to have lower $\delta^{18}O$. Reasons for relatively higher $\delta^{18}O$ values include evaporation of standing water, particularly in open vegetation or arid settings (A); mixing of high $\delta^{18}O$ ocean water with freshwater sourced in precipitation (B); enhanced evaporation of leaf water in sunnier, windier open settings (G). Reasons for relatively lower $\delta^{18}O$ values include reduced evaporation of leaf water in shady, still understory settings (C); collection of precipitation from higher elevations having lower $\delta^{18}O$ values (D); more humid air and saturated soils (E). $\delta^{18}O$ values of ponds and lakes are variable but are likely to be higher than $\delta^{18}O$ of local precipitation, except when very small or when located in very humid settings. $\delta^{18}O$ of a trunk river (I) will depend on the source and hydrologic history of all the waters collected and mixed into it at any given point. Because combinations of different factors such as regional climate, hydrology, and plant type may all act to modify the general pattern presented here, this figure alone should not be used to interpret isotope data from bonebed remains.

$\delta^{18}O$ of the remaining vapor becomes progressively lower. The resulting patterns in $\delta^{18}O$ of precipitation ($\delta^{18}O_{pt}$) include a regular decrease in $\delta^{18}O_{pt}$ as air masses cool while rising over mountains, moving away from coastal areas, or moving from tropical source areas to polar sinks (Epstein and Meyada, 1953; Dansgaard, 1964; Rozanski et al., 1993; Gat, 1996). In tropical regions, where vertical convection results in cooling, a correlation also occurs between the amount of precipitation and $\delta^{18}O_{pt}$ (Dansgaard, 1964; Araguas-Araguas et al., 1998).

Although regional temperatures and rainout patterns play a major role in determining the oxygen isotope ratio of precipitation at any given locality, terrestrial vertebrates generally do not ingest precipitation directly. Instead, they ingest water from surface water reservoirs such as streams, lakes, and leaves. In turn, these reservoirs may have oxygen isotope ratios that differ significantly from those of local precipitation due to a variety of local hydrological processes (Fig. 8.4). For example, small ponds and

streams in humid areas may hold local precipitation with little isotopic modification, but larger lakes and soil waters, especially in arid regions, may undergo evaporation that modifies their oxygen isotope ratio via the preferential incorporation of ^{16}O into the vapor phase. Similarly, $\delta^{18}O$ values of leaf water ingested by herbivorous animals can be shifted to higher values relative to precipitation as a result of evaporation at the surface of a leaf, particularly in less-humid environments (Sternberg, 1989). Lastly, precipitation from large areas and over long periods of time can be mixed together during the formation of lakes, soil water, groundwater, and larger rivers.

Bioapatite as a Record of Isotope Variability over an Ancient Landscape

Animals record the isotopic characteristics of ancient landscapes when they ingest organic material and drink from surface water reservoirs. After being eaten by an herbivore, organic compounds in plants are metabolized and carbon is incorporated into a number of different phases, including dissolved CO_2 and HCO_3^-, which ultimately come to reside in the herbivore's bioapatite (Koch et al., 1994; Koch, 1998; Cerling and Harris, 1999; Passey et al., 2005). Carbon isotope fractionations associated with these processes result in $\delta^{13}C$ values of both bioapatite carbonate and organic materials that are significantly higher than those of ingested plant matter. For large wild mammals, the offset between $\delta^{13}C$ of bioapatite and bulk diet is approximately 12–15‰ (Koch, 1998; Cerling and Harris, 1999; Kohn and Cerling, 2002; Hoppe et al., 2004a; Passey et al., 2005), for rodents the offset is approximately 10‰ (DeNiro and Epstein, 1978; Ambrose and Norr, 1993), for large birds the offset between $\delta^{13}C$ of eggshell carbonate and bulk diet is about 14–16‰ (Schaffner and Swart, 1991; Johnson et al., 1998), and for dinosaurs the offset between $\delta^{13}C$ of bioapatite and presumed bulk diet is estimated to be roughly 18‰ (Fricke et al., in review). The reason for these different amounts of offset is not clear, although they may be related to differences among taxa regarding (1) the biogeochemical processes that take place as carbon from plants is incorporated into bioapatite and organic materials of herbivores, or (2) which organic compounds in a plant (i.e., proteins, carbohydrates, lipids) are actually utilized by the animal (DeNiro and Epstein, 1978; Krueger and Sullivan, 1984; Lee-Thorp et al., 1989; Ambrose and Norr, 1993; Gannes et al., 1998; Koch, 1998; Hedges, 2003; Jim et al., 2004; Passey et al., 2005).

Carbon isotope ratios of modern terrestrial carnivores have also been studied (both bioapatite and bone proteins; Ambrose and DeNiro, 1986;

Lee-Thorp et al., 1989; Hilderbrand et al., 1999; Roth and Hobson, 2000; Roth, 2002; Bocherens and Drucker, 2003; Kohn et al., 2005; Fox-Dobbs et al., 2006), but not as extensively as in herbivores. Paleoecological and dietary interpretations of bioapatite isotope data from carnivores are difficult to make because there is a lack of empirical data regarding the isotopic offset between $\delta^{13}C_{herbivore}$ and $\delta^{13}C_{carnivore}$, and an incomplete understanding of why and how these offsets occur. Lee-Thorpe et al. (1989) calculated isotopic offsets between carnivores and other organisms from a South African ecosystem where C4 plants were presumed to be absent. They observed that $\delta^{13}C$ of herbivore meat is ~2.5‰ higher than that of consumed plants and calculated an offset of ~9‰ between carnivore bioapatite and herbivore meat. Accordingly, they calculated that the carbon isotope offset between carnivore bioapatite and local plants is ~11.5‰, a value similar to that observed between herbivore bioapatite and plants living in the same area. In contrast, Fox-Dobbs et al. (2006) analyzed remains of coexisting wolves and large herbivores from C3 ecosystems of North America, and observed that carnivore $\delta^{13}C$ was consistently ~1‰ lower than herbivore $\delta^{13}C$.

In order to understand these offsets and whether they should be expected to remain constant in the face of taxonomic, dietary, or environmental changes, researchers have used experimental approaches (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Balasse et al., 1999; Passey et al., 2005; Zazzo et al., 2005) and modeling (Hedges, 2003). If the results of Lee-Thorpe et al. (1989) or Fox-Dobbs et al. (2006) are confirmed by future research, then $\delta^{13}C_{herbivore} - \delta^{13}C_{carnivore}$ values may be useful in identifying the dietary choices of ancient carnivores and, thus, ancient trophic relationships.

Oxygen in vertebrate bioapatite has sources primarily in ingested water and atmospheric oxygen that contribute to blood/metabolic water (Longinelli 1984; Luz and Kolodny, 1985; Bryant and Froelich, 1995; Kohn, 1996; Kohn and Cerling, 2002). $\delta^{18}O$ of atmospheric oxygen has remained relatively constant over time and space with a value of ~23‰ (Kohn, 1996). Thus, it probably does not influence oxygen isotope variations in bioapatite of vertebrates living in different places or drinking different waters (Fig. 8.4).

The isotopic offset between ingested surface water and both phosphate and carbonate phases that are present in biogenic apatite is controlled by (1) body temperature, which determines the isotopic fractionation between apatite and body water, and (2) fractionations that occur during the formation of body water from ingested water. Where body temperature is known and constant (i.e., homeothermic mammals and birds), both of these factors can be considered together using physiological models that account for the fluxes of oxygen into and out of the body, and the

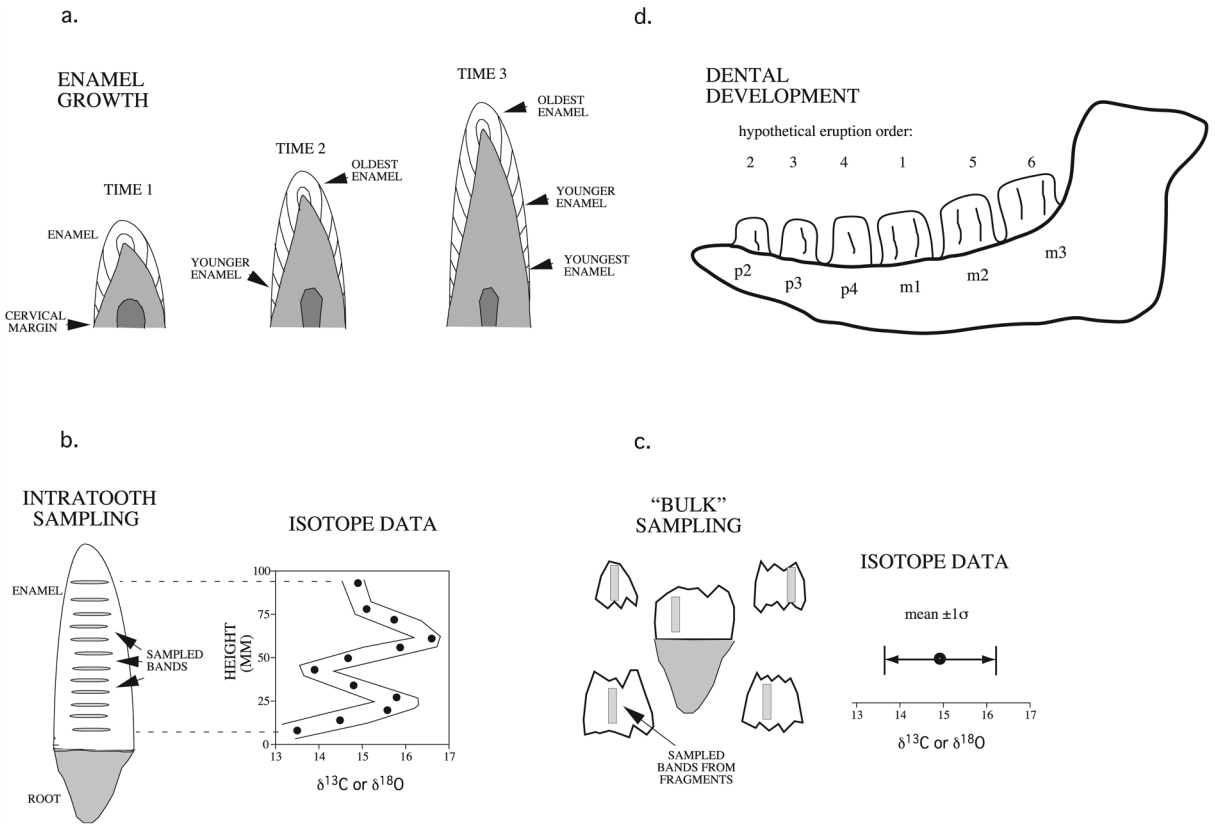
oxygen isotope fractionations associated with each metabolic process (Bryant and Froelich, 1995; Kohn, 1996). Alternatively, direct empirical relations between $\delta^{18}O$ of bioapatite and $\delta^{18}O$ of meteoric water may be used for different kinds of animals. In general, both methods of determining the isotopic offset between $\delta^{18}O$ of bioapatite (or carbonate in the case of eggshell) and $\delta^{18}O$ of ingested waters provide similar results for large mammals and birds that do not rely on ingested leaf water or rely on unique water conservation strategies (Schaffner and Swart, 1991; Johnson et al., 1998; Koch, 1998; Iacumin and Longinelli, 2002; Kohn and Cerling, 2002; Hoppe et al., 2004a; Hoppe, 2006).

Determining the offset between $\delta^{18}O$ of bioapatite and $\delta^{18}O$ of ingested waters is not straightforward if body temperature is unknown (e.g., dinosaurs). In such cases, only relative differences in $\delta^{18}O$ of ingested waters may be inferred. Alternatively, if body temperature varies in response to environmental conditions (e.g., ectothermic reptiles, amphibians, fish), then environmental temperature, which is used to estimate the $\delta^{18}O$ of ingested waters, must be determined independently.

