CHAPTER 5

Isotopic study of the biology of modern and fossil vertebrates

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Introduction

Naturally occurring variations in the stable isotope composition of fossil vertebrates have been studied since the late 1970s. Isotopic data from vertebrate fossils are sometimes used as proxies for environmental factors, such as temperature or precipitation, with biological processes viewed as annoying "vital" effects that must be filtered out to obtain a pure environmental signal. Yet knowledge of these biological factors, which include diet, digestive physiology, reproductive state, thermoregulatory or osmoregulatory physiology, habitat preference, and migration, deepen our understanding of the ecology and evolution of ancient vertebrates. Research on these biological issues was spearheaded by paleoanthropologists studying ancient humans and their kin (see reviews by van der Merwe 1982; DeNiro 1987; Schwarcz & Schoeninger 1991; Ambrose & Krigbaum 2003b), but has exploded in the last decade in animal ecology and physiology (see reviews by Gannes et al. 1998; Hobson 1999; Kelly 2000). The growing body of work on modern vertebrates offers a foundation for more nuanced paleobiological interpretations. In addition, technological advances have made isotopic analysis more routine and allowed study of new systems, new substrates, and extremely small samples.

Isotopic data are used in vertebrate paleobiology in two ways. Because of differences in their mass, isotopes of light elements (e.g., H, C, N, O, or S) are sorted (or fractionated) by chemical and physical processes (see Sulzman, this volume, pp. 00–00 for definitions and conventions for reporting isotopic data and fractionations). In some studies, the extent of isotopic fractionation is used to monitor the magnitude or rate of a process. For example, the process of evaporation is associated with preferential loss of water enriched in ^{16}O and ^{1}H. Experiments on rock doves showed that the δD value of body water is positively correlated with the fraction of water lost by evaporation (McKechnie et al. 2004). Alternately, isotopic differences among substances can serve as natural labels to trace the flow of these substances into vertebrates. This type of research can involve either light or more massive elements (e.g., Sr, Nd, Pb). For example, water and tissues in saguaros have unusual isotopic values relative to other resources available to desert animals. Wolf & Martínez del Rio (2003) exploited these differences to assess the

impact of carbon and water from saguaros at the community level, showing that saguaros support a diverse guild of frugivorous, granivorous and insectivorous birds.

Here, I will explore recent advances in the study of isotopic variations in vertebrates, focusing on studies of fossils. Table 5.1 offers summary information about the isotopic systems to be discussed. After considering the vertebrate tissues that are found in the fossil record, I will introduce the main isotopic systems, focusing on physiological and environmental controls on isotopic values in vertebrate tissues. I will then briefly consider the reliability (or lack thereof) of vertebrate fossils as recorders of biogenic isotopic compositions. Paleobiological applications are presented last, including examples of studies of diet, thermal physiology, reproductive biology, habitat preference, and migration. These examples are not meant to provide an exhaustive review; the literature on these subjects has become too vast to cover in a single paper.

Vertebrate tissues in the fossil record

Types of tissue

Vertebrate bodies are constructed from tissues with different macromolecular and elemental compositions, different styles of growth and turnover, and different potentials for post-mortem preservation (Table 5.2). Soft tissues such as skin, muscle, hair, and feathers contain protein and lipids, and well-preserved soft tissues persist for 10^3 to 10^4 years in unusual settings (e.g., mummification in dry environments, permafrost). Soft tissues are preserved in exceptional cases for up to 10^8 years, but often only as impressions or pseudomorphs composed of phosphate minerals or fossilized bacteria (Martill 1995; Briggs et al. 1997). A report of organic preservation in a Cretaceous dinosaur hints that the potential for excellent preservation in more typical depositional environments may have been underestimated (Schweitzer et al. 2005). Immunohistochemical and amino acid analyses suggest that traces of keratin (feather protein) survive in Mesozoic fossils (Schweitzer et al. 1999), but this material has not been isolated for isotopic analysis.

Mineralized tissues such as bone, tooth enamel and dentin, eggshell, and otoliths have much greater potential for preservation in deep time. Bone and tooth dentin and enamel are composites of mineral, protein, and lipid. The mineral is a highly substituted form of hydroxylapatite ($Ca_{10}[PO_4]_6[OH]_2$) I will call bioapatite. Bioapatite has a few weight percent carbonate substituting for hydroxyl and phosphate groups, and various cations (e.g, Sr, Pb) substituting for calcium (Simkiss & Wilbur 1989). Bioapatite readily adsorbs carbonate, rare earth elements, amino acids, and nucleic acids on crystal surfaces (Beshah et al. 1990; Hedges 2002; Trueman & Tuross 2002). Bone is com-

Table 5.1 Vertebrate isotope systems and their applications.

Element	Isotope	Fractional abundance	Standard for δ value calculation	Isotopic range in vertebrates*	Applications†
Hydrogen	^{1}H	0.999844	Standard Mean	−175 to +70‰	Migration, habitat use, diet, trophic level, osmoregulatory physiology
	^{2}H	0.000156	Ocean Water (SMOW)	Kelly et al. (2002) Wolf & del Rio (2000)	
Carbon	^{12}C	0.98889	Pee Dee	−60 to +5‰	Diet, digestive physiology, habitat use, migration
	^{13}C	0.01111	Belemnite Limestone (PDB)	Doucett et al. (2002) Kohn & Cerling (2002)	
Nitrogen	^{14}N	0.99634	Air	−30 to +30‰	Trophic level, diet, habitat use, migration, starvation, reproduction
	^{15}N	0.00366		Hare et al. (1991)	
Oxygen	^{16}O	0.99755	SMOW or PDB	0 to +35‰	Habitat use, migration, diet, thermoregulation, osmoregulation
	^{17}O	0.00039		Kohn & Cerling (2002)	
	^{18}O	0.00206			
Sulfur	^{32}S	0.9493	Canyon Diablo Troilite	−15 to +20‰ Lott et al. (2003)	Habitat use, migration, diet
	^{33}S	0.0076			
	^{34}S	0.0429			
	^{36}S	0.0002			
Calcium	^{40}Ca	0.96941	NIST 915a (CaF_2)	−3.1 to +1.8‰ Skulan & DePaolo (1999)	Trophic level, habitat use, migration
	^{42}Ca	0.00647			
	^{43}Ca	0.00135			
	^{44}Ca	0.02086			
	^{46}Ca	0.00004			
	^{48}Ca	0.00187			
Strontium	^{84}Sr	0.0056	Bulk Earth (ε) or Sea Water (δ)	0.7043–0.7583 Price et al. (2000) Nelson et al. (1986)	Habitat use, migration, diet
	^{86}Sr	0.0986			
	^{87}Sr	0.0700			
	^{88}Sr	0.8258			

* The range of values is defined by data in cited papers.
† Study of migration is a potential application of all isotope systems.

Table 5.2 Summary information on materials used as substrate for isotopic analysis of vertebrates.

Tissue	Component	Signal window	Isotope systems	Preservation window (years)
Hair	Keratin	Accretion	H, C, N, O, S	10^4
Feather	Keratin	Accretion	H, C, N, O, S	10^4
Bone	Bioapatite	Years	CO_3–C, O	10^3 (10^6)
			PO_4–O	10^4 (10^8)
			Ca	10^7–10^8
			Sr, Nd, Pb	10^3
	Collagen	Years	H, C, N, O, S	10^5–10^6 (10^8)
	Lipid	Weeks–months	H, C	
Enamel	Bioapatite	Accretion	CO_3–C, O	10^8
			PO_4–O	10^8
			Ca	10^7–10^8
			Sr, Nd, Pb	10^7
Dentin	Bioapatite	Accretion	Same as bone	Same as bone
	Collagen	Accretion	H, C, N, O, S	Same as bone
Egg shell	Carbonate	Days–weeks	C, O	10^7–10^8
	Protein	Days–weeks	C, N	10^4
Otoliths	Carbonate	Accretion	C, O	10^7
Compound type	Signal window		Isotope systems	Preservation window
Amino acid	Depends on tissue type		H, C, N	10^5?
Cholesterol	Weeks–months		H, C	10^6?
Fatty acid	Weeks–months		H, C	10^4?

posed of tiny bioapatite crystals ($100 \times 20 \times 4$ nm) intergrown with an organic matrix (chiefly composed of the protein collagen) that comprises ca. 30 percent of its dry weight (Simkiss & Wilbur 1989). Enamel is much less porous than bone. It contains <5 weight percent organic matter (chiefly non-collagenous proteins) and has much larger crystals ($1000 \times 130 \times 30$ nm) with fewer substitutions (LeGeros 1991). The crystal size, organic content, and organic composition of tooth dentin resemble bone, whereas its porosity is intermediate between enamel and bone (Lowenstam & Weiner 1989).

Bird and crocodile eggshells are composed of tiny crystals secreted around a honeycomb of fibrous organic sheets. The crystalline portion of shells is almost entirely calcite. Mineral in bird eggshells occurs in three distinct layers that are covered externally by cuticle and anchored internally to a shell membrane (Simkiss & Wilbur 1989). The organic matrix comprises ca. 3 percent of the mass of bird eggshell and is largely protein (ca. 70%) with lesser amounts of carbohydrate and lipid (Burley & Vadehra 1989). A range

of microstructures occurs in dinosaur eggshells, including some quite similar to those of bird eggshell (Mikhailov et al. 1996).

Otoliths are mineralized bodies in the vertebrate inner ear (Panella, 1980). Otolith mineralogy varies among vertebrates – bioapatite occurs in agnathans, aragonite occurs in jawed fish and amphibians, and calcite occurs in amniotes (Simkiss & Wilbur 1989). In teleosts, calcification occurs on a preformed organic matrix rich in non-collagenous proteins and mucopolysaccharides (Panella 1980).

Individual organic compounds

Most soft tissues decay rapidly, and even the organic matter in mineralized tissues degrades by various processes (Bada et al. 1999; Collins et al. 2002). Yet under favorable circumstances, individual organic molecules (or diagenetic products that can be directly related to biogenic molecules) can be isolated from fossil vertebrates by gas or liquid chromatography. Fatty acids occur in characteristic relative abundances in different organisms, as do amino acids in different types of protein (Tuross et al. 1988; Smith et al. 1997; Evershed et al. 1999). Abundance patterns can be used to trace sources and to ensure that the organic residues extracted from fossils are not exogenous (Tuross et al. 1988; Bada et al. 1999). Sometimes, particular molecules are highly specific to a class of organisms, making them especially reliable molecular substrates for isotopic analysis. For example, vertebrate collagen contains a relatively high abundance of the amino acid hydroxyproline, which is uncommon in other proteins from terrestrial organisms (Tuross et al. 1988). Similarly, the steroidal lipid cholesterol does not occur in plants, but does occur in relatively high concentrations in the bodies and bones of vertebrates (Stott et al. 1999).

If sufficient quantities of organic matter are available, the products of chromatographic separation can be collected and analyzed by standard, dual-inlet mass spectrometry, as was done in early studies of amino acids from bone collagen (Gaebler et al. 1966; Tuross et al. 1988; Hare et al. 1991) or with an elemental analyzer interfaced with a mass spectrometer. In many cases, however, only traces of organic compounds remain. By coupling a gas chromatograph (GC) to a combustion, pyrolysis, or reduction furnace, and then feeding the effluent to an isotope-ratio-monitoring mass spectrometer on a carrier gas stream, it is possible to separate minute quantities of individual organic molecules and measure their δD, $\delta^{13}C$, $\delta^{15}N$, or $\delta^{18}O$ values. Systems whereby organic molecules in the effluent of a liquid chromatograph are converted to CO_2 for carbon isotope analysis are available as well.

Three classes of compounds from vertebrates have been examined using these methods: fatty acids, sterols such as cholesterol, and amino acids. Lipids (e.g., fatty acids and sterols) are rich in carbon and hydrogen. Amino acids, in contrast, contain abundant carbon and nitrogen, and lesser amounts of

hydrogen, oxygen, and sulfur. Early work on individual amino acids using dual-inlet mass spectrometry examined both $\delta^{13}C$ and $\delta^{15}N$ values (Tuross et al. 1988; Hare et al. 1991). Yet nearly all the papers on vertebrates using GC methods have examined only $\delta^{13}C$ values of individual compounds (e.g., Evershed et al. 1999; Fogel & Tuross 2003). There is a growing literature on $\delta^{15}N$ and δD values of individual molecules measured via GC methods from enriched-tracer studies in biomedical research (e.g., Scrimgeour et al. 1999; Metges et al. 2002), as well as work on the $\delta^{15}N$ values of individual amino acids in invertebrates (e.g., McClelland & Montoya 2002), but this approach has yet to impact ecological or paleontological studies of vertebrates.

Growth and turnover time

The time represented by an isotopic sample is dependent on the mode of growth and turnover time of each tissue, as well as the sampling strategy. Bone growth is complex, involving both ossification of cartilage and accretionary growth, which can be interrupted by lines of arrested growth (LAGs). Bone is also remodeled by dissolution and reprecipitation (Lowenstam & Weiner 1989). Remodeling is active in mammals, birds, and other rapidly growing vertebrates and in any bone under substantial load (Reid 1987; Chinsamy & Dodson 1995; Padian et al. 2004). A bulk sample of bone mineral or collagen thus contains material that may have formed over several years of growth, though it will be weighted towards periods of rapid growth. In portions of bone that show incremental features or LAGs, it is possible to obtain samples that represent a time series in the life of an animal, albeit one that is smoothed by bone turnover. In contrast, cholesterol in bone turns over more rapidly, and probably represents an average of at most a few months (Stott et al. 1999).

Dentin grows by accretion and experiences little post-depositional remodeling (Lowenstam & Weiner 1989). Dentin exhibits incremental laminations at a variety of temporal scales, from daily to annual (Carlson 1990), and sequential analysis of samples taken from these increments provides a time series of body chemistry (e.g., Koch et al. 1989). Enamel also bears incremental laminae indicating accretionary growth (striae of Retzius). Workers have tried to obtain sub-annual samples of body chemistry by collecting sequential samples from these increments (e.g., Bryant et al. 1996; Fricke & O'Neil 1996; Kohn et al. 1998; Sharp & Cerling 1998). Yet as noted by Fisher & Fox (1998), incremental laminae mark the front of organic matrix apposition, not the mineralization front. Complete enamel mineralization may lag organic matrix apposition by months, and the mineralization front need not parallel the organic apposition front (Balasse 2002; Hoppe et al. 2004; Zazzo et al. 2005). Thus even samples collected along incremental laminae will be time averaged. Passey & Cerling (2002) offered a mathematical model for dealing with this problem, and it may be minimized by examining enamel

from ever-growing teeth (Stuart-Williams & Schwarcz 1997; Fox & Fisher 2004) or teeth that mature rapidly (Straight et al. 2004). In all mammals except those with ever-growing teeth, mineralization takes place early in the animal's life. In contrast, fish, amphibians, reptiles and some non-mammalian synapsids replace their teeth continuously throughout their lives.

Growth of feathers and hair may be continuous or episodic, but these tissues are typically replaced within a year or two. Eggshell crystallization occurs rapidly, lasting only 20 hours in chickens and perhaps 36 hours in ostriches (Burley & Vadehra 1989). Teleost otoliths exhibit incremental laminae formed at a variety of temporal scales (i.e. daily to lunar to annual) that are used to study age and growth rate (Panella 1980; Campana & Neilson 1985). Overall, we might expect that eggshell carbonate and proteins represent very short time intervals, that depending on sampling strategy hair and feather samples can represent weeks to months, and that otoliths offer a relatively complete record of body chemistry that can be microsampled for time series or bulk sampled for a life-time average.

Controls on the isotopic composition of vertebrate tissues

Controls on the isotopic composition of vertebrates have been reviewed repeatedly over the past two decades. Key reviews will be noted at the start of each section. The discussion in each section draws on these reviews, with citations only for more recent studies or topics not covered by the reviews.

Carbon isotopes

Key reviews of controls on carbon isotopes in vertebrates are van der Merwe (1982), Schoeninger (1985), DeNiro (1987), Schwarcz & Schoeninger (1991), Schoeninger & Moore (1992), Koch et al. (1994), Pate (1994), Koch (1998), Kelly (2000), Kohn & Cerling (2002), and Ambrose & Krigbaum (2003b).

Carbon in biominerals

Food is the source of carbon in the mineral and organic substrates of terrestrial vertebrate bones, teeth, and eggshells, yet each tissue differs in $\delta^{13}C$ value from diet by a characteristic amount. The fractionations among dissolved carbon dioxide (which is largely derived from oxidation of food), body fluid bicarbonate, and carbonate-bearing minerals at mammal and bird body temperatures are such that a ^{13}C-enrichment of 9–10‰ is expected between food and bioapatite or calcium carbonate. In terrestrial herbivores, the $\delta^{13}C$ value of bioapatite shows a strong 1:1 correlation with the $\delta^{13}C$ value of bulk diet, with a ^{13}C-enrichment of 9–11‰ for laboratory rodents and 12–14‰ for wild ungulates (Cerling & Harris 1999: Balasse 2002: Howland et al. 2003: Jim et al. 2004: Passey et al. 2005). Little is known about the diet-to-bioapa-

tite fractionation in carnivores, but the value is thought to be ca. 9‰. The difference between bird eggshell carbonate and diet is quite large (14–16‰).

Differences in diet-to-mineral fractionation among terrestrial animals may have a number of sources, but modeling and laboratory experiments strongly suggest that greater degrees of enrichment occur in animals that obtain nutrients from microbial fermentation (Hedges 2003; Passey et al. 2005). Fermentation produces very ^{13}C-depleted CH_4, which escapes the body, and ^{13}C-enriched CO_2, which may diffuse from the gut to the blood stream, thereby labeling body fluid bicarbonate and mineral carbonate pools.

Diet-to-bioapatite fractionations in marine mammals are similar to those for terrestrial mammals (Clementz & Koch 2001). In aquatic ectotherms, carbon in bioapatite is derived from both respiration (i.e., food) and ambient water (Vennemann et al. 2001; Biasatti 2004). Carbon in otolith aragonite is also a mixture supplied by ambient water and respiration (Thorrold et al. 1997; Wurster & Patterson 2003). As a consequence, diet-to-mineral fractionations in these ectotherms are sensitive to any factor that alters the fluxes of metabolic versus ambient bicarbonate to body fluids (i.e., temperature, metabolic or growth rate, activity level, etc.).