Bones, Teeth, and Scales

Bioapatite is a major component of several different skeletal elements found in vertebrates, including teeth, tusks, bones, and body scales. Both teeth and tusks consist of two main kinds of materials: enamel and dentine. The former forms a hard shell around the softer dentine core. Body scales of some fish are analogous to teeth in that a carapace of enamel-like bioapatite (ganoine) covers dentine-like material. Teeth and body scales grow incrementally for a limited period early in the life of an animal, whereas tusks continue to grow until an animal dies. In all three cases, once bioapatite is deposited it is no longer open to chemical modification via biological or physiological processes. Thus, teeth, tusks and scales "lock in" the isotopic ratios that existed during bioapatite deposition. Bone, however, can be remodeled over the entire life of an organism, and stable isotope ratios in its bioapatite may simply reflect conditions prior to death.

Kohn and Cerling (2002) provide an excellent overview of the mineralogy and biogeochemistry of these materials, and only key factors are repeated here. In living vertebrates, enamel, dentine, and bone differ primarily in (1) the size of apatite crystals and (2) the amount of organic collagen originally present. Bone has very small apatite crystals tens of nanometers in length, and a framework of organic collagen that makes up ~35% of unaltered bone. Tooth dentine is characterized by similar crystal


a.
ENAMEL GROWTH
TIME 1
ENAMEL
CERVICAL MARGIN
TIME 2
OLDEST ENAMEL
YOUNGER ENAMEL
TIME 3
OLDEST ENAMEL
YOUNGER ENAMEL
YOUNGEST ENAMEL
d.
DENTAL DEVELOPMENT
hypothetical eruption order:
2 3 4 1 5 6
p2 p3 p4 m1 m2 m3
b.
INTRATOOTH SAMPLING
ENAMEL
SAMPLED BANDS
ROOT
ISOTOPE DATA
HEIGHT (MM)
100 75 50 25 0
13 14 15 16 17
δ13C or δ18O
c.
"BULK" SAMPLING
SAMPLED BANDS FROM FRAGMENTS
ISOTOPE DATA
mean ±1σ
13 14 15 16 17
δ13C or δ18O

sizes, but less collagen (~20%). In contrast, enamel is made up of larger apatite crystals hundreds of nanometers in length and only contains <5% original organic material. These differences influence porosity and, thus, the quality of preservation of isotopic information over time (see below).

One final detail to consider is that teeth of many vertebrates grow incrementally over the span of months to years (Fig. 8.5a; Hillson, 1986) and, thus, tooth enamel and tusks can capture short-term isotopic variations in carbon and oxygen isotope ratios of ingested food and water (Fig. 8.5b; Koch et al., 1989; Fricke and O'Neil, 1996; Stuart-Williams and Schwarcz, 1997; Fricke et al., 1998a; Kohn et al., 1998; Sharp and Cerling, 1998; Wiedemann et al., 1999; Gadbury et al., 2000; Fox and Fisher, 2001, 2004; Passey and Cerling., 2002a; Feranec 2004a; Higgins and MacFadden, 2004; Hoppe et al., 2004b; Rinaldi and Cole, 2004; Straight et al., 2004; Nelson, 2005; Zazzo et al., 2005). In fact, both carbon isotope ratios of plants and oxygen isotope ratios of surface water can vary over the course of a year in response to changes in environmental conditions such as temperature, humidity, and precipitation. Furthermore, vertebrate herbivores may occupy different parts of an ecosystem and consume different plants and waters depending on the time of year. Thus, the intratooth isotope variability that results must be quantified in order to effectively compare data between and among populations of animals. Ultimately, such variability may be useful in studying seasonal changes in climatic variables or seasonal shifts in animal behavior (see below).

STABLE ISOTOPE RATIOS AND DIAGENESIS

In order for stable isotope ratios of vertebrate fossils from bonebeds to provide useful information regarding environments or ecological relations

Figure 8.5. A. Enamel and dentine formation occurs incrementally as a tooth erupts past the cervical margin. Thus, a continuous time series of isotopic information is preserved. Note that bands of enamel form at an angle to the enamel and dentine contact. Because it is difficult to measure the isotopic composition of only a single time band, some mixing, or dampening, of isotopic signals may occur. B. Isotopic data ($\delta^{18}O$ of water and $\delta^{13}C$ of plants) from sampled bands of enamel along the direction of growth provide information about the magnitude of seasonal variations in environmental conditions. Using these data it is also possible to estimate rates and season of tooth growth in an individual. C. Seasonal variations in isotope ratios can also be assessed by sampling enamel from isolated tooth fragments. Assuming no major climatic changes over time, average $\delta^{18}O/\delta^{13}C$ values for a population of fragments approximates the annual mean isotope ratio, and the standard deviation approximates the seasonal variability (Clementz and Koch, 2001). D. Teeth in a jaw do not all erupt through the cervical margin at the same time, and the eruption rate may vary from tooth to tooth. It may be possible to quantify these rates of dental development by conducting intratooth sampling of all the teeth in a jaw and comparing their isotopic patterns (e.g., Kohn et al., 1998; Gadbury et al., 2000; Nelson, 2005).

of the past, primary isotopic information must be obtained. Therefore, the topic of diagenesis (postdepositional alteration) of original isotope ratios is of tremendous importance. There is now broad consensus on a number of diagenetic issues. First, enamel, dentine, and bone have different susceptibilities to diagenetic alteration, with enamel regarded as the least susceptible and, thus, the most preferred for analysis. Secondly, mechanisms of geochemical alteration may be different for stable isotopes than for trace elements, including rare earth elements (REE), and therefore a decoupling of isotopic and REE alteration is possible. Lastly, although there is no unambiguous way to determine whether diagenetic alteration of primary isotope ratios has occurred, it is still possible to obtain useful paleoenvironmental and paleoecological information using stable isotope ratios as long as the potential for some degree of alteration is recognized and materials suitable for analysis are identified.

Differing Susceptibilities to Alteration

Biogenic apatite that makes up hardparts of vertebrate fossils is far more common in the geologic record than organic material, and this fact reflects its general resistance to decomposition over a wide range of geochemical conditions. Nevertheless, there are both physical and chemical variations within bioapatite that may result in differing susceptibilities to alteration. For example, the size of apatite crystals and the original amount of organic matter present are not uniform among skeletal elements. Furthermore, the isotopic ratios of two chemical components found in bioapatite, PO_4 and CO_3, are commonly studied, and these may be affected differently even when exposed to the same diagenetic conditions.

In general, diagenetic alteration of bioapatite is thought to occur by two end-member processes: (1) isotopic exchange between biogenic apatite and surrounding fluids containing H_2O, HCO_3^-, CO_2, CH_4, and (2) dissolution and/or addition of secondary apatite and carbonate (Zazzo et al., 2004). The former requires that P-O or C-O bonds of anionic complexes within apatite are broken and then reformed so that isotope exchange may occur. In order for the isotope ratio of carbonate or phosphate in recrystallized apatite to differ significantly from initial ratios (and thus be recognized as resulting from diagenesis), the temperature of this diagenetic process must be significantly different than that of the original bioapatite formation, or the isotopic exchange must occur in the presence of C and O from external sources that have an isotope ratio much different than that found in the primary phosphate or carbonate complex.

In the case of secondary mineral precipitation, biogenic apatite may retain its original isotope ratios but the primary signal can be overwhelmed by that of secondary precipitates. In these cases, the degree of isotopic alteration observed will depend on the percentage of secondary mineral present. Secondary carbonate and apatite minerals are often precipitated from groundwater that is isotopically different than body water and at temperatures that are much different than those in the body of an animal. Accordingly, secondary minerals often have an isotope ratio that is significantly different from that of original (and unaltered) biogenic apatite.

A basic understanding of these processes is important because skeletal remains with high porosities can be subjected to great fluxes of exogenous fluids, while those with small apatite crystals will have much more surface area available to undergo isotopic exchange and more volume available for precipitation of secondary phosphates and carbonates. Of the common skeletal materials, bone has very small apatite crystals and a high porosity potential due to the high percentage of organic collagen that is likely to be altered or removed soon after burial. In contrast, enamel is made up of much larger crystals and has much less porosity potential. Thus, although bone is most likely to be susceptible to all diagenetic processes (Nelson et al., 1986; Kolodny et al., 1996; Kohn and Cerling 2002; Trueman et al., 2003), tooth dentine is less likely to be affected, and tooth enamel is the least likely to be affected. For this reason, it is strongly recommended that bone be avoided in favor of tooth enamel as a substrate for isotopic analysis.

In the case of chemical differences within enamel bioapatite, P-O bonds are stronger and more resistant to breaking during inorganic reactions than C-O bonds (Tudge, 1960; Zazzo et al., 2004). In general, therefore, phosphate oxygen in enamel is considered better suited for isotopic research than carbonate carbon and oxygen in enamel (see review by Kohn and Cerling, 2002), but ratios from both should be considered suspect unless there is convincing evidence that alteration did not occur.

There are also important exceptions to these generalities. In the presence of microbial activity, enzyme-mediated reactions may break P-O bonds (Blake et al., 1997; Lécuyer et al., 1999), and may do so to an even greater extent than C-O bonds (Zazzo et al., 2004). In contrast, skeletal remains buried quickly in clay-rich sediment, or buried in very arid settings, may be effectively sealed off from exposure to groundwater and microbial activity. For this reason, alteration needs to be considered on a case-by-case basis, and it is necessary to have testable means of identifying whether primary isotope information is preserved.

It must also be stressed that the mechanisms of stable isotope alteration are not the same as those that cause changes in REE signatures of

bioapatite (Trueman, Chapter 7 in this volume). Alteration of REE values is due primarily to cation substitution of elements for Ca in the crystal lattice and/or the adsorption of these elements onto surfaces of small apatite crystals, the effects of which may be enhanced by recrystallization of apatite (Kohn et al., 1999; Trueman and Tuross, 2002; Trueman, Chapter 7 in this volume). Neither cation substitution nor apatite recrystallization result in the breaking of C-O and P-O bonds that make up anionic complexes in this mineral, thus neither process will necessarily be associated with diagenetic alteration of stable isotope ratios of enamel bioapatite (Trueman et al., 2003).

Because there is no direct geochemical link between REE and stable isotope diagenesis, the occurrence of each must be addressed independently. It is quite possible that REE signatures of vertebrate fossils will reflect geochemical conditions of early depositional environments due to the ease with which cation substitution occurs during diagenesis (Kohn et al., 1999; Trueman and Tuross, 2002; Trueman, Chapter 7 in this volume), while at the same time primary stable isotope ratios related to animal behavior and biology will be preserved. In such an ideal case, combined stable isotope and REE analysis of bonebed remains can be amazingly powerful, having the potential to provide insight into both environmental conditions and ecological relations while the animals were living (stable isotopes; see below) and information regarding depositional environments and taphonomic processes after their deaths (REE).