Carbon in proteins and individual amino acids

Proteins are comprised of amino acids. Most amino acids have a central α-C atom to which is bonded (i) a hydrogen atom, (ii) a carboxyl group (—COOH), (iii) an amino group (—NH_3^+), and (iv) a distinct side chain, or R-group, which is often rich in carbon. The R-groups of essential amino acids (and non-essential amino acids whose sole precursors are essential) must originate from dietary protein. Non-essential amino acids may be ingested, or they may be assembled within the animal, and so may contain carbon from any dietary source (e.g., proteins, lipids, or carbohydrates) (Fogel et al. 1997). In collagen, ca. 20 percent of carbon atoms are essential and must be routed from dietary protein (Howland et al. 2003). Feeding experiments suggest that on relatively high protein diets, the $\delta^{13}C$ value of collagen is controlled by that of dietary protein, whereas on low protein diets, contributions from dietary carbohydrate and lipid are evident (Ambrose & Norr 1993; Tieszen & Fagre 1993; Howland et al. 2003; Jim et al. 2004). Associations between diet type and diet-to-tissue fractionation have also been detected in birds and fish (Bearhop et al. 2002; Pearson et al. 2003; Gaye-Siessegger et al. 2004).

Because different compound classes in diet can come from sources with different $\delta^{13}C$ values, the effects of carbon routing complicate dietary interpretations for omnivores if they are based on bulk collagen $\delta^{13}C$ values (Fogel & Tuross, 2003). For relatively committed herbivores or carnivores, in contrast, the picture is clearer. The bulk diet-to-collagen fractionation is 3–5‰

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in mammals and birds. I am aware of no published controlled feeding experiments on fish or reptiles where collagen was analyzed, but data from ecological and paleoecological studies suggest a fractionation of similar magnitude (e.g., Sholto-Douglas et al. 1991; Ostrom et al. 1994; Dufour et al. 1999). In mammals, the diet-to-hair fractionation is 1–3‰ (Tieszen & Fagre 1993; Roth & Hobson 2000; O'Connell et al. 2001; Sponheimer et al. 2003a). In birds, the diet-to-feather fractionation is ca. 3–4‰ (Hobson & Clark 1992; Bearhop et al. 2002; Gaye-Siessegger et al. 2004), and the diet-to-eggshell matrix protein fractionation is ca. 2‰.

Studies of individual amino acids are shedding light on dietary routing and laying the groundwork for paleobiological analysis. Individual amino acids from collagen or muscle can differ greatly in $\delta^{13}C$ value, though consistent patterns of variation are emerging (Hare et al. 1991; Fogel et al. 1997). With respect to routing of dietary carbon, experiments on pigs with diets of variable protein content show that the $\delta^{13}C$ values of non-essential amino acids in collagen (e.g., glutamate and alanine) correlate well with the $\delta^{13}C$ value of bulk diet, whereas the $\delta^{13}C$ values of some important essential amino acids in collagen (e.g., leucine and phenylalanine) correlate with the same amino acid in diet without substantial isotopic fractionation (Howland et al. 2003). If these results hold for other taxa, analysis of glutamate or alanine could be used to assess the $\delta^{13}C$ value of bulk diet (i.e., calories), whereas analysis of leucine and phenylalanine could be used to assess the $\delta^{13}C$ value of dietary protein. The study by Fogel & Tuross (2003), which did not include feeding experiments, suggested substantial fractionations from plant diet-to-herbivore collagen for most essential amino acids, including phenylalanine. More experimental work is clearly needed, particularly for animals with different digestive physiologies.

Carbon in lipids, fatty acids, and cholesterol

The lipids within an animal's body are comprised of different fatty acids, some of which can be synthesized and some of which are essential and must be ingested. Lipids are ^{13}C-depleted relative to bulk diet and other body tissues (DeNiro & Epstein 1978). Few isotopic data have been reported on bulk lipids extracted from modern or fossil bones, perhaps because of concerns about differential degradation of different classes of lipids, differences between essential and non-essential lipids, and contamination (Koch et al. 2001; Collins et al. 2002). With the development of GC-C-IRMS, individual lipid molecules, especially cholesterol, have become targets for analysis.

Analyses of herbivorous mammals subjected to controlled feeding suggest that the $\delta^{13}C$ values of non-essential fatty acids and cholesterol are tightly correlated to the $\delta^{13}C$ value of bulk diet, and that the $\delta^{13}C$ values of essential fatty acids, such as linoleic acid, are directly related to the value of that fatty acid in diet without substantial isotopic fractionation (Stott et al. 1997, 1999;

Howland et al. 2003; Jim et al. 2004). Thus compound-specific isotope analysis of lipids may offer data on both bulk diet and dietary lipids.

Environmental controls on carbon isotopes

Carbon isotope differences among vertebrates largely reflect differences in the $\delta^{13}C$ values of primary producers at the base of the food web. In terrestrial ecosystems, the dominant control on the $\delta^{13}C$ value of plants is photosynthetic pathway. Basic physiological controls on the $\delta^{13}C$ values of plants using different pathways have been reviewed elsewhere (Ehleringer & Monson 1993) and will not be discussed here. The C3 pathway is the most common, occurring in all trees, most shrubs and herbs, and grasses in regions with a cool growing season. C3 plants have a mean $\delta^{13}C$ value of ca. –27‰ (range –22 to –35‰). C4 photosynthesis occurs in grasses from regions with a warm growing season, and in some sedges and dicots. C4 plants have higher $\delta^{13}C$ values (mean ca. –13‰, range –19 to –9‰). Crassulacean acid metabolism (CAM) is the least common pathway, occurring in succulent plants. CAM plants exhibit $\delta^{13}C$ values that range between the values for C3 and C4 plants.

There are strong abiotic influences on the distribution and isotopic composition of plants using different pathways. Among grasses, C4 species abundance and biomass increase with growing season temperature and wetness (Epstein et al. 1997). CAM plants and C4 dicots are most abundant in arid regions. Variations in light level, water and osmotic stress, nutrient levels, temperature, and P_{CO_2} produce predictable variations in the $\delta^{13}C$ values of C3 plants (reviewed by Tieszen 1991; Ehleringer & Monson 1993; Heaton 1999). C3 plants may also show large differences in $\delta^{13}C$ value related to plant functional type. In dense, closed-canopy forests, the $\delta^{13}C$ value of forest floor leaves may be ^{13}C depleted by up to 8‰ relative to leaves from the top of the canopy, owing to recycling of ^{13}C-depleted carbon dioxide and the effects of low irradiance. Because of their efficient method of carbon fixation, C4 plants show little environmental variability in $\delta^{13}C$ values (Ehleringer et al. 1997). CAM plants in arid regions have $\delta^{13}C$ values similar to those of C4 plants, whereas in wetter regions, CAM plants have values intermediate between those of C3 and C4 plants.

Marine primary producers exhibit strong spatial $\delta^{13}C$ gradients due to differences in (i) the rate of photosynthesis, (ii) the taxon and size of phytoplankton and bacteria fixing carbon, (iii) the $\delta^{13}C$ value of dissolved inorganic carbon, and (iv) water mass properties that influence factors i–iii (see discussion and references in Burton & Koch (1999) and Clementz & Koch (2001)). Primary producers are enriched in ^{12}C relative to starting substrates, and in areas of the ocean where primary and export production strip carbon from the ocean surface, primary producers have high $\delta^{13}C$ values. These conditions occur in highly productive regions (i.e., upwelling zones) and in oligotrophic waters, but not in regions where nutrient supply far exceeds photosynthetic

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demand, such as at high latitudes. Overall, primary producer $\delta^{13}C$ values increase from offshore to nearshore ecosystems, peaking in macrophytic ecosystems (i.e., kelp and seagrass beds). There are also strong gradients across different current systems (e.g., Rau et al. 1991). In estuarine and freshwater ecosystems, mean $\delta^{13}C$ values for primary producers are typically lower but much more variable than in marine systems.

Given these physiological and environmental controls, $\delta^{13}C$ values in vertebrates will vary with diet (photosynthetic pathway; marine vs. freshwater vs. terrestrial feeding), location, and ecosystem properties related to plant type and carbon cycling. They may shed light on digestive physiology and metabolic rate.

Nitrogen isotopes

Key reviews exploring nitrogen isotopes in vertebrates are Schoeninger (1985), DeNiro (1987), Ambrose (1991), Schwarcz & Schoeninger (1991), Schoeninger & Moore (1992), Koch et al. (1994), Pate (1994), Koch (1998), Kelly (2000), and Ambrose & Krigbaum (2003b).

Nitrogen in proteins and individual amino acids

Unlike carbon, which has multiple macromolecular dietary sources, nitrogen in animal protein is supplied almost entirely by dietary protein. A diet-to-tissue fractionation of ca. 3‰ has been observed or assumed in many studies. In general, most body and shell proteins have similar $\delta^{15}N$ values. This trophic level fractionation is thought to relate to excretion of urea and other nitrogenous wastes that are enriched in ^{14}N relative to body nitrogen pools (e.g., Parker et al. 2005), perhaps due to fractionation associated with deamination of glutamate (which yields one of the NH_4^+ molecules in urea) or cleavage of arginine to yield urea (Fogel et al. 1997). Feeding experiments on mammals and birds have shown that the magnitude of fractionation increases with increasing protein content in the diet (Pearson et al. 2003; Sponheimer et al. 2003b, 2003c), and field observations suggest that fractionation is greater in mammals inhabiting arid habitats (Schwarcz et al. 1999). The causes of these variations in fractionation are debated, and variously attributed to changes in urea concentration, recycling of urea, or lack of nitrogen balance (Sponheimer et al. 2003c). Finally, for animals that are out of nitrogen balance, there is evidence that diet-to-tissue fractionation decreases for animals in anabolic states (growth) and increases for animals in catabolic states (fasting, starvation). Fuller et al. (2005) reviews these issues and offer compelling data on ^{15}N-enrichment in pregnant women with morning sickness.

To understand why trophic fractionation might vary with the protein content of diet, consider a simple open-system isotope mass balance model with the following properties:

1 a flux of dietary nitrogen into the body (F_d) with a value $\delta^{15}N_d$;
2 a single pool of body nitrogen with a value $\delta^{15}N_b$;
3 a flux of urea nitrogen out of the body (F_u) with a value $\delta^{15}N_u = \delta^{15}N_b + \varepsilon_{bu}$, where ε_{bu} is the net fractionation between body tissue and urea nitrogen associated with deamination and urea synthesis;
4 a flux of tissue nitrogen out of the body (chiefly fecal nitrogen, F_f) with a value $\delta^{15}N_f = \delta^{15}N_b$ (Figure 5.1).

At steady-state (i.e., with a body nitrogen pool of fixed size), input and output isotopic fluxes must be equal, so $F_d\delta^{15}N_d = F_u\delta^{15}N_u + F_f\delta^{15}N_f$. After conversion of actual fluxes to proportional fluxes (by dividing both sides of the equation by F_d), substitution of equations involving $\delta^{15}N_b$ for $\delta^{15}N_u$ and $\delta^{15}N_f$, and algebraic rearrangement, we obtain the following equation: $\delta^{15}N_b = \delta^{15}N_d - \varepsilon_{bu}X_u$, where X_u (= F_u/F_d) is the proportional flux of nitrogen lost as urea. Figure 5.1 plots $\delta^{15}N_b$ and $\delta^{15}N_u$ at different values of X_u with $\varepsilon_{bu} = -6‰$ and $\delta^{15}N_d = 0‰$.

For animals on diets rich in proteins, daily nitrogen intake far exceeds requirements for nitrogen balance. These animals catabolize the carbon skeletons of amino acids as fuel and shed the stripped amine groups as urea, leading to high proportional loss of body nitrogen as urea and high diet-to-tissue fractionations. Animals on low protein diets use most of their dietary nitrogen to build body protein, and consequently have a lower urea nitrogen flux, which reduces the diet-to-tissue fractionation. Models of greater complexity have been developed to investigate trophic fractionation of nitrogen isotopes (Schoeller 1999; Hedges & van Klinken 2000; Olive et al. 2003). The key points to note here are that fractionation of nitrogen isotopes between diet and tissue may vary among animals at steady-state, and that fractionation associated with urinary nitrogen loss may be a primary driver even when the $\delta^{15}N$ value of urea is not substantially lower than that of diet (*contra* Sponheimer et al. 2003c), as long as most nitrogen is lost via urine.

The controls on $\delta^{15}N$ values in individual amino acids are related to biosynthetic and catabolic pathways. The amine group is reversibly exchangeable by transamination for all amino acids except threonine and lysine, and so for all other amino acids, amine group nitrogen may be homogenized in the circulating amino acid pool. Several amino acids have nitrogen in their R-groups, and for essential amino acids, such as lysine and histidine, these must be supplied by diet. Most amino acids in collagen are enriched in ^{15}N relative to the same amino acid in diet, with the strongest enrichment for glutamate, which plays a central role in transamination reactions and the urea cycle (Hare et al. 1991; Fogel et al. 1997; see also McClelland & Montoya (2002) for a similar result involving invertebrates). Threonine in collagen shows very strong ^{15}N-depletion relative to threonine in diet, presumably because of an unusual fractionation associated with catabolism (Hare et al. 1991). Lysine and arginine also have low $\delta^{15}N$ values. For arginine, this may occur because it receives an NH_4^+ from deamination of glutamate. The fact

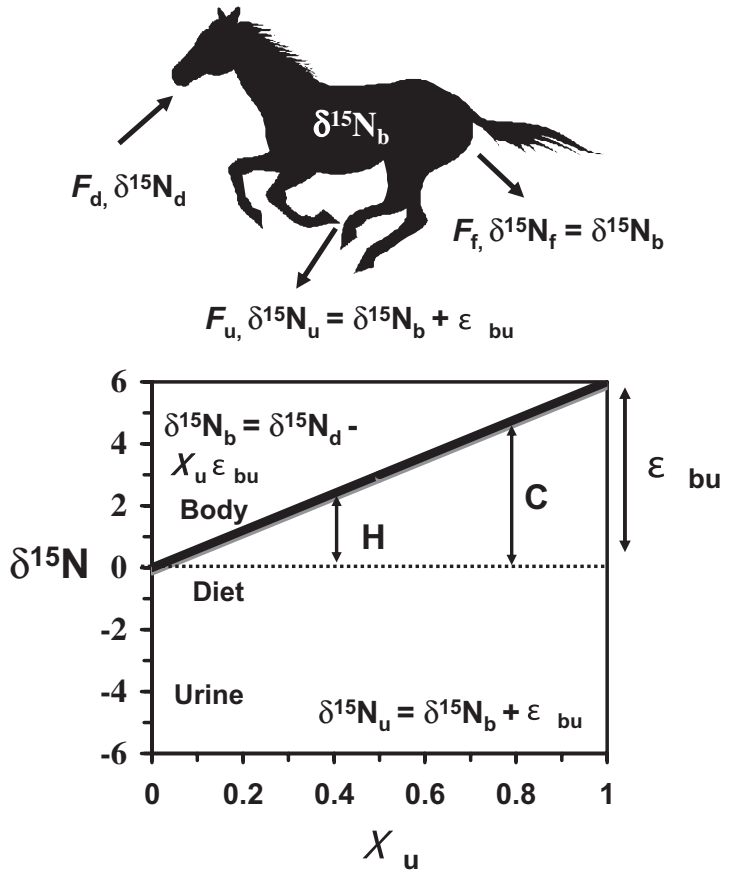

Figure 5.1 A steady-state nitrogen isotope mass balance model for mammals. The arrows into and out of the horse identify the major nitrogen fluxes and their isotopic compositions. The bivariate plot shows how the $\delta^{15}N$ values of body tissue (dark line) and urine (gray line) change as the proportion of body nitrogen lost as urine (X_u) changes, assuming that the main fractionation in this system occurs between body tissue and urine (ε_{bu}) and that the $\delta^{15}N$ value of diet is 0‰ (dotted line). Carnivores (C) consume protein for calories, and hence take in much more nitrogen than is needed to maintain mass balance. They shed excess nitrogen in ^{15}N-depleted urine and therefore show a relatively large ^{15}N-enrichment in body tissues relative to diet. In contrast, to maintain mass balance an herbivore (H) on a protein-poor diet must shed less nitrogen as waste/urine; it loses more nitrogen through unavoidable shedding of body protein as hair, milk, enzymes, and especially gut wall. As a consequence, herbivore body tissues exhibit a smaller ^{15}N-enrichment relative to diet. The abbreviations for all variables are defined in the text.

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that nitrogen in lysine is entirely sourced from dietary lysine may buffer it from the ^{15}N-enrichment affecting most of the body amino acid pool (Hare et al. 1991; Fogel et al. 1997). As such, lysine is the best target for monitoring the $\delta^{15}N$ value of dietary protein.

Environmental controls on nitrogen isotopes

Controls on $\delta^{15}N$ values in primary producers at the base of food webs are complex. Nitrogen in most plants is taken up from soils, and soil and plant $\delta^{15}N$ values vary geographically depending on soil pH, moisture, and atmospheric nitrogen deposition (Nadelhoffer & Fry 1994; Högberg 1997). Most processes by which nitrogen is lost from soils (denitrification, ammonia volatilization, loss of dissolved species) lead to ^{15}N-enrichment of soil nitrogen, generating trends associated with soil age and maturity (Nadelhoffer & Fry 1994; Hobbie et al. 1998). The $\delta^{15}N$ value of foliar nitrogen is negatively correlated with rainfall abundance globally (Handley et al. 1999), though recent work in southern Africa suggests this relationship may be driven entirely by C3 plants (Swap et al. 2004). Plant $\delta^{15}N$ values are negatively correlated with soil moisture at a local scale as well (Evans & Ehleringer 1994). These correlations may reflect greater ^{15}N-enrichment in dry soils due to higher rates of nitrogen loss. The $\delta^{15}N$ value of plants that do not fix N_2 is higher in coastal regions, perhaps owing to deposition of marine nitrate (Heaton 1987). Finally, within sites, plants show consistent differences among growth forms related to differences in rooting depth, symbioses with nitrogen-fixing bacteria, and mycorrhizal associations. For example in boreal ecosystems, nonmycorrhizal plants (graminoids, clubmosses, forbs) have higher $\delta^{15}N$ values than mycorrhizal plants (chiefly trees and shrubs), mosses and lichens (Schulze et al. 1994; Nadelhoffer et al. 1996; Michelsen et al. 1998).