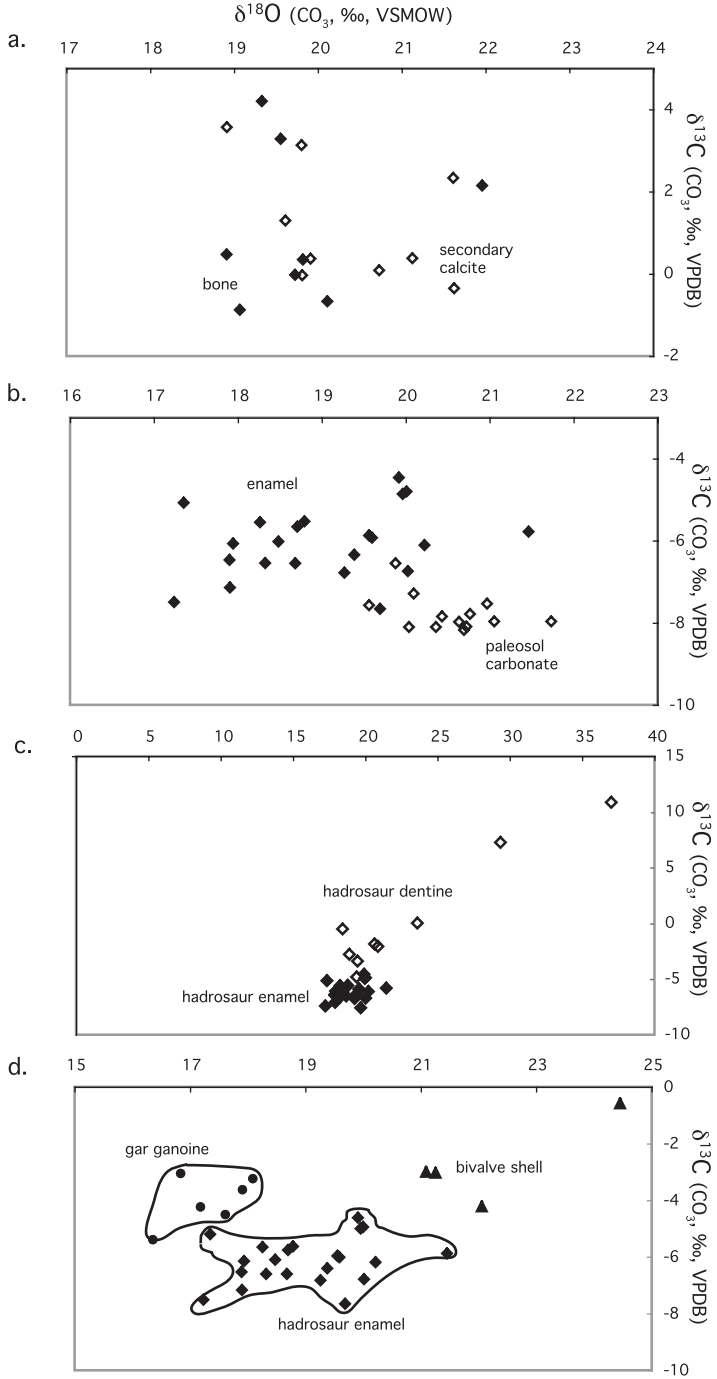
Identifying Effects of Isotopic Alteration

Although reasonable arguments can made that certain kinds of vertebrate remains are more or less susceptible to diagenetic alteration, it should never be assumed that original stable isotope information is or is not preserved for any given material from any bonebed. Instead, attempts should be made to resolve whether a primary signal remains. Many different methods of doing so have been proposed, but none provide unambiguous results (Sharp et al., 2000; Kohn and Cerling, 2002; Fricke et al., in review). Therefore, it is suggested here that several different tests for diagenetic alteration be applied with the goal of obtaining consistent results. Tests emphasized here involve comparing isotope data from bioapatite in enamel with isotope data from (1) associated authigenic carbonates and phosphates and (2) associated dentine or bone. A third test involves comparing isotope data from bioapatite in enamel or biocarbonate of shell among different taxa.

The first approach is based on the premise that authigenic minerals found in sediments hosting vertebrate remains should have isotope ratios that reflect those of diagenetic fluids and temperatures. Thus, any evidence of an isotopic relation between data sets, such as the formation of an isotopic mixing relationship can be used to identify effects of diagenetic overprinting of primary isotope ratios (Fig. 8.6a), whereas lack of mixing trends indicate that alteration is perhaps minimal (Fig. 8.6b; Barrick and Showers, 1994, 1995; Quade et al., 1992). It is difficult to determine, however, if the timing of authigenic mineral formation and the geochemical conditions reflected by authigenesis are, in fact, the same as the diagenetic processes that may be influencing the isotope ratios of bioapatite. In other words, authigenic mineral formation and isotopic alteration may be geochemically decoupled, as are processes of REE alteration and isotope alteration (Trueman and Tuross, 2002; Trueman, Chapter 7 in this volume).

Because of this uncertainty, it is better to compare isotope data between different skeletal components such as enamel, dentine, and bone (Fig. 8.6c; Wang and Cerling, 1994). Here, the assumptions are that (1) alteration of these biogenic materials occurred at the same time and (2) any isotopic difference between enamel, dentine, or bone reflects differences in crystal size and porosity, and thus susceptibility to isotopic exchange and precipitation of secondary minerals during diagenesis. In this test, if isotope ratios of tooth enamel are observed to have significantly different average values and variances than dentine or bone, then it can be concluded that the latter have been altered to some degree by diagenetic processes (Fig. 8.6c). Such a result does not rule out alteration of enamel, but it does provide strong evidence that enamel has not been affected by diagenesis to the same degree as dentine or bone.

Additional support for preservation of primary isotope information in enamel can be gathered by comparing isotope data from fossil remains of different taxa (Fig. 8.6d). Differences in the mean and/or variance for populations of different animals have been observed for a number of taxa from different time periods and localities (Feranec and MacFadden, 2000, 2006; Clementz et al., 2003; Cerling et al., 2004; Kohn et al., 2005), and are expected if the taxa are characterized by different physiologies and/or ecological behaviors. For example, a semiaquatic herbivore that drinks river water and eats plants that have not experienced stress due to limited water availability should have lower $\delta^{18}O$ and $\delta^{13}C$ than herbivores that rely on leaf water and eat stressed plants from drier parts of the local environment. Resulting isotopic offsets among taxa would not exist if isotopic alteration was extensive. In such a case isotopic exchange with groundwaters or secondary precipitation of apatite during diagenesis should result


a.
δ18O (CO3, ‰, VSMOW)
17
18
19
20
21
22
23
24
δ13C (CO3, ‰, VPDB)
4
2
0
-2
bone
secondary
calcite
b.
16
17
18
19
20
21
22
23
δ13C (CO3, ‰, VPDB)
-4
-6
-8
-10
enamel
paleosol
carbonate
c.
0
5
10
15
20
25
30
35
40
δ13C (CO3, ‰, VPDB)
15
10
5
0
-5
-10
hadrosaur dentine
hadrosaur enamel
d.
15
17
19
21
23
25
δ13C (CO3, ‰, VPDB)
0
-2
-4
-6
-8
-10
gar ganoine
bivalve shell
hadrosaur enamel

in uniform isotope ratios. It is possible, of course, that isotopic overlap will occur between taxa whose remains are unaltered due to similarities in the isotopic composition of animal diet, drinking water, or physiological processes. Thus, a lack of isotopic offset between fossil taxa does not necessarily imply diagenesis.

A slightly different way of constraining the amount of oxygen isotope alteration that may have occurred involves a comparison of $\delta^{18}O$ values from CO_3 and PO_4 components from the same skeletal sample. Modern mammal bone and enamel show a consistent offset in $\delta^{18}O$ between these phases of ~9‰ (Bryant et al., 1996b; Iacumin et al., 1996; Iacumin and Longinelli, 2002), which reflects differences in isotopic fractionation associated with carbonate and phosphate precipitation in bioapatite. If the offset in $\delta^{18}O$ values of CO_3 and PO_4 components of fossil mammal bioapatite vary from this modern norm, it can be concluded that diagenetic alteration of CO_3 or PO_4 or both, has occurred. However, exactly which component is affected, and to what degree, is difficult to quantify (Zazzo et al., 2004). Furthermore, even among modern samples the offset in $\delta^{18}O$ between CO_3 and PO_4 may vary by several per mil within a single population of animals. In the absence of a method to differentiate normal variation from that introduced during diagenesis, the usefulness of this approach as an unambiguous means of identifying isotopic alteration must be regarded as limited.

Lastly, studies of bioapatite using infrared spectroscopy (IR) allow for the investigation of elements in specific sites within a crystal lattice, such as phosphate and carbonate groups, and for a characterization of how they are modified during diagenesis. Integration of these methods with isotopic analysis of oxygen and carbon from these groups may ultimately allow changes in the crystallographic structure of bioapatite to be tied directly

Figure 8.6. There are several possible ways of identifying whether significant diagenetic alteration of carbon and oxygen isotope ratios has occurred. One is a comparison of bioapatite and diagenetic carbonate. A. Complete overlap of isotopic ratios between bone (filled) and secondary calcite (open) indicates that primary bone values have been overprinted and now reflect diagenetic conditions (data from Trueman, personal communication, 2006). B. Limited overlap and a lack of a linear mixing relationship between tooth enamel (filled) and paleosol (i.e., diagenetic; open) carbonate is not consistent with diagenetic alteration of tooth enamel carbonate (data from Fricke et al., in review). Another is comparisons of isotope data from tooth enamel and dentine of the same tooth (C). Increased isotopic variability and different isotope ratios are evidence that one of the bioapatite phases (most likely dentine) have been adversely affected to a larger degree by diagenesis (see text). Lastly, isotopic differences between coexisting taxa can be used (D). Extensive overprinting of primary isotope signals by diagenetic processes should result in uniform isotope ratios that reflect temperatures and fluid conditions of diagenesis. The lack of such uniformity, as shown by the occurrence of taxonomic differences, is evidence that complete isotopic resetting did not occur.

to changes in isotope ratios. Although this type of research may allow for unambiguous interpretations of isotopic preservation to be made in the future, at present, the technique has limited usefulness (e.g., Sponheimer and Lee-Thorpe, 1999a).

Application of some or all of the above approaches to a suite of bonebed samples may allow for the independent confirmation that bioapatite of some material, most likely enamel, has not been altered significantly during diagenesis. That said, such a result begs the question of how much evidence for or against isotopic alteration is sufficient to render a study viable or impracticable. A large part of the answer depends on the type of questions being asked. If the goal is to test whether different herbivores partitioned food or water resources by eating plants and ingesting waters from different parts of the ecosystem, then the preservation of isotopic offsets in bone—even in the face of shifts in mean values or population variance—may be sufficient. However, if the paleoenvironmental and paleoecological investigations being conducted require more precise estimates of isotopic means and variances for a given population, then analysis of bone will likely be insufficient. Instead, enamel should be the focus of study, and as many methods as possible should be applied to increase confidence in the results.

APPLICATIONS: WORKING WITH BONEBED SAMPLES

Because stable isotope ratios of vertebrate remains are influenced by environmental conditions, animal behavior, and animal physiology, it is possible to study all of these subjects using stable isotope data from bonebeds. However, the feasibility of addressing questions related to these topics depends on the number of individuals sampled from a bonebed, the temporal relationship of these individuals (are they contemporaneous?), and the degree to which seasonal isotopic variability is captured by the sample. In turn, these factors are strongly influenced by the taphonomic history, taxonomic diversity, and geology of a given bonebed (e.g. Eberth et al., Chapter 5 in this volume). These general issues are discussed below.

Accounting for Seasonal Isotope Variations

It is critical to identify any seasonal bias that may exist in isotope data sets before comparing isotope data from within or between assemblages at one or more bonebeds. By so doing, any observed isotopic similarities

and differences within and between assemblages can be more confidently attributed to biology, behavior, or local environment.