As with carbon in marine primary producers, there are strong $\delta^{15}N$ gradients in some regions due to differences in (i) the rate of nitrogen uptake, (ii) the extent of nitrate utilization or denitrification, (iii) the type of phytoplankton and bacteria fixing nitrogen, and (iv) water mass properties that influence factors i–iii (Michner & Schell 1994; Voss et al. 1996). In many regions of the ocean, primary producers remove all nitrogen from surface waters, so annually integrated production must have the same $\delta^{15}N$ value as the combined sources of nitrogen to the ocean surface. Spatial gradients may be produced by the differential mixing of these sources, particularly if deeper water that is ^{15}N-enriched due to denitrification is involved (e.g., along the eastern Pacific margin, Altabet et al. 1999). In regions where nitrogen utilization is not complete, the extent of ^{15}N-enrichment will correlate with degree of nitrogen utilization (e.g., from the Southern Ocean south to the sub-Antarctic, Altabet & François 2001). In any case, these strong spatial gradients in marine $\delta^{15}N$ values offer a natural tag of position for ecological and paleoecological studies (e.g., Schell et al. 1998, McClelland et al. 2003).

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Given these physiological and environmental controls, it is clear that the $\delta^{15}N$ values of terrestrial vertebrates will vary with trophic level, marine vs. terrestrial feeding, factors affecting nitrogen balance (starvation, lactation, pregnancy, etc.), the type of plant food taken, rainfall abundance and location. In the ocean, all these factors except rainfall abundance affect vertebrate $\delta^{15}N$ values.

Sulfur isotopes

Key reviews exploring sulfur isotopes in vertebrates are Krouse (1989) and Richards et al. (2003).

Sulfur in proteins and biominerals

Sulfur in animals is primarily found in proteins (in the amino acids cysteine and methionine) and in bioapatite as sulfate (SO_4^{2-}) substituting for phosphate. Methionine is the most common sulfur-bearing amino acid in collagen (5 residues per 1000), whereas both cysteine (112 residues per 1000) and methionine (5 residues per 1000) are more abundant in hair. The proteinaceous organic matrix of otoliths contains 1–3 percent cysteine and methionine (Weber et al. 2002). Methionine is an essential amino acid and is the source of sulfur for cysteine biosynthesis.

In natural systems, the difference between potential foods and animal tissues is small, suggesting a small sulfur isotope fractionation. This inference has been verified by feeding experiments on bears and pigs, which found a small positive fractionation between diet and tissue (González-Martín et al. 2001; Felicetti et al. 2003). It is more difficult to assess the results of a feeding experiment on horses (Richards et al. 2003), because horses switched to low protein diets may not have fully equilibrated with the new diet.

Environmental controls on sulfur isotopes

Plants take up sulfur derived from (i) weathering of bedrock, which can vary widely in $\delta^{34}S$ value, (ii) wet atmospheric deposition (sea spray, acid rain), (iii) dry atmospheric deposition (SO_2 gas), and (iv) microbial processes in soils. As a consequence, the $\delta^{34}S$ value of terrestrial plants varies with location, with values ranging from −22 to +22‰ (Peterson & Fry 1987). In their study of grizzly bears, Felicetti et al. (2003) detected a large within-ecosystem difference in $\delta^{34}S$ value between pine nuts and all other plant and animal foods available to bears. They offered no explanation for the strong ^{34}S-enrichment in pine nuts, but it may relate to differences in rooting depth or soil properties near the edge of the tree line where whitebark pine occur. In rivers and lakes, differences in the extent of anaerobic sulfate reduction (which produces sulfate extremely depleted in ^{34}S) leads to a similarly wide

range of $\delta^{34}S$ values (Peterson & Fry 1987). Sulfur in marine phytoplankton is relatively uniform, with a mean value of ca. 20‰.

Given the minor physiological impacts on $\delta^{34}S$ values, sulfur isotope data from vertebrates can be used to reconstruct marine vs. terrestrial vs. freshwater feeding (in the many settings where non-marine and marine values do not overlap), location, and type of plant consumed.

Oxygen isotopes

Key reviews of controls on oxygen isotopes in vertebrates are Schwarcz & Schoeninger (1991), Schoeninger & Moore (1992), Koch et al. (1994), Koch (1998), and Kohn & Cerling (2002).

Oxygen in biominerals

Oxygen in bioapatite phosphate and carbonate and in calcium carbonate has been used in isotopic studies of vertebrates. Some analytical methods extract all the oxygen in the bioapatite, including that in hydroxyl groups, into a combined pool (Kohn et al. 1996; Sharp & Cerling 1996), but to my knowledge, there are no studies that have isolated bioapatite hydroxyl oxygen for isotopic analysis. The $\delta^{18}O$ value of a biomineral depends on the temperature at which it forms and the $\delta^{18}O$ value of the body fluid from which it precipitates. For homeothermic mammals, there is a constant offset between the $\delta^{18}O$ value of body water and phosphate (ca. 18‰), and between the phosphate and carbonate components of bioapatite (ca. 8‰), close to values predicted for oxygen isotope equilibrium at body temperatures. Bird eggshell carbonate has values that range from near equilibrium with body water to values ^{18}O-enriched by 3‰ relative to equilibrium. For heterothermic animals (fish, reptiles, etc.), the oxygen isotope fractionation between body water and bone, tooth, or scale bioapatite phosphate and carbonate or otolith carbonate increases as temperature drops. Aquatic heterotherms form biominerals in isotopic equilibrium with ambient water at body temperature. For turtles, Barrick et al. (1999) noted that the fractionation between bone and environmental water (a chief source of body water oxygen) varies less than expected with environmental temperature, perhaps because turtles regulate their body temperatures within a narrow window behaviorally, or because bone growth occurs predominantly within a narrow thermal window.

Physiology affects the $\delta^{18}O$ value of body water by altering the magnitude of fluxes of oxygen into and out of the body, as well as fractionations associated with transport and/or transformation of oxygen-bearing compounds. Major oxygen fluxes into terrestrial mammals include drinking and diet water (>50%), which are not fractionated during uptake, and inhalation of atmospheric oxygen gas (ca. 25%) and water vapor (ca. 15%), which undergo isotopic fractionation during diffusion across the lung lining. Fluxes of

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oxygen out of the body include respired carbon dioxide (ca. 25%), water and organic matter in feces and urine (ca. 40%), and water lost during sweating, transcutaneous evaporation, and exhalation (ca. 35%). Oxygen in respired carbon dioxide and water lost by exhalation or transcutaneous evaporation are fractionated relative to body water. For aquatic mammals, ca. 98 percent of the oxygen flux into the animal may come from diffusion of water across the skin, and fluxes out of the body are fewer in number and less subject to strong fractionation because of high humidity. These physiological factors are relatively constant within species, leading to taxon-specific relationships between the $\delta^{18}O$ value of ingested water and body water that are somewhat predictable from body size, taxonomy, or habitat, and that have been successfully approximated with mass balance models of varying degrees of complexity.

Oxygen in proteins

I am aware of only one paper and two abstracts examining oxygen isotope variations in vertebrate protein (deHart & Wooller 2004; Hobson et al. 2004; Wooller & O'Brien 2004). As such, basic physiological controls remain to be explored. One of the oxygen atoms in the carboxyl group of amino acids can be supplied by body water via hydrolysis of the peptide bonds that link amino acids in proteins. Oxygen also occurs in carboxyl, hydroxyl, and amide groups in the R-groups of some non-essential amino acids (e.g., serine, tyrosine, aspartate/asparagines, glutamate/glutamine, hydroxyproline) and one essential amino acid (threonine). Examination of the tricarboxylic acid cycle and amino acid biosynthetic pathways indicates that oxygen can enter these sites from food (largely carbohydrate), body water, phosphate, or oxygen gas (D.M. O'Brien, pers. comm.). Experiments on shrimp suggest that muscle protein is most strongly labeled by ambient water oxygen, not food oxygen (Epp et al. 2004). In general, animal proteins seem to be ^{18}O-enriched relative to drinking or ambient water.

Environmental controls on oxygen isotopes

Environmental factors that shift the $\delta^{18}O$ value of vertebrate body water will impact the $\delta^{18}O$ value of biominerals and protein. For terrestrial animals, ingested drinking water is supplied by meteoric water. Meteoric water varies in $\delta^{18}O$ value geographically and temporally, with higher values in warm regions or seasons, and lower values in colder regions or seasons. Larger reservoirs of water will exhibit damped (or no) seasonal fluctuations. In addition, evaporation will lead to progressive ^{18}O-enrichment of environmental water. Vertebrates also get substantial amounts of oxygen from water in food. Water in stems has a $\delta^{18}O$ value relatively close to that of meteoric water. In contrast, the water in leaves may be highly enriched in ^{18}O relative to

meteoric water due to evapotranspiration, with increasing enrichment with decreasing relative humidity. Because of differences in water-use efficiency and anatomy, the $\delta^{18}O$ values of leaf water differ such that C3 dicots < C3 grass < C4 grass (Helliker & Ehleringer 2000). The $\delta^{18}O$ value in leaf water also varies with height in the canopy, with higher $\delta^{18}O$ values higher in the canopy. The flux of oxygen from food dry matter is relatively small, and the $\delta^{18}O$ value of atmospheric oxygen gas is globally homogeneous, so neither is a major contributor to environmental variability in vertebrate $\delta^{18}O$ values. Finally, as mentioned above, the oxygen flux in aquatic vertebrates is strongly dominated by environmental water. Consequently, aquatic vertebrate $\delta^{18}O$ values should vary spatially in freshwater systems, due to regional differences in meteoric water, but should be relatively homogeneous in marine vertebrates. Of course, biomineral from marine heterotherms will exhibit variance in $\delta^{18}O$ values related to differences in growth temperature (Thorrold et al. 1997; Vennemann et al. 2001).

In summary, vertebrate $\delta^{18}O$ values will vary with marine vs. freshwater vs. terrestrial habitat use, location, diet, and thermoregulatory and osmoregulatory physiology.

Hydrogen isotopes

Controls on hydrogen isotope variations in vertebrates are discussed briefly in Schwarcz & Schoeninger (1991).

Hydrogen isotopes in organic molecules

To my knowledge, hydrogen from the hydroxyl site in bioapatite has not been used for isotopic analysis. There is a small body of work on hydrogen isotope variations in bone collagen (Cormie et al. 1994a, 1994c; Birchall et al. 2005) and a growing literature on hydrogen isotopes in feathers, hair, and lipids (Estep & Dabrowski 1980; Chamberlain et al. 1997; Hobson & Wassenaar 1997; Hobson et al. 1999; Sharp et al. 2003; Cryan et al. 2004; deHart & Wooller 2004). Pioneering work by Estep & Dabrowski (1980) suggested that the δD value of body tissues was chiefly controlled by the δD value of food dry matter. Subsequent experiments revealed that organic molecules contain exchangeable hydrogen (bonded to oxygen or nitrogen), as well as non-exchangeable hydrogen bonded to carbon (Schimmelmann et al. 1993; Cormie et al. 1994b; Chamberlain et al. 1997; Hobson et al. 1999; Sharp et al. 2003). By equilibration with vapors of known composition, the δD value and amount of non-exchangeable hydrogen can be calculated. Lipids have nearly 100 percent non-exchangeable hydrogen, whereas the amount of non-exchangeable hydrogen in proteins ranges from 75 to 90 percent. Of the non-exchangeable hydrogen in feathers and hair protein, 18–32 percent comes from water, with the remainder coming from food

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(Hobson et al. 1999; Sharp et al. 2003). In the case of collagen, ca. 25 percent of the non-exchangeable hydrogen is present in essential amino acids, and therefore must come from food (Birchall et al. 2005). There are two steps in the tricarboxylic acid cycle where hydrogen from water is added to carbon, and they occur immediately prior to the formation of two key intermediates in amino acid metabolism (α-ketogluterate and oxaloacetate), so labeling of non-exchangeable hydrogen in protein with hydrogen from water is not unexpected. Finally, Birchall et al. (2005) discovered a strong, trophic level hydrogen isotope fractionation in collagen from herbivore/omnivore to carnivore in both terrestrial and aquatic birds and mammals. The cause of this fractionation is unclear.

Environmental controls on hydrogen isotopes

Environmental controls on hydrogen isotopes are similar to those on oxygen isotopes, but generally produce signals of greater magnitude. δD values vary in meteoric water in space and time and evaporation leads to ^{2}H-enrichment of surface waters and leaf water, though of proportionally lower magnitude than for ^{18}O. Leaf water δD values differ depending on plant photosynthetic pathway in some settings, such that C3 < C4 < CAM, probably due to differences in evapotranspiration among the pathways (Ziegler 1989). Differences in leaf tissue δD values follow the same order, but are of much greater magnitude, suggesting differences in water-to-plant isotope fractionation among the pathways (Sternberg 1989; Ziegler 1989).

Vertebrate δD values are primarily used to study location and diet in modern animals, and it should be possible to assess marine vs. freshwater vs. terrestrial habitat use and trophic level.

Calcium isotopes

Measurement of δ^{44}Ca values has become more common with the development of the double-spike method by Skulan et al. (1997), but data are still sparse. Choice of a material for a calcium isotope reference standard is still in progress; a CaF_2 (NIST 915a) has been proposed (Hippler et al. 2003). Once a final decision is made, data from earlier papers that are standardized relative to different substances (e.g., carbonate rock, sea water) will need to be recalibrated. Calcium isotope systematics are reviewed by DePaolo (2004).

Calcium isotopes in biominerals

Calcium in terrestrial vertebrates is supplied chiefly by diet. In marine systems, ingestion of seawater might contribute to the body's calcium budget, though at least for carnivorous marine mammals, water is obtained chiefly from prey body fluids and metabolic water. Soft tissues have the same δ^{44}Ca

value as diet or, in the case of marine invertebrates, as seawater. In contrast, biominerals are ^{44}Ca-depleted relative to diet by 1–1.5‰ (Skulan et al. 1997; Skulan & DePaolo 1999). Progressive ^{44}Ca-depletion with each trophic step has been demonstrated for both marine and terrestrial vertebrates, though this effect may depend on consumption of bone or shell (Clementz et al. 2003a). Inorganic synthesis of calcium carbonate and culturing experiments on foraminifera demonstrate that the fractionation is largely related to mineral precipitation, not an enzymatic or biological transport reaction, but there is disagreement about the physico-chemical mechanism producing fractionation (Gussone et al. 2003; Lemarchand et al. 2004). In addition, there is debate about the extent to which calcium isotope fractionation is sensitive to temperature, and the causes of this sensitivity (Marriott et al. 2004).

Environmental controls on calcium isotopes

Inorganic environmental variation in calcium isotope ratios is low. Most igneous rocks have a value ca. 0‰ (relative to the ultrapure calcite standard of Skulan et al. (1997)) and marine carbonate has a value of +1‰. As a consequence, δ^{44}Ca values in marine food webs are 1‰ higher than those in terrestrial food webs at a similar trophic level.
In summary, vertebrate δ^{44}Ca values can be used to reconstruct trophic level and marine vs. terrestrial resource use.

Strontium isotopes

Controls on strontium isotope variation in mammals were reviewed by Beard & Johnson (2000) and Kohn & Cerling (2002). Similar assumptions and processes apply for other high mass isotope systems in which a stable daughter isotope is produced by radioactive decay of a parent isotope (e.g., the U–Pb and Sm–Nd systems). The strontium isotope system has been applied the most widely to vertebrates, and so will be used as an example here. Finally, the δ notation is sometimes used to report strontium isotope data relative to modern seawater ($^{87}Sr/^{86}Sr_{\text{sea water}} = 0.7092$) (Capo et al. 1998), or the ε notation is used, where relative to $\varepsilon^{87}Sr = ([^{87}Sr/^{86}Sr_{\text{sample}}]/[^{87}Sr/^{86}Sr_{\text{bulk earth}}] - 1) \times 10^4$ and $^{87}Sr/^{86}Sr_{\text{bulk earth}} = 0.7045$. More often workers just report the $^{87}Sr/^{86}Sr$ ratio; we follow that convention here.

Strontium isotopes in biominerals

To date, there is no evidence for fractionation of strontium isotopes by biochemical processes, so the isotopic composition of a biomineral is assumed to be identical to that of the source of strontium and is passed up the food chain without modification.

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Environmental controls on strontium isotopes

Continental rocks exhibit a large range in $^{87}Sr/^{86}Sr$ ratios that varies with rock type and age (average for rock type 0.702 to 0.716) (see Capo et al. (1998) for a review of controls on strontium isotopes in ecosystems). ^{87}Sr is produced by radioactive decay of ^{87}Rb. Older rocks with high initial Rb/Sr ratios (e.g., continental granites) have the highest $^{87}Sr/^{86}Sr$ ratios, whereas younger rocks, or rocks with low Rb concentrations (e.g., limestones, basalts) have lower ratios. Soil $^{87}Sr/^{86}Sr$ ratios are controlled by bedrock and by atmospheric deposition of strontium as dust and precipitation. Plants have $^{87}Sr/^{86}Sr$ ratios that match those of the soluble or available strontium in soils. Because of differences in rooting depth, and differences in the isotopic composition within soil weathering profiles, different plants within an ecosystem may have different $^{87}Sr/^{86}Sr$ ratios. The $^{87}Sr/^{86}Sr$ ratio of modern seawater (0.7092) is globally uniform because the residence time of marine strontium is much longer than the time required for oceanic mixing. Since the origin of vertebrates in the Cambrian, the seawater $^{87}Sr/^{86}Sr$ ratio has fluctuated between 0.7095 and 0.707 due to differences in continental weathering and hydrothermal alteration of oceanic basalt. Finally, $^{87}Sr/^{86}Sr$ ratios in estuaries are controlled by mixing. Strontium concentrations are much lower in freshwater than in seawater, so $^{87}Sr/^{86}Sr$ ratios of estuarine waters are quickly dominated by marine inputs (Bryant et al. 1995)

Strontium isotope data can be used to study location, marine vs. terrestrial vs. freshwater foraging, and perhaps diet if there are persistent strontium isotope differences among local plant types.

Preservation of biogenic isotope compositions by vertebrate fossils

Preservation of biogenic compositions is a prerequisite for most isotopic studies of fossil vertebrates. The subject has been contentious, with substantial disagreement among different research communities about the reliability of different tissues. In addition, preservation varies with mineralogy, tissue type, element, and depositional environment, so generalizations are hard to come by. The discussion below is based largely on my own research and reviews (Koch et al. 1994, 1997, 2001), as well as the recent review in Kohn & Cerling (2002).

Preservation of biogenic isotope compositions in biominerals

At least five post-mortem processes may lead to isotopic alteration of vertebrate biominerals.