One way to characterize seasonal isotope variability is to undertake intratooth sampling, which involves the collection of multiple samples from along the length of a single tooth. This method works because enamel is added incrementally during tooth growth, creating a time series of isotope data (Fig. 8.5b). Depending on the rate of tooth growth and the amount of tooth wear, this sampling approach can allow median $\delta^{13}C$ and $\delta^{18}O$ values as well as maxima and minima values to be determined precisely for time periods of up to several years (Fig. 8.5b). However, intratooth sampling of a single tooth provides information for only a one- to two-year period in the life of a single individual. Studies of multiple teeth from more than one individual will more likely provide a representative sample of the environmental conditions experienced by a population of animals that lived and died together (mass-death assemblages) or during a relatively brief period of geologic time (time-averaged assemblages). Among mass-death assemblages, an additional benefit of doing a multitooth multi-individual study is that a comparison of yearly isotope records can provide insight into annual variability in isotope ratios and isotope ranges.

Another approach to characterizing seasonal variations in tooth enamel isotope ratios involves the analysis of many bulk samples of tooth enamel from the same taxon. Such bulk samples, usually obtained from worn or broken tooth fragments, will contain only a small part of the seasonal isotope record, and any single $\delta^{13}C/\delta^{18}O$ value is unlikely to represent the annual median or seasonal maxima/minima. Statistical models suggest, however, that the range in $\delta^{13}C/\delta^{18}O$ for a large population of bulk samples will accurately reflect the seasonal isotopic variability experienced by that population of animals, whereas mean $\delta^{13}C/\delta^{18}O$ values will reflect the annual median isotope ratio (Fig. 8.5c; Clementz and Koch, 2001). In this bulk-sampling approach it is not possible to obtain precise records of seasonal isotope variations for single years.

Bonebed End-members

The number of specimens available for study, the taxonomic diversity of the assemblage, and the amount of time represented by the specimens are the three most important factors influencing the degree to which isotopic data from bonebeds effectively and accurately reflect original biological and paleoenvironmental conditions. Here, these factors are considered in the context of two bonebed end-members: those that yield low-diversity,

mass-death assemblages, and those that yield high-diversity, time-averaged assemblages.

Mass-death assemblages often consist of small to modest numbers of articulated or partially articulated skeletons that were deposited and buried over short periods of time (e.g., Ashfall Fossil Beds [Voorhies, 1985; Rogers and Kidwell, Chapter 1 in this volume]). This type of assemblage is often limited in its taxonomic diversity (monotaxic to monodominant; Eberth et al., Chapter 3 in this volume), making it more difficult to obtain isotope samples for comparative analysis. Moreover, teeth in jaws and other exceptional specimens of entire teeth in collections are usually off limits to the destructive sampling techniques of isotopic analysis. Mass-death assemblages often do not consist of paleofaunas or yield isotopic data that accurately reflect long-term paleoenvironmental or paleoecological trends. However, they do have tremendous potential to provide information about the paleobiology of the most abundant animals present, including habitat preferences and tooth eruption rates and patterns. Also, as indicated above, analyses of these assemblages can provide important insight into seasonal climatic variability.

At the other end of the spectrum are high-diversity (multitaxic) assemblages that include isolated remains that were deposited and possibly reworked over time (e.g., channel lag and ravinement surface deposits [Brinkman, 1990; Eberth, 1990; Rogers and Kidwell, 2000]). In many cases, these types of assemblages preserve large numbers of individuals representing multiple taxa, and many fragmentary specimens that are of little value in morphological analyses. Accordingly, collections from bonebeds that yield these assemblages are frequently available for destructive sampling techniques.

By analyzing remains of different animals in multitaxic, time-averaged assemblages, a larger part of the isotopic variability present in any given ecosystem is likely to be sampled due to differences in dietary choices and drinking behaviors of different taxa and individuals. For example, different species of mammalian herbivores often partition plant resources and have different strategies for obtaining water (e.g., directly from water bodies; indirectly from leaves). Thus, analyses of many mammalian taxa from a bonebed, including carnivores and herbivores, have the potential to record more of the extant carbon and oxygen isotope variability associated with different plants, photosynthetic pathways, and microenvironments that make up any ecosystem (e.g., Fig. 8.7; Bocherens et al., 1996, Cerling and Harris, 1999; Sponheimer and Lee-Thorp, 1999c; Sponheimer et al., 2003; Passey et al., 2005). Similarly, by collecting data from large numbers of individuals, the impact of climatic anomalies, such as unusually cold or warm years, on isotopic means and ranges for a given

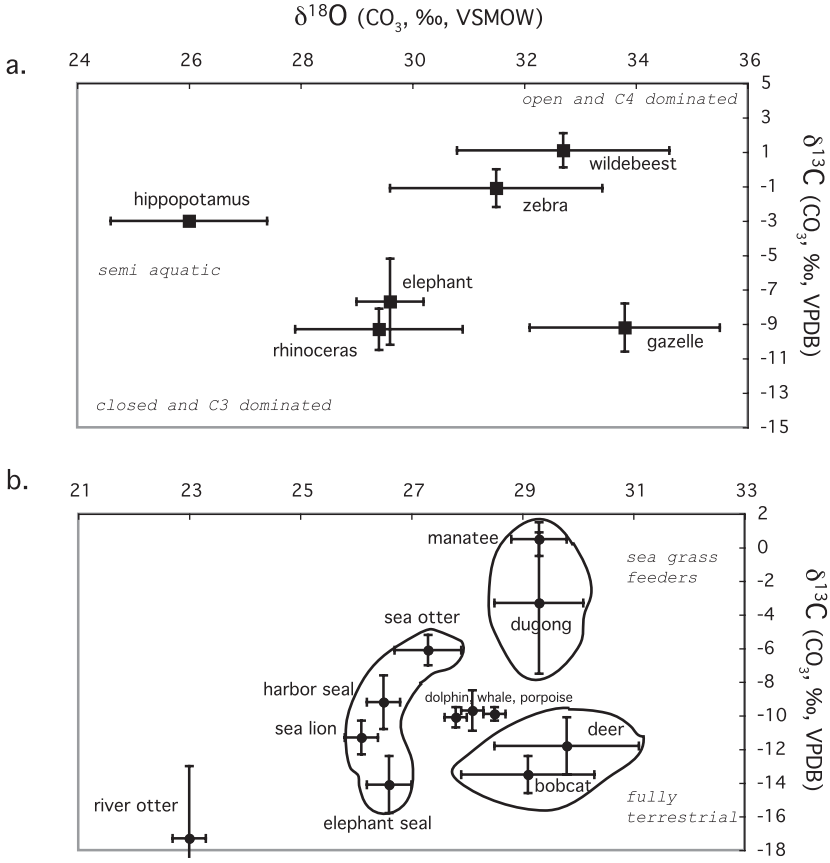


Figure 8.7. Carbon and oxygen isotope data from modern mammals illustrate how vertebrates living in the same terrestrial (A) and marine (B) ecosystems can have significantly different isotope ratios. The distribution reflects differences in environmental conditions, water sources, and plant types between microhabitats. For example, terrestrial herbivores that are more likely to obtain water from leaves (e.g., gazelle, deer) are expected to have higher $\delta^{18}O$ values due to evaporation at leaf surface. This evaporative effect will be magnified in more open and arid habitats but may be negligible for semiaquatic herbivores that eat aquatic plants (e.g., hippopotamus) or for carnivores (e.g., bobcat). In the case of carbon, animals eating plants that utilize the C4 pathway (e.g., zebra) or sea grass (e.g., manatee) are expected to have higher $\delta^{13}C$ values. Aquatic taxa are generally characterized by less isotopic variability than terrestrial taxa as fewer microenvironments exist to accommodate these forms (Clementz and Koch, 2001). Data from Bocherens et al. (1996), Clementz and Koch (2001), and Clementz et al. (2003). Symbols represent population means and bars represent one standard deviation away from the mean.

population is decreased. Similarly, large sample sizes decrease the impact of behavioral variation over time or between individuals. In general, multitaxic bonebeds provide greater opportunities to more thoroughly characterize past environments and animal behavior compared to bonebeds containing low-diversity, mass-death assemblages.

Working with Multiple Bonebeds

Isotopic analyses of vertebrate remains from bonebeds of different age or from different areas offer a number of exciting research possibilities. Data can be used to establish a regional isotope record of paleoenvironmental change through time, which, because they are linked to records of ecological interactions, may allow for the study of how plant-animal and animal-animal interactions responded to changing climatic or hydrologic conditions. Focusing instead on a single time slice, isotope data from multiple bonebeds can be used to establish spatial patterns in isotopic variation. These spatial pattern "maps" can then be used to infer relative differences in paleoenvironmental conditions (e.g., temperature, humidity), explore plant type and animal dietary choices (e.g., C3/C4), and reconstruct regional patterns in air-mass rainout.

Bonebeds must be spaced at semi-regular to regular stratigraphic intervals in order to document and quantify temporal changes in paleoenvironments. Alternatively, bonebeds must occur within narrow stratigraphic limits if valid paleogeographic comparisons are to be made. These limitations place investigators at the mercy of the geologic record and chronostratigraphic data. Because most bonebeds are not closely associated with rocks that yield absolute ages, their chronostratigraphic relationships are usually determined in the context of broadly constrained time intervals based on paleomagnetic, biostratigraphic, and sequence stratigraphic data. Thus, the time intervals within which some bonebeds are compared may represent hundreds of thousands to millions of years. Furthermore, researchers may choose to combine bonebeds from different geologic time intervals in order to increase the number of comparable sites. For example, Fricke and Rogers (2000) lumped together localities from the "Campanian/Maastrichtian boundary interval" that may have spanned several million years. Similarly, Fricke (2003) compared isotope data from Eocene fossils of "Wasatchian" land mammal age, with possible differences in age of several million years. In both cases, climate records from marine sediment cores were used to argue that climate change during these intervals was minimal.

The effect that spatial-temporal lumping of bonebeds may have on the interpretation of stable isotope data must be addressed on a case-by-case basis. If it can be argued, using independent lines of data, that climate did not change significantly during the specified time interval, then spatial differences in isotope ratios can be interpreted as reflecting geographic differences in paleoenvironmental and paleoecological factors.

Complementing Bonebeds: Authigenic Minerals and Organic Material

Bonebeds may be associated with nonbiogenic minerals and other sedimentary material that also contain stable isotope information of paleoenvironmental and paleoecological importance. For example, carbon isotope ratios of soil carbonates may accurately reflect the average $\delta^{13}C$ for all plants that lived in the area as the carbonates were forming (Fox and Koch, 2003, 2004). By comparing $\delta^{13}C$ values of the carbonates to those from associated vertebrate remains, it is theoretically possible to reveal an animal's dietary and ecological preferences for plants with either high or low carbon isotope ratios. In the case of oxygen, evaporation can affect $\delta^{18}O$ of soil waters, particularly in arid settings, and oxygen isotope fractionation during carbonate formation is more temperature dependent than carbon isotope fractionation. Therefore, $\delta^{18}O$ of soil carbonates is not an ideal monitor of $\delta^{18}O$ of surface waters, but it may confirm the occurrence of arid or nonarid conditions.

Sedimentary organic matter such as wood, charcoal, or leaf cuticle, which is sourced from plants that once lived in the area of study, can provide useful isotopic data when collected from a bonebed, particularly if such organic matter is taxonomically identifiable. As carbon isotope ratios of such organic matter are the most direct representation of herbivore diet that can be obtained, they can be used to (1) determine which carbon isotope offsets between diet and bioapatite are reasonable for a given vertebrate, (2) describe variability in isotope ratios of different plants living in an ancient ecosystem, and (3) identify specific kinds of plants that were eaten by ancient vertebrates. Finely comminuted plant material ("coffee grounds") present in a bonebed is different in that it most likely provides a homogenized average $\delta^{13}C$ value reflecting contributions from many plants. Lastly, it is also possible that a preservational bias exists against certain plant remains and organic molecules with high carbon isotope ratios. In such cases, $\delta^{13}C$ of the sedimentary organic matter in a bonebed is generally high relative to that for the actual plant communities in the area (e.g., Fogel and Tuross, 1999; Krull and Retallack, 2000; Wynn et al., 2005).