1 Precipitation of secondary minerals in and around biogenic crystals. If diagenetic minerals have a different chemistry or crystallography than the

biomineral, it may be possible to isolate unaltered mineral through pretreatment. In bones, infilling by secondary minerals may occur rapidly, even sub-aerially, and may be facilitated by the high porosity of bone and the rapid loss of the organic matrix (Nielsen-Marsh & Hedges 2000; Trueman et al. 2004). Furthermore, small poorly organized bone crystals undergo simultaneous dissolution and growth, such that large crystals grow at the expense of smaller ones. The new apatite may have isotope compositions unlike biogenic values due to incorporation of pore fluid ions at surface temperatures and it may be difficult or impossible to isolate bioapatite under such conditions.

2 Adsorption of ions at sites on the surface of crystals or in poorly organized hydration layers around crystals. This process affects even modern samples during preparation for isotopic analysis. Adsorbed ions may be released by leaching.

3 Solid-state exchange (i.e., diffusion of ions into the mineral lattice). For most crystallized materials, this process is too slow at Earth surface conditions to permit substantial alteration. The situation for bone is more complex, however.

4 Exchange of ions or atoms at lattice sites exposed on crystal surfaces. An exchange process of this sort may occur commonly in fossilized biominerals, and it may be facilitated by microbial activity (Zazzo et al. 2004a). For many minerals, surface-to-volume ratios are low, so this process cannot lead to wholesale resetting of biogenic isotope values. However, bone crystals are very small (only a few unit cells thick) with an extremely large surface area. Consequently surface exchange and even solid-state diffusion over very small distances could completely reset biogenic isotope values in bone mineral. It is not possible to strip away diagenetic mineral in this situation, so isotopic preservation is only possible if the ions in the fully exchanged bone came from the bone itself (not the sediments) and if there is minimal isotopic fractionation during exchange. This may explain why oxygen isotope analysis of fossil bone calcium and phosphate oxygen isotopes sometimes yields reliable biogenic isotope values.

5 Wholesale resetting by dissolution/reprecipitation or recrystallization. If the original biomineral is completely lost and replaced either by a similar mineral or an entirely different mineral, there is little hope that biogenic isotope values are preserved. Such wholesale resetting is typically obvious mineralogically (i.e., the transformation of aragonitic otoliths into calcite), crystallographically, or optically.

Kohn & Cerling (2002) discussed six types of tests used to assess isotopic preservation in bioapatite. They include: retention of expected levels of multi-sample isotopic variation within or among individuals; retention of expected isotopic differences among sympatric species; retention of expected isotopic differences among different tissues from the same specimen;

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retention of expected isotopic differences among different ions from the same tissue; retention of original crystallinity; and retention of biogenic isotope values following deposition in a sedimentary environment with very different values.

Some generalizations have emerged about isotopic preservation in biominerals from these studies.

1 Enamel is much more likely to retain biogenic isotope values than bone or dentin. Bone $^{87}Sr/^{86}Sr$ ratios and $\delta^{18}O$ and $\delta^{13}C$ values from bone carbonate are often completely reset, even on Holocene–Pleistocene time scales (Hoppe et al. 2003). Enamel has been shown to carry biogenic values for these systems at least to the early Cenozoic, and perhaps to the Triassic (Botha et al. 2005), but even enamel is not completely closed to exchange and alteration. These conclusions from empirical studies of fossils are supported by experimental work showing extremely rapid exchange of bone carbonate and phosphate oxygen with pore solutions (Zazzo et al. 2004a). There may be settings where biogenic isotope values survive for these isotope systems if diagenetic processes impacting bone bioapatite lock in original values (Lee-Thorp & Sponheimer 2003). Likewise, bone phosphate sometimes retains biogenic $\delta^{18}O$ values. Still, for any isotopic study of bone bioapatite, preservation must be carefully demonstrated on a case-by-case basis; it cannot be assumed. In the very limited tests conducted to date, bone seems to carry biogenic $\delta^{44}Ca$ values in Cenozoic- and Cretaceous-aged specimens (Skulan et al. 1997; Clementz et al. 2003a).

2 With respect to oxygen in bioapatite, the long-standing assumption that phosphate is a more reliable substrate than carbonate may not be valid. This assumption is based on bond strength differences between the two ions. Experiments show that while phosphate is more resilient to inorganic isotope alteration than carbonate, when microbes are involved in the alteration process, phosphate oxygen alters much more rapidly than carbonate oxygen (Zazzo et al. 2004a). Zazzo et al. (2004b) offered a clever (albeit labor intensive) method for determining which type of alteration (if any) has occurred and for correcting back to biogenic oxygen isotope values in bioapatite.

3 At present, there are no crystallographic or chemical analyses that provide an unambiguous independent test of isotopic fidelity in bioapatite or eggshell calcite.

4 With respect to teleost otoliths, it has long been assumed that if original, aragonitic mineralogy is preserved, biogenic isotope values are as well. Reasonable isotopic data have been retained by aragonitic otoliths at least to the Jurassic (Patterson 1999).

5 With respect to eggshell carbonate, $\delta^{13}C$ values show mean values and trends congruent with those for enamel bioapatite and soil carbonate in specimens as old as the Miocene (Stern et al. 1994), and Cretaceous-aged shells yield plausible $\delta^{13}C$ values.

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Preservation of biogenic isotope compositions in organic fossils

Post-mortem processes may alter isotopic values in proteins extracted from fossils. Since different amino acids in a protein have different isotopic compositions, if hydrolysis and amino acid loss are non-random, the isotopic composition of residual protein in bone may be shifted relative to that of unaltered protein. In addition, most tissues contain multiple proteins that may degrade at different rates, yet extraction protocols lump them as a single sample. For example, while collagen is the dominant protein in bone, non-collagenous proteins (albumin, osteocalcin) do occur. These non-collagenous proteins may have different isotopic compositions, thus isotopic trends may occur if diagenesis alters the proportions of proteins in bones. Bacterial or fungal proteins may be introduced during weathering and alter the isotopic composition of protein extracted from bones. Finally, amino acids and protein fragments may condense with exogenous organic matter (e.g., humic substances) during decomposition.

If chemical integrity can be demonstrated, then bulk protein from fossils is likely to yield biogenic isotope values. The simplest indicator is protein yield. Workers have noted anomalous isotope values when yield drops too low and developed rules-of-thumb based on these observations. The most common indicator is demonstration that the molar or atomic ratio of carbon to nitrogen (C/N) in the residue is biogenic or nearly so (Ambrose 1990). Quantitative amino acid analysis is the most robust indicator of chemical integrity (Tuross et al. 1988; Macko et al. 1999). If a bulk extract has biogenic amino acid abundances, then it is likely to have biogenic isotope values, as long as the materials subject to isotopic and amino acid analysis are identical. We note this last caveat because if humic substances are not removed from a bulk extract, even an extract with collagenous amino acid abundances, humics may contaminate the isotopic analysis but not the amino acid analysis. Finally, with respect to isotopic analysis of individual amino acids, the chief concern is contamination by sources from outside the fossil. Well-preserved collagen is common in many settings from Holocene-aged fossils, but becomes increasingly more rare with increasing age. Well-preserved collagen is present in Arctic fossils up to 100,000 years old, and is more sporadically present in older Pleistocene fossils. Claims of good collagen preservation in fossils much older than 100,000 years must be supported by stronger verification and testing.

Few studies have examined the state of preservation of lipids in fossil vertebrates. Most fatty acids are lost from bones quickly in sub-aerial and burial environments (Koch et al. 2001). In contrast, archaeological bone contains enough cholesterol for compound-specific isotope analysis (Stott et al. 1999), and cholesterol may be preserved in 100,000 year old Stellar sea cow bones (Clementz et al. 2003c). Cholesterol may degrade by reduction and oxidation, but if its presence can be demonstrated, then it was derived

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from a metazoan and will have biogenic isotope values. The chief concern is that bone cholesterol may be contaminated by cholesterol from saprotrophic organisms.

Paleobiological applications

Paleodietary reconstruction

Paleodietary reconstruction is the most common way that isotopes are used to study ancient vertebrates. The ability to discriminate between consumption of resources from C3 versus C4 food webs by carbon isotope analysis has allowed extensive study of the use of maize and other C4 plants by humans around the globe (van der Merwe 1982; Schwarcz & Schoeninger 1991). It has shown that sympatric camelids, equids, and proboscideans partitioned resources in Pleistocene North America (Connin et al. 1998; Koch et al. 1998; Feranec 2004a), as well as the surprising fact that many horses with high-crowned teeth were browsing, not grazing (MacFadden et al. 1999; Koch et al. 2004) (Table 5.3). Isotopic records from eggshells have revealed the impact of dietary preferences on differential survival of flightless birds in the late Pleistocene of Australia (Miller et al. 2005). Isotopic evidence has shown that australopithecines obtained up to 20 percent of their calories from C4 food webs. Either they ate C4 plant parts (perhaps underground storage organs) or they hunted or scavenged open country animals (Sponheimer & Lee-Thorp 2003). Finally, data from mammal teeth or bird eggshells have been used to examine climatically, atmospherically, or tectonically driven changes in the balance between C3 and C4 plants (MacFadden et al. 1994; Cerling et al. 1997; Koch et al. 2004; Miller et al. 2005). For example, eggshell isotope records have documented the rise to dominance of C4 plants in south Asia in the Miocene (Stern et al. 1994), and the lack of C4 dominance in the Miocene of Namibia (Segalen et al. 2002).