BONEBED APPLICATIONS: EXAMPLES

Having considered background issues and how sampling influences study design, it is now time to describe the types of paleoenvironmental, paleoecological, and paleobiological investigations that can be undertaken

using isotope data from bonebeds. The selection of research highlighted below includes studies that focus on multiple animals and/or taxa. It is fully recognized that some, but not all, of these studies utilize samples derived from bonebeds. Research topics are presented separately so that the reader can focus on (1) the assumptions involved in each kind of study and (2) the kinds of interpretations that are possible. The following examples do not represent all potential research topics, rather, they reflect the background of the author and are intended to show how stable isotope analyses can be profitably included in a bonebed research program.

For general reviews of applications see Koch et al. (1994), Gröcke (1997), Gannes et al. (1998), Koch (1998), Kohn and Cerling (2002), and Lee-Thorp and Sponheimer (2005). Examples focusing on marine vertebrates include Lécuyer et al. (1993, 1996, 2003), Vennemann and Hegner (1998), Vennemann et al. (2001), and Pucéat et al., (2003). Lastly, the archaeological and paleoanthropological literature is rich with studies aimed at unraveling the diets and ecological preferences of primates and hominids using isotope data (Schwarcz and Schoeninger, 1991; Schoeninger, 1996; Sponheimer and Lee-Thorpe, 1999b; Schoeninger et al., 2001; Lee-Thorpe et al., 2003; Sponheimer et al., 2005).

Paleoenvironmental Conditions

The successful study of environmental conditions at the scale of a single ecosystem relies on (1) accounting for any seasonal bias in $\delta^{13}C$ and $\delta^{18}O$ of vertebrate remains, (2) a careful consideration of isotopic offsets between vertebrate remains and ingested food and water, and (3) interpreting both the absolute values and ranges of $\delta^{13}C$ and $\delta^{18}O$. Once obtained, isotopic data can be utilized in several different ways.

Vegetation Structure

By providing a snapshot of isotopic variability in plants, data from vertebrate remains in bonebeds can be used to document the presence of a variety of vegetation structures, such as closed- and open-canopy forest, open grassland, or some combination of these. However, because such documentation requires a record of all isotopic variability in plants and surface water (e.g., Fig. 8.7), it is essential that $\delta^{13}C$ and $\delta^{18}O$ be determined for as many taxa as possible in a bonebed.

Many studies of this type have focused on Pleistocene landscapes. For example, Kohn et al. (2005) analyzed tooth enamel carbonate from six

herbivore taxa that lived in South Carolina 115 ky ago (Fig. 8.8a). Ranges in $\delta^{13}C$ for an individual taxon such as horses (~6‰) or tapirs (~1‰) were small, but together $\delta^{13}C$ values from these six common taxa ranged from –16‰ to –1‰ and presented a more complete picture of vegetation structure. In particular, this broader range indicated that C3 plants were affected by a variety of environmental conditions in forested areas, whereas high values pointed to the existence of C4 grasslands. Furthermore, a positive correlation between $\delta^{13}C$ and $\delta^{18}O$ indicated that grassland areas were more open and subject to greater evaporation (Kohn et al., 2005). Other mixed C3–C4 forest-grassland paleoenvironments have been described using multiple-taxa isotope data from the Pleistocene of Florida (Koch et al., 1998) and from the Miocene of Kenya (Cerling et al., 1997b), and these studies demonstrate well how isotope data can complement other means of reconstructing vegetation structure.

In the absence of C4 plants, there is less potential for carbon isotope variability across a landscape, making it more difficult to resolve differences in vegetation type. Nevertheless, such resolution is still possible. In a recent study of a modern closed-canopy C3 forest in Africa, Cerling et al. (2004) observed a range in $\delta^{13}C$ of 12‰ for tooth enamel carbonate from more than a dozen vertebrate taxa living in and below closed-forest canopy. Similar ranges in isotope ratios were also observed in plants due to the canopy affect. These results indicate that isotope data of mammal remains have the ability to capture aspects of the vertical structure of forested areas. To date, applications of isotopic methods to ancient C3 ecosystems have been limited (MacFadden and Higgins, 2004; Botha et al., 2005; Feranac and MacFadden, 2006), but they do reveal this potential. Feranec and MacFadden (2006) observed dietary differences among five herbivorous taxa of Miocene age (Fig. 8.8b). The relatively small range in $\delta^{13}C$ of ~5‰ is not consistent with a closed-canopy C3 forest (Cerling et al., 2004); rather, variability in $\delta^{13}C$ and $\delta^{18}O$ is attributed to animals occupying different parts of a more open C3 forest, some of which were more open or closer to flowing water than others. These insights into the physical structure of ancient forests, such as spacing of trees and occurrence of open areas, and into animal behavior are otherwise difficult to obtain using more traditional paleobotanical or sedimentological techniques.

As a complement to fossil remains, isotope data from paleosol carbonates in some cases can provide an even more complete isotopic picture of ancient landscapes. For example, analysis of paleosol carbonates by Fox and Koch (2003, 2004) indicate the presence of C4 grasses, which suggest a more open landscape. Because this interpretation was not supported by isotope data collected from the tooth enamel of the associated fossil equids,

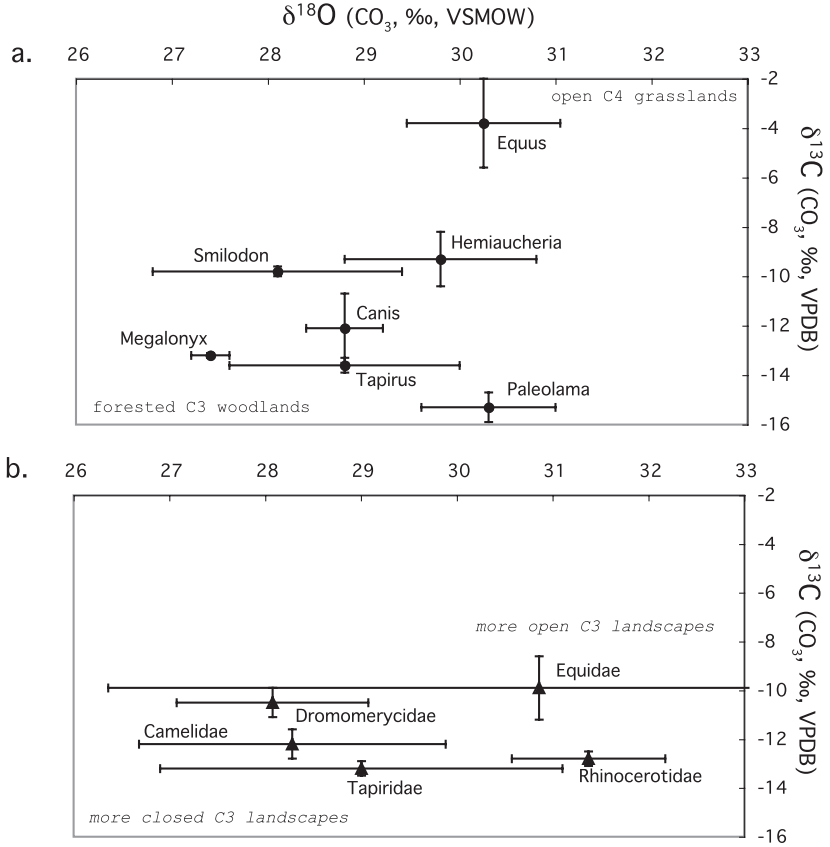


Figure 8.8. Carbon and oxygen isotope data from fossils of coexisting mammals can be used to reconstruct vegetation structure and to identify niche and resource partitioning between taxa. Higher $\delta^{13}C$ and $\delta^{18}O$ are generally associated with more open environments and evaporatively modified water sources. A. Samples from a Pleistocene terrestrial locality in South Carolina are interpreted to represent a mix of C3 and C4 plants eaten by herbivores. Isotopic differences suggest that carnivores also hunted in different parts of the ecosystem (Kohn et al., 2005). B. Samples from a Miocene terrestrial locality in Florida where only C3 plants were present (Feranec and MacFadden, 2006). A limited range in $\delta^{13}C$ implies a lack of closed canopy forest, while significant isotopic differences among some taxa illustrate preferences for more open or closed microhabitats. Symbols represent population means and bars represent one standard deviation away from the mean.

they concluded that isotope ratios of vertebrates are not necessarily a direct reflection of all vegetation and water present. Rather, isotope ratios from vertebrates reflect only the plants and water that are actually ingested.

Climatic Conditions

The link between rainout of moisture from air masses, $\delta^{18}O$ of resulting precipitation and surface water, and $\delta^{18}O$ of vertebrate remains in

bonebeds can be used to study several different aspects of paleoclimate. In particular, it may be possible to estimate mean annual temperature (MAT) and the amounts of precipitation. Records of climatic conditions in terrestrial environments are generally sparse, thus studies of bonebed remains with this goal in mind will be of interest to climate modelers and paleontologists alike.

Because of the unreliability in applying modern relations between δ18O of precipitation and MAT to the past (Boyle, 1997; Fricke and O'Neil, 1999; Rowley et al., 2001; Fricke and Wing, 2004), this author believes that the best isotopic estimates of MAT are those that rely on the temperature-sensitive fractionation of oxygen isotopes between apatite and water at the scale of local paleoenvironments. This approach uses two or more physiologically distinct, but coexisting, taxa (collected from a single bonebed) that are assumed to have been ingesting the same waters (e.g., a terrestrial mammal with a constant body temperature and a freshwater fish or invertebrate; Fig. 8.9). Because biogenic apatite in the mammal forms at a

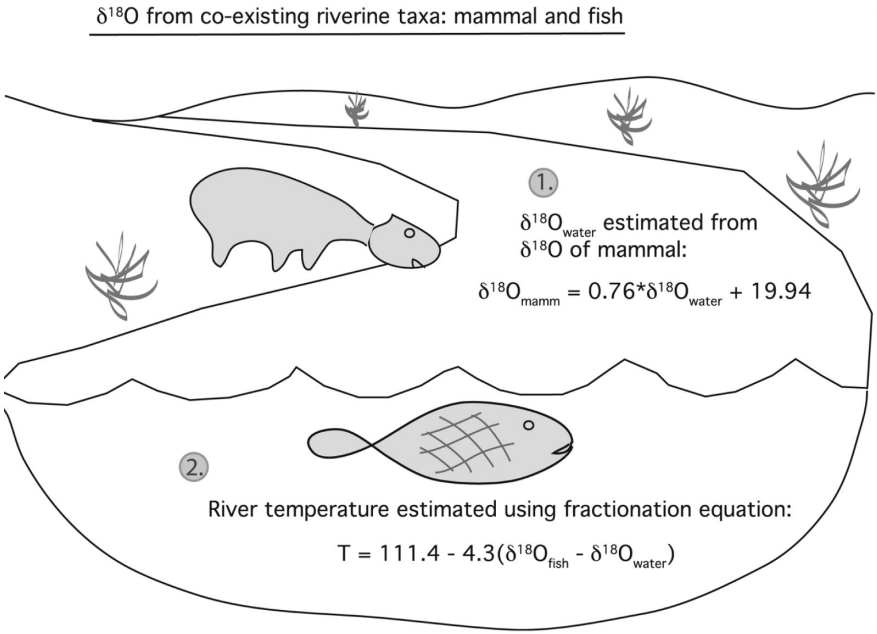


Figure 8.9. The two-taxa approach to estimating temperature in terrestrial environments (after Fricke and Wing, 2004). 1. Biogenic apatite in mammals is formed at a constant temperature. Using a physiological model (Kohn [1996] for biogenic phosphate), δ^{18}O of river water can be estimated. 2. The value calculated above is then substituted along with measured δ^{18}O values from fish into carbonate/phosphate-water fractionation equations to estimate river water temperature, which is assumed to be similar to that of the overlying atmosphere.

constant body temperature, its δ18O can be used (along with a physiological model; e.g., Kohn, 1996) to estimate $\delta 18O_{water}$. In contrast, δ18O of fish scales is dependent on δ18O of water and on water temperature (in the case of phosphate, $T_{river} = 111.4 - 4.3\ [\delta 18O_{water} - \delta 18O_{bp}]$; Longinelli and Nuti, 1973). $\delta 18O_{water}$ values estimated from mammalian phosphate and δ18O values from fish are substituted into this phosphate-water fractionation equation and are used to estimate river water temperature, which is very close to that of the overlying atmosphere at present (Fricke and Wing, 2004).

A test of the "two-taxa approach" was conducted by Fricke and Wing (2004). In that study a comparison of standard paleobotanical and two-taxa isotope methods for estimating MAT in the Paleogene of Wyoming showed similar results, thus indicating that data from large-mammals and fish can be combined to provide useful estimates of ancient MATs. Therefore the two-taxa approach represents a potentially powerful paleoclimate tool (Kolodny et al., 1983; Fricke et al., 1998b; Barrick et al., 1999; Grimes et al., 2003, 2004a, 2005). In the case of Grimes et al. (2003), an oxygen isotope data set was obtained from rodent teeth, fish scales and otoliths, and several aquatic invertebrates of Eocene age from Britain. In turn, these were used to produce an internally consistent set of paleotemperature estimates during periods of skeletal growth for the different taxa. Overall, these results from the Eocene are very encouraging and will hopefully lead to similar studies over a wider range of time and space.

Seasonality

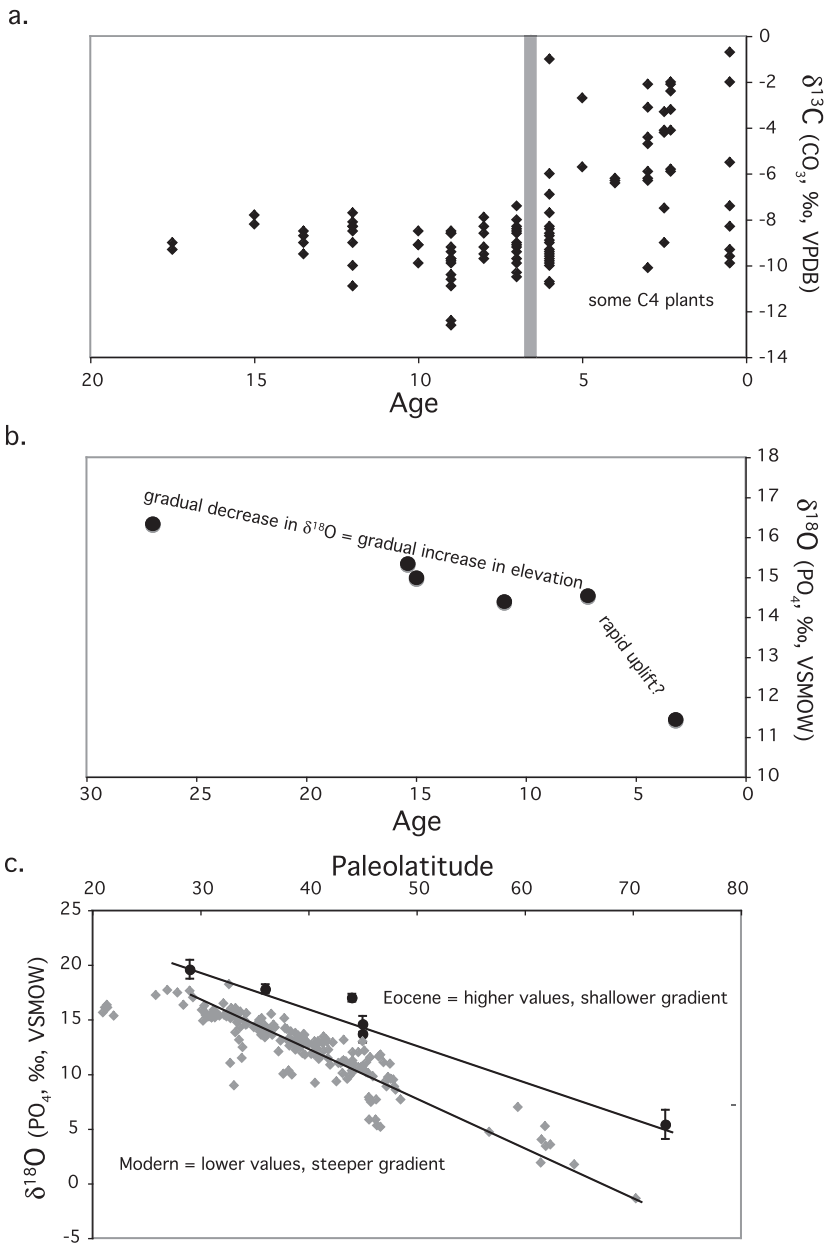
The ability of tooth enamel from single teeth to record seasonal variations in isotope ratios (Fig. 8.5a and 8.5b) represents a special climatic application. At this time, however, it appears difficult to use seasonal variations in isotope ratios to study climatic conditions quantitatively. One problem is that the isotopic measure of seasonality preserved in tooth enamel is generally less than that which actually exists in plants and waters ingested by an animal. This discrepancy occurs because body water represents a time-averaged reservoir of carbon and oxygen with a turn over time of weeks to months (Kohn and Cerling, 2002; Passey and Cerling, 2002 et al., 2002a; Balasse et al., 2003; Ayliffe et al., 2004; Hoppe et al., 2004a; Zazzo et al., 2005). Therefore, carbon and oxygen isotope ratios of enamel forming at any one time reflect a homogenized pool of carbon and oxygen in which shorter-term seasonal extremes in isotope ratios of ingested materials are dampened. Furthermore, contemporaneous layers

of tooth enamel are deposited at variable angles to the tooth surface (Fig. 8.5a), and when enamel is sampled via drilling or laser ablation, enamel of different ages is combined, resulting in isotopic mixing. Lastly, even if a true reflection of seasonal variations in $\delta^{13}C$ and $\delta^{18}O$ of ingested plants and waters can be reconstructed using tooth enamel data, the fact remains that changes in animal behavior may also occur over the course of a year that *also* impact $\delta^{13}C$ and $\delta^{18}O$ of ingested food and water. Thus, it is not valid to attribute all isotopic variability to climatic factors alone, and unambiguous interpretations of seasonal isotope variations are difficult to make.

Despite these problems, the simple observation that intratooth ranges in isotope ratios do or do not vary over time, from place to place, or among taxa can provide useful qualitative paleoenvironmental and paleoecological information. For example, oxygen isotope data from the teeth of mammals and dinosaurs have also been used to investigate seasonal patterns in the amount of precipitation and in humidity from the Pleistocene to the Cretaceous (Higgins and MacFadden, 2004; Straight et al., 2004). These studies note that surface temperature and the amount of air-mass rainout can both influence $\delta^{18}O$ of precipitation in climatic regimes characterized by marked wet and dry seasonality. Depending on the interplay of these factors, different seasonal patterns in $\delta^{18}O$ of precipitation are produced. By comparing predictions with observed intratooth patterns in $\delta^{18}O$ from theropod teeth, Straight et al. (2004) inferred that the Late Cretaceous in Alberta was often characterized by several months of high rainfall and high humidity during tooth growth. Similarly, Higgins and MacFadden (2004) inferred a rainy season for southwestern North America during the late Pleistocene based on the isotopic analysis of horse and bison teeth. Many other excellent studies have utilized intratooth variations in $\delta^{18}O$ to infer local climatic conditions (Stuart-Williams and Schwarcz, 1997; Gadbury et al., 2000; Fox and Fisher, 2001, 2004; Stanton-Thomas and Carlson, 2003; Nelson, 2005).

Inferences Drawn from Multiple Bonebeds

Comparisons of isotope data from multiple bonebeds may underscore environmental changes through time, or paleogeographic variation in paleoenvironmental conditions during a given time interval in the past. The key assumption in these approaches is that isotopic differences are due only to differences in paleoenvironmental conditions, not the basic biology of a given taxon. For this reason, it is best to compare isotope data from closely related taxa, and, preferably, from within a species. Furthermore,


a.
0
-2
-4
-6
-8
-10
-12
-14
δ13C (CO3, ‰, VPDB)
some C4 plants
20
15
10
5
0
Age
b.
18
17
16
15
14
13
12
11
10
δ18O (PO4, ‰, VSMOW)
gradual decrease in δ18O = gradual increase in elevation
rapid uplift?
30
25
20
15
10
5
0
Age
c.
Paleolatitude
20
30
40
50
60
70
80
25
20
15
10
5
0
-5
δ18O (PO4, ‰, VSMOW)
Eocene = higher values, shallower gradient
Modern = lower values, steeper gradient

only those taxa that are known to have been widely distributed in time and space will be useful in making intrabonebed comparisons.

The most straightforward application of isotope data from bonebeds of different age is the creation of temporal records of vegetation change or climate change in a given area. One of the best examples of this approach is the study of grassland expansion during the Neogene (Fig. 8.10a; Quade et al., 1992; Cerling et al., 1993, 1997a; Wang et al., 1994; Passey et al., 2002). By analyzing vertebrate remains—generally those of horses—of different ages, an obvious increase in $\delta^{13}C$ at ~8 Ma is visible. In turn, this increase is interpreted to reflect the expansion of C4 plants, particularly grasses, over the landscape. However, because many of these studies rely on the analysis of only one taxon, feeding behavior must be considered; if the studied taxon did not consume C4 grass, then no record of the grassland would be preserved in the taxon's isotope ratios. In the case of climate, multiple bonebeds have been used to document (1) changes in mean annual temperature across the Paleocene-Eocene boundary that capture short-term warming in Wyoming associated with the Paleocene-Eocene Thermal Maximum (Fricke and Wing, 2004; Koch et al., 1995), (2) possible climatic stability across the Eocene-Oligocene boundary (Grimes et al., 2005; Kohn et al., 2004; Bryant et al., 1996a), and (3) changes in the seasonal variation in $\delta^{18}O$ in the Late Cretaceous of Alberta (Straight et al., 2004).

Another application of isotope data from multiple bonebeds has been the study of changes in paleoelevation (and paleoenvironment) through time (Fig. 8.10b). This research takes advantage of the fact that rainout of air masses occurs as they are forced over topographic barriers, with a resulting decrease in $\delta^{18}O$ and the formation of a rain shadow or a monsoonal circulation pattern. For example, Kohn et al. (2002) observed that $\delta^{18}O$ of vertebrate remains decreased over time on the leeward side of the Cascade Mountain and related this decrease to the timing of mountain uplift. Similarly, vertebrate remains from Tibet, China, and Nepal have been used to investigate the uplift history of the Himalayan Mountains

Figure 8.10. Data from multiple bonebeds can be used to study elevation changes over time, grassland evolution over time, and spatial distributions in isotope ratios for a single time period. A. The occurrence of $\delta^{13}C$ values of equid tooth enamel greater than –8‰ in bonebeds younger than ~8 Ma provides evidence for the expansion of C4 grasslands over North America at this time (Passey et al., 2002). B. $\delta^{18}O$ of tooth enamel from vertebrates living on the leeward side of the Cascade mountains gradually decreases with time in response to increasing elevation and hence air-mass rainout until ~7 Ma when rapid uplift is inferred (Kohn et al., 2002). C. $\delta^{18}O$ of tooth enamel from mammals living over a wide latitudinal range during the Eocene can be used to reconstruct paleohydrologic gradients. In this case, $\delta^{18}O$ of Eocene mammals (black circles) are higher and latitudinal gradients slightly steeper than calculated for mammals drinking modern river water (gray diamonds; from Fricke, 2003).

(Dettman et al., 2001; Wang et al., 2006), and the impact of uplift on average and seasonal variations in temperature and monsoonal precipitation (Dettman et al., 2001).

Other studies have focused on differences in isotope ratios between bonebeds of the same age. Here the goal is to reconstruct paleohydrology, paleoclimate, paleovegetation distributions, and topographic patterns at one instant in geologic time (Fig. 8.10c). In the case of vegetation, MacFadden et al. (1999a) used the distribution of $\delta^{13}C$ in horse tooth enamel to reconstruct the distribution of C3 and C4 grasses across North and South America during the Pleistocene. Because of the relation of $\delta^{18}O$ of precipitation to air-mass rainout, comparisons of $\delta^{18}O$ of mammals over a wide latitudinal range can be used to study the transport of water vapor from tropics to poles under different climatic regimes such as those of the Eocene (Fricke, 2003) and the Late Cretaceous (Amiot et al., 2004). When paired with isotope data from coexisting fish, data from these localities can be used to estimate MAT and, thus, reconstruct latitudinal temperature gradients for these same time intervals (Fricke and Wing, 2004). Lastly, more accurate descriptions of paleoelevation and paleorelief are possible if isotope data are collected from both windward and leeward sides of paleomountains. Such efforts have been undertaken for Eocene age Laramide ranges of western North America (e.g., Fricke, 2003) and the Miocene to recent Sierra Nevada Mountains (Koch and Crowley, 2005).

Ecological Relations

The way in which organisms interact with each other and their environments is fundamentally related to their behavior. In the case of extant animals, behavior can be observed, whereas the behavioral activities of extinct animal are only rarely preserved in the fossil record (Brinkman et al., Chapter 4 in this volume) and are much more often inferred by comparing their skeletal and dental morphology and microwear patterns with modern relatives. Even so, such comparisons become increasingly more difficult to undertake and accurately resolve as one moves back through the geologic record. In this context, stable isotope data from fossil remains can provide important complementary information that relates to food choices, sources of water, and habitats. In those cases where different paleoecological niches are characterized by different paleoenvironmental conditions (e.g., water sources, plant types), it may be possible to resolve these differences using stable isotope data. For this reason, stable-isotope-based

paleoecological studies focus on isotopic differences between vertebrate taxa, which reflect differences in the kinds of plants and waters ingested.

Before describing paleoecological applications, it is important to clarify two additional aspects of this type of research. First, because isotopic offsets can also result from biological differences between taxa, it is important to carefully consider isotopic offsets between ingested food and water, and vertebrate remains. To do so, it must be known whether the animal was an herbivore, a carnivore, or an omnivore. Secondly, isotopic overlap between fossil taxa does not necessarily imply behavioral similarities; it is possible that animals ate different plants and obtained water from different sources that were isotopically similar. In such cases, isotope data cannot resolve ecological differences.

Resource Partitioning and Habitat Preferences

To date, most paleoecological research using stable isotope ratios has centered on mammalian herbivores and interpretations based on isotopic similarities or differences among taxa. At the most basic level, the mere existence of isotopic offsets between herbivores is strong evidence that they were partitioning dietary resources. To the extent that certain kinds of plants live on certain parts of the landscape, these same isotopic offsets can also be used to identify differences in habitats preferred, or at least frequently occupied, by groups of herbivores.

For example, carbon and oxygen isotope data from all common mammals characteristic of a single ecosystem can be described from the perspective of isotopic differences, and systematically higher or lower isotopic ratios between taxonomic pairs can be interpreted appropriately (Ambrose and DeNiro, 1986; Tieszen and Boutton, 1989; Bocherens et al., 1996; Cerling et al., 1997b; Koch et al., 1998; Sponheimer and Lee-Thorpe, 1999c, 2001; Sponheimer et al., 2001, 2003; Palmqvist et al., 2003; Kohn et al., 2005). Studies using carbon and oxygen isotope data to focus on the dietary choices of one or two herbivores reveal similar kinds of isotopic offsets (Quade et al., 1995; McFadden and Cerling, 1996; MacFadden and Shockey, 1997; MacFadden, 1998; Cerling et al., 1999; MacFadden et al., 1999b; Feranec and MacFadden, 2000, 2003; Zazzo et al., 2000; Fox and Fisher, 2001, 2004; Harris and Cerling, 2002). A common interpretation in all these studies is that high $\delta^{13}C$ and $\delta^{18}O$ values are indicative of a diet of C4 grasses and a preference for more open habitats (e.g., North American horses, bison, and mammoths; African elephants). Conversely, lower $\delta^{13}C$

and $\delta^{18}O$ in coexisting herbivores indicate a preference for C3 plants from a denser forest setting (e.g., North American tapirs, mastodons, African giraffes). The lowest $\delta^{18}O$ values are indicative of a preference for riparian habitats and the ingestion of nonevaporated river water (e.g., hippopotamus; Figs. 8.7 and 8.8). Even when C4 plants are not inferred to be present, isotopic differences have revealed dietary preferences for understory plants versus canopy plants or more closed versus more open microenvironments (Fig 8.8b; Cerling et al., 2004; Feranec and MacFadden, 2006). Although an offset between $\delta^{13}C$ of bioapatite and bulk diet of ~12–15‰ is assumed in all these cases, Grimes et al. (2004b) demonstrated that this may not always be true, particularly in the case of rodents, and that paleoecological interpretations depend on this choice.

The dietary preferences of carnivores can also be inferred, although fewer data are usually available. In this approach, the isotope systematics of all common herbivores and potential prey living in an area must be described, and the offset in carbon isotope ratio that corresponds to changes in trophic level (Lee-Thorpe et al., 1989; Fox-Dobbs et al., 2006) must be assumed correct. Feranec (2004a, 2005) and Kohn et al. (2005) used the offset of Lee-Thorpe et al. (1989) and inferred the identity of common prey for carnivores living in several different regions during the Pleistocene. In turn, isotope data from the herbivores in each region were used to determine which part of the landscape (i.e., open areas, dense forests, transitional settings) made up the hunting grounds for these carnivores (Fig. 8.8a; Kohn et al., 2005).

To date, most stable-isotope-based paleoecological research involving vertebrates has focused on Neogene-age terrestrial herbivores, in particular, those of Pleistocene age. There is no a priori reason, however, that material from older bonebeds cannot be studied, and there is now a growing suite of research projects involving bones and teeth from older bonebeds. Barrick et al. (1992), Thewissen et al. (1996), Clementz and Koch (2001), Clementz et al. (2003), and MacFadden et al. (2004) have all used stable isotopes to study preferences of early Cenozoic mammals from coastal, semiaquatic, and fully marine habitats (Fig. 8.11a). Carbon and oxygen isotope offsets between herbivorous dinosaur taxa of the Late Cretaceous have also been observed (Fig. 8.11b) and are interpreted as reflecting dietary differences. It even appears possible to resolve ecological differences among nonmammalian therapsids of Triassic age using stable isotope data (Botha et al., 2005). In fact, stable isotope studies aimed at understanding behaviors and dietary preferences of ancient animals with no anatomically similar modern relatives are arguably the most intriguing, given that few other methods can address such questions.

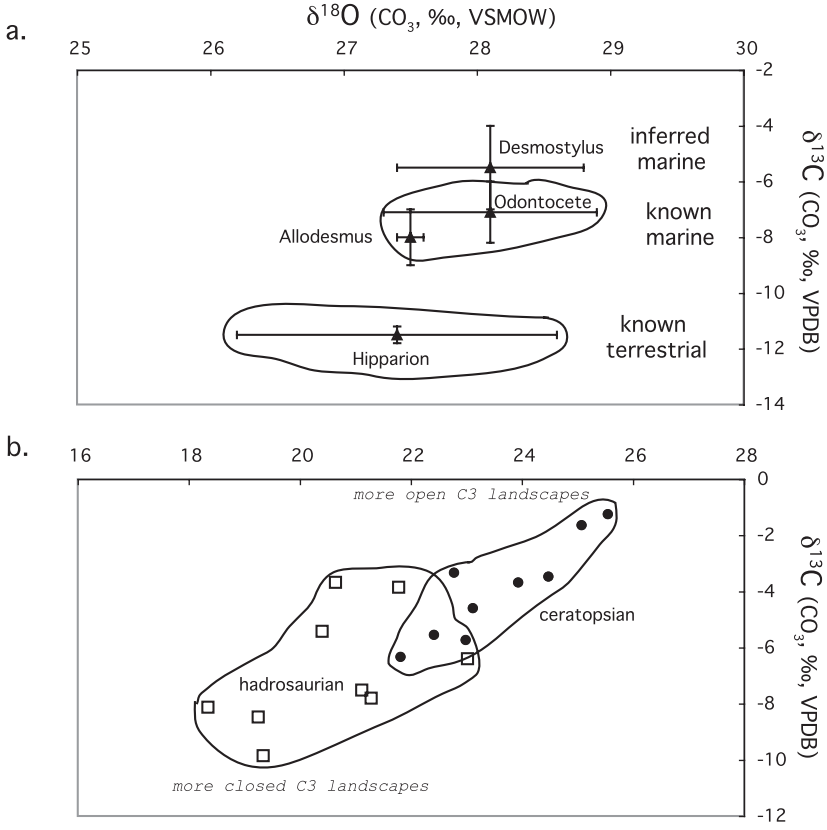


Figure 8.11. Investigations of ecological niche and dietary resource partitioning are not limited to the Neogene or to terrestrial settings. A. Data from early Cenozoic fossils are used to study ecological preferences of different mammals living in coastal settings. Symbols represent population means and bars represent one standard deviation away from the mean. Higher $\delta^{18}O$ and $\delta^{13}C$ values are characteristic of mammals adapted to marine environments, thus making it possible to infer a marine preference for *Desmostylus* (Clementz et al., 2003). B. Higher $\delta^{18}O$ and $\delta^{13}C$ values for herbivorous ceratopsian dinosaurs versus herbivorous hadrosaurian dinosaurs from the Hell Creek Formation, North Dakota, indicate that hadrosaurs utilized forest vegetation while ceratopsians ate a mix of forest plants and plants living in more open, dry or salt-stressed habitats (Fricke, unpublished data).

Inferences Drawn from Multiple Bonebeds

If remains from multiple individuals of the same or closely related taxa can be studied from a number of contemporaneous bonebeds, then it should be possible to determine if isotopic relations and ecological interactions between taxa are similar from place to place, or if these relations and interactions are dependent on paleoenvironmental conditions. Studies of this type have focused on the dietary habits of Pleistocene mammoths and mastotodons, and other large herbivores from eastern and southern North

America (Koch et al., 1989, 1998; Feranec and MacFadden, 2000, 2003; Feranec, 2004b). The results of these studies suggest that mammoths preferred a diet of grasses, compared to the more mixed plant assemblage preferred by mastodons. Furthermore, the proportion of C3 to C4 grasses ingested by both taxa changed with floral availability and climatic conditions. Similarly, in a study of Late Pleistocene herbivore communities along an east-to-west gradient in southwestern North America, Connin et al. (1998) documented an increase in C3 plant intake by all animals, and attributed this change to differences in rainfall along the gradient.

Similar kinds of isotopic data can be brought to bear on the question of animal movement and migration between bonebed localities, a question that is otherwise difficult to address using fossil morphology alone. For example, if populations of a mobile terrestrial vertebrate taxon are characterized by different isotope ratios at two bonebed sites that are separated by tens to hundreds of kilometers, then a strong argument can be made that these animals did not eat the same food or drink the same water. In turn, it can be concluded that there was no intermingling, or migration, of animals between the sites. Unfortunately, the occurrence of isotopic overlap for two populations of a single taxon found in separate areas is not as easy to interpret; it is possible that two or more populations of a taxon (represented by separate bonebed assemblages) each consumed plants and water from geographically separate localities where isotope ratios happened to be similar to one other. In such a case, the two populations could be misinterpreted as being subsets of a single larger population that migrated between the two areas. In a study of mammoth migration, Hoppe (2004) used a combination of stable and radiogenic isotope data from mammoth tooth enamel to conclude that these animals from the Great Plains did not undertake large migrations of over 600 km but may have migrated distances of several hundred kilometers or less (see also Fig. 8.12). This uncertainty in distance of migration makes it clear that the better the spatial resolution of a suite of contemporaneous bonebeds, the more precise such descriptions of animal movement will be.

Lastly, studies of closely related taxa from a stratigraphic succession of bonebeds can reveal behavioral change through time. MacFadden (2000) provides a review on the topic that covers the entire Cenozoic and illustrates how isotope data can be integrated with morphological information. Other excellent examples of the isotopic approach focus on the evolution of grazing behavior in Neogene herbivores. Because the unique $\delta^{13}C$ values of C4 grasses are easy to track in vertebrate remains, many studies have used the C4 "signal" to document changes in herbivore diet as C4 grasslands expanded during the Miocene, and to study possible relationships

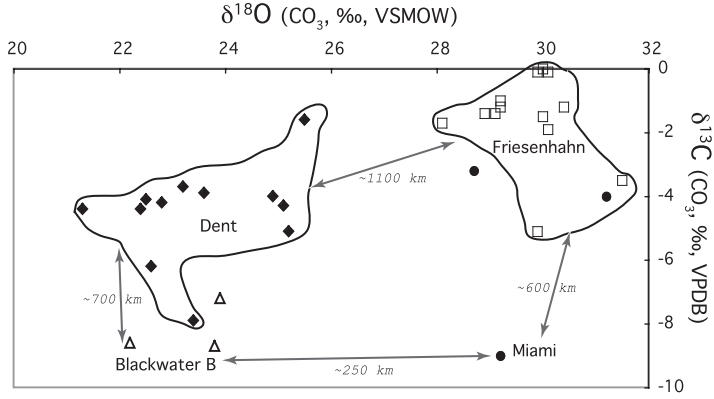


Figure 8.12. Isotope data from contemporaneous remains of late Pleistocene mammoths found in four separate areas in western North America along with approximate distances between them (Hoppe, 2004). Because there is no overlap in stable isotope data from mammoths at Dent, Colorado, and Friesenhahn, Texas, it is reasonable to infer that they did not consume the same plants and water. In turn, these data suggest that these groups of mammoths did not migrate between these localities. It is more difficult to make unambiguous interpretations in the case where there is an overlap in data.

between these changes in diet and tooth morphology (Quade et al., 1992; Cerling et al., 1993, 1997a; MacFadden, 1994, 1998; Wang et al., 1994; MacFadden and Cerling, 1996; MacFadden et al., 1999b; Passey et al., 2002; Feranec and MacFadden, 2003). Equally important research has centered on the adaptation of land mammals to semiaquatic and fully marine habitats during the early Cenozoic by identifying changes in diet through time (Barrick et al., 1992; Thewissen et al., 1996; Roe et al., 1998; Clementz and Koch, 2001; Clementz et al., 2003; MacFadden et al., 2004).

Paleobiology

Soft tissues of vertebrates can provide a unique window into animal physiology, but they are rarely preserved in the rock record. Hence, detailed knowledge of metabolic processes, body temperatures, and growth rates in extinct taxa is very difficult to obtain. Fortunately, stable isotope ratios of bioapatite may be influenced by biological processes in identifiable ways that provide paleobiological insight. The potential exists to obtain much more paleobiological information if carbon and nitrogen isotope data are also available from organic remains (Gannes et al., 1998, and references therein), however descriptions of these applications are beyond the scope of this chapter.

Thermoregulation

Body temperatures of vertebrates can be influenced by several factors including (1) internal metabolic processes that result in approximately constant temperatures (endothermy), (2) varying environmental temperatures that are mirrored by the body (ectothermy), (3) behavioral activities such as basking that keep temperatures relatively constant, and (4) large body size, which can result in relatively constant body temperature (inertial homeothermy; gigantothermy). Because the oxygen isotope fractionation that occurs during the precipitation of skeletal apatite from body water is sensitive to body temperature, it is possible to design a study of bonebed remains that can shed some light on thermoregulatory strategies utilized by ancient vertebrates.

In the case of dinosaurs, Barrick and Showers (1994, 1995) and Barrick et al. (1996) measured $\delta^{18}O$ values of bones from body cores and extremities of several dinosaur taxa and observed no statistical differences in the values regardless of the element examined. Based on these results, they argued that dinosaurs were homeothermic and able to maintain a constant temperature throughout their bodies. Using another approach, Fricke and Rogers (2000), and more recently Amiot et al. (2004), compared $\delta^{18}O$ values of coexisting crocodiles, turtles, and theropod dinosaurs from a number of contemporaneous sites across a wide latitudinal range. They observed a steeper latitudinal gradient in $\delta^{18}O$ for dinosaurs, which is consistent with an interpretation of homeothermy for this group, and ectothermy for crocodiles and turtles.

Rates of Dental Development

Tooth enamel can be used as a temporal marker to measure the rate of tooth growth, the order in which teeth erupt, and overall rates of dental development (Fig. 8.7b and 8.7d). Investigations of bioapatite from tusks may provide similar information as well as insight into season of death (Fisher, 1987). Comprehensive studies of this type require intratooth sampling of all the teeth from one jaw or a serial sampling of an entire tusk. Vertebrates with high-crowned teeth are best suited for this type of approach, whereas low-crowned teeth may not be as well suited due to spatial limitations in sampling and inherent limitations in temporal resolution. In this approach it is best to analyze teeth from the jaws of many individuals in order to confirm growth rates and to document variation.

Several isotope-based studies of the rate of tooth growth and schedule of tooth eruption in modern mammals have been confirmed by direct

observation (Balasse et al., 2003; Passey et al., 2005; Zazzo et al., 2005). However, far less is known about extinct animals. Investigations aimed at filling this gap include those of teeth from theropod dinosaurs (growth rate approximately 55 mm/year [Straight et al., 2004]), hadrosaur dinosaurs (growth rate approximately 38 mm/year [Stanton-Thomas and Carlson, 2003]), sabre-toothed cats (growth rate approximately 60–80 mm/year [Feranec et al., 2004a; Feranec, 2005]), Miocene equids (growth rate approximately 47 mm/year [Nelson, 2005]), and beavers (growth rate approximately 270–365 mm/year [Stuart-Williams and Schwarcz, 1997; Rinaldi and Cole, 2004]). In the case of tusks, serial sampling of those from extinct proboscidean has documented rates of growth, and seasons of birth and death (Fisher, 1987; Koch et al., 1989; Fox and Fisher, 2001).

SUMMARY

The most compelling reason for incorporating stable isotope analyses of vertebrate hardparts into bonebed studies is that these data can shed light on paleoenvironmental conditions and on the behavior and biology of extinct animals. The power of stable isotope ratios of carbon and oxygen to provide insight into these factors lies in their source: carbon from ingested plants and other foods, and oxygen from ingested surface waters. Variation in carbon isotope ratios typically reflect variations in the degree to which leaf stomata are open or closed, which, in turn, is influenced by paleoenvironmental variations across a given landscape. Similarly, oxygen isotope ratios are related to ingested surface waters and typically reflect differences in the amount of evaporation of these waters across a given landscape. In addition to local environmental conditions, animal behavior and physiology play roles in determining stable isotope ratios of vertebrate remains. Given the relation between isotope ratios of vertebrate remains and these factors, isotopic data have the potential to provide insights that are otherwise difficult to obtain via traditional fossil analysis. In turn, these kinds of data may help confirm or refute paleoecological or paleobiological hypotheses based on other types of data or inference (e.g., facies associations, morphological traits, phylogenetic associations).

The challenge associated with such research is making unambiguous interpretations of stable isotope data in the face of multiple environmental, ecological, and biological influences on isotope ratios of vertebrate remains. To overcome this problem and to strengthen interpretations, two things must be done. First, the focus should be less on interpreting absolute

isotope ratios and more on interpreting isotopic similarities or differences, regardless of whether comparisons are made between taxa found in a single bonebed, or between taxa found in spatially or temporally separated bonebeds. Second, studies must be designed so that any isotopic similarities or differences observed can be reasonably attributed to environmental, ecological, or biological factors. This is critical because doing so provides a context for the consideration and interpretation of data. For example, if a single herbivorous taxon has an average $\delta^{13}C$ value of –10‰, multiple interpretations are possible: this could indicate that all plants available in the ecosystem were water stressed, that this particular taxon preferentially occupied such stressed habitats, or that this animal was eating a mixture of C3 and C4 plants. By comparing these data with data from other herbivorous taxa, however, specific dietary and niche preferences should become apparent.

This example also illustrates the importance of study design. By making the reasonable assumptions that all herbivores are behaviorally similar in respect to diet (i.e., they eat plants) and physiologically similar, then the source of isotopic differences can be assumed to derive mostly from the behavioral—i.e., ecological—differences among them. In contrast, if the research question centers on environmental variability over time or space, then sampling should be restricted to a single taxon. The assumption in this case is that the animals are behaviorally and physiology similar regardless of location, and that any isotopic similarities or differences among bonebeds reflects climatic or hydrological differences. Lastly, to address paleobiological questions, an intertaxonomic comparison of data from an environment where isotopic variability is low (e.g., open C3 forest) may make it reasonable to assume that physiological differences between taxa are the cause of any observed isotopic offsets.

In the end, there are many kinds of questions that a bonebed researcher can strive to answer by measuring stable isotope ratios of vertebrate remains. Keeping in mind site-specific limitations associated with taphonomic factors that control the kinds and numbers of vertebrate remains present, and with due consideration of potential diagenetic alteration of isotopic ratios, here are some suggested starting points. Use oxygen isotope data from mammals and fish to estimate temperatures for a single time period in the past. Make maps of such estimates by analyzing contemporaneous bonebed remains, or alternatively, strive to construct a record of temperature change over time. Such maps and records are of particular interest to climate modelers hoping to test their simulations, and to paleoclimatologists trying to resolve regional versus global climatic change events. If your interests lie in the realm of paleoecology, explore

dietary niche partitioning for herbivores, omnivores, and carnivores at the ecosystem scale. This avenue of research is wide open, especially for pre-Pleistocene time periods. A related topic of particular interest to paleontologists is the possibility of intrataxonomic changes in diet in the face of changes in environmental conditions, vegetation structure, or competition, and it is one that can be addressed by comparing isotopic data between spatially or temporally separated bonebeds. Lastly, isotopic data may be useful in characterizing animal migratory patterns, especially if several contemporaneous bonebeds are present over a small geographic range. Questions such as these, which are difficult, if not impossible, to address using traditional approaches, are waiting to be explored using stable isotope data, especially when these data are derived from well-understood bonebed data sets.

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