Isotopic studies of paleodiet in terrestrial C3-dominated ecosystems are more rare. In Eurasia, Beringia, and California, carbon and nitrogen isotope differences among Pleistocene herbivores seem to be related to differences in diet, though differences in digestive physiology and secular trends in environmental isotope values must be carefully evaluated as potential contributors to inter-specific differences (Bocherens et al. 1997; Iacumin et al. 1997; Drucker et al. 2003; Richards & Hedges 2003; Coltrain et al. 2004). Surprising results on C3-dominated Pleistocene Eurasian ecosystems come from the work of Bocherens, Drucker, and colleagues. They argued that extinct cave bears were highly herbivorous, whereas other co-occurring bears were more omnivorous (Bocherens et al. 1997). They also examined the diets of Neanderthals and early modern humans in Eurasia (Drucker & Bocherens 2004; Bocherens et al. 2005; Drucker & Henry-Gambier 2005). They argued that

Table 5.3 Carbon isotope values for tooth enamel bioapatite from Quaternary mammals. Data are for Rancholabrean- and Holocene-aged mammals, from Connin et al. (1998), Koch et al. (1998, 2004, unpublished data) and Feranec (2003), and are reported as the mean ± one standard deviation (in units of ‰ relative to VPDB), with the number of samples in parentheses.

		Texas	Florida	Missouri	$\delta^{13}C$†	%C4†
Cuvieronius	Gomphothere	−7.2 (1)	−6.1 (1)		2	97
Mammut	Mastodon	−10.5 ± 0.6 (25)	−11.0 ± 0.9 (41)	−11.4 ± 0.6 (37)	1	90
Mammuthus	Mammoth	−2.4 ± 1.4 (64)	−1.6 ± 1.8 (29)	−1.6 ± 0.4 (6)	0	83
Equus	Horse (inland)	−4.4 ± 1.8 (36)	−4.0 ± 3.2 (14)	−2.0 ± 0.8 (2)	−1	77
Equus	Horse (coastal)	−0.3 ± 0.4 (4)	−5.5 ± 2.7 (10)	n.a.	−2	70
Tapirus	Tapir	−11.2 ± 0.5 (7)	−12.9 ± 0.8 (9)	−12.8 ± 0.8 (2)	−3	63
Mylohyus	Pecarry	−9.9 ± 0.1 (2)	−10.3 ± 1.4 (5)		−4	57
Platygonus	Pecarry	−9.0 ± 0.2 (3)	−8.3 (1)		−5	50
Camelops	Camel	−4.8 ± 4.5 (17)			−6	43
Hemiauchenia	Llama		−8.5 ± 4.4 (25)		−8	30
Paleolama	Llama	−11.1 ± 0.8 (5)	−14.8 (1)		−9	23
*Bison**	Bison	−1.0 ± 1.5 (22)	−0.7 ± 2.5 (8)	−0.7 ± 1.9 (2)	−10	17
Bootherium	Muskox			−11.3 ± 0.2 (5)	−11	10
Cervalces	Stag moose			−12.1 ± 0.3 (2)	−12	3
*Odocoileus**	Deer	−12.2 ± 1.2 (17)	−13.6 ± 1.4 (15)	−14.4 ± 0.3 (2)	−13	−3
Casteroides	Giant beaver	−11.3 (1)				

* Genus survived in North America.

† Percent C4 estimates for given $\delta^{13}C$ values for mammalian bioapatite. Percent C4 was calculated from $\delta^{13}C$ values assuming a diet-to-apatite fractionation of 14‰ and a mixing model, assuming end-member $\delta^{13}C$valuesof −26.5‰ for C3 plants and −11.5‰ for C4 plants in the late Pleistocene (Koch et al. 2004). Enamel $\delta^{13}C$ values below −12.5‰ yield negative percent C4 estimates, and potentially to feeding under a closed canopy forest.

Neanderthals were highly carnivorous, focusing on large open country herbivores, such as mammoths and woolly rhinoceros. Modern humans appeared in Europe around 45,000 years ago. Isotopic data indicate that they too were heavily reliant on large, open country herbivores, offering little support for the hypothesis that diet breadth increased in modern humans in Europe long before the Pleistocene–Holocene boundary (Drucker & Bocherens 2004; Drucker & Henry-Gambier 2005). Work on tropical Miocene and subtropical Eocene C3-dominated ecosystems has revealed resource partitioning among herbivores related to position in the canopy and plant functional group (Grimes et al. 2004; MacFadden & Higgins 2004), and comparisons of dinosaur eggshell isotope and trace element data with sedimentologic and paleosol carbonate data revealed the paleoenvironmental context of the late Cretaceous sites yielding eggshells (Cojan et al. 2003). Finally, Botha et al. (2005) have pushed back the temporal window on paleodietary research to the middle Triassic in their study of tooth enamel from non-mammalian cynodonts (*Cynognathus*, *Diademodon*). Carbon isotope data indicated that, as expected, both taxa fed in a C3-dominated ecosystem. Yet consistent differences between the taxa in both $\delta^{13}C$ and $\delta^{18}O$ values suggested resource or habitat partitioning.

There is a vast archaeological literature attempting to quantify the proportions of marine versus terrestrial foods in human diets using $\delta^{13}C$ and $\delta^{15}N$ values, and a growing body of work using $\delta^{34}S$ values and $^{87}Sr/^{86}Sr$ ratios (e.g., Sealy et al. 1991; Macko et al. 1999; Richards et al. 2003). In a non-archaeological application involving Pleistocene vertebrates, Chamberlain et al. (2005) used $\delta^{13}C$ and $\delta^{15}N$ data to show that a substantial fraction of the California condors from the La Brea tar pits consumed marine mammals (Figure 5.2). In deeper time, Clementz et al. (2003b) used $\delta^{13}C$ values to demonstrate that desmostylians, an extinct group related to elephants and sea cows, foraged on sea grass and kelp, whereas horses, proboscideans, and rhinos from the same marginal marine deposits foraged on C3 land plants.

There is a vast ecological literature examining diet and trophic relationships among modern marine vertebrates using $\delta^{13}C$ and $\delta^{15}N$ values, but work on the diets of ancient marine vertebrates is sparse. Clementz et al. (2003a) analyzed $\delta^{44}Ca$ values in Miocene marine toothed whales, seals, and desmostylians. As expected, high-trophic-level whales had the lowest values and fossil seals were reconstructed as mollusk-feeders (as suggested by morphological analysis), but calcium isotopes were unable to distinguish between herbivory and mollusk-consumption for desmostylians.

Despite the long-recognized promise of compound-specific isotope analysis, it is just now being used to answer paleodietary questions. Fogel & Tuross (2003) measured $\delta^{13}C$ values in essential versus non-essential amino acids from North American humans with and without corn in their diets. C4 carbon from corn labeled non-essential amino acids in humans, but essential amino acids reflected the C3 protein sources available to these people. Corr D2

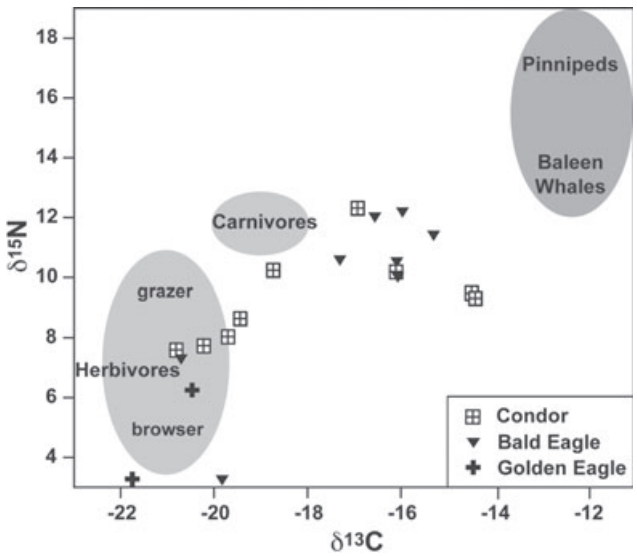

Figure 5.2 Isotopic reconstruction of the dietary preferences of Pleistocene birds from the La Brea tar pits. Estimates of the isotope composition of different dietary resources are provided by the labeled, gray oval fields. Isotope data from bone collagen for bald eagles, golden eagle, and California condors are plotted, after correcting these data to account for trophic level fractionation between diet and collagen. As expected golden eagle diets were strongly terrestrial, whereas bald eagle had a substantial amount of marine food in their diets. California condor diets were variable, but most are so enriched in both ^{13}C and ^{15}N that they must have contained a significant fraction of marine food. Even specialized predation on trapped carnivores (saber toothed cats and dire wolves), would not provide a dietary source with great enough heavy isotope enrichment to explain isotope values from condors.

et al. (2005) tackled the vexing problem of estimating the consumption of marine resources in arid coastal regions with C4 vegetation. In these settings, terrestrial foods have high $\delta^{15}N$ and $\delta^{13}C$ values that overlap values for marine protein. Corr et al. (2005) noted that the $\delta^{13}C$ value of the amino acid glycine is very ^{13}C-enriched in marine foodwebs. In contrast, the $\delta^{13}C$ value of phenylalanine (an essential amino acid) ultimately must track primary producers in marine or terrestrial ecosystems. They calculated a ratio of the $\delta^{13}C$ value of glycine to phenylalanine; this ratio was much higher in marine mammals than in terrestrial C3 or C4 feeders and was also high in Holocene humans from coastal South Africa thought to consume substantial amounts of marine protein. Finally, Clementz et al. (2003c) studied cholesterol in different fossil marine mammals; kelp consumption was apparent in $\delta^{13}C$ values from some fossil sea cows, but was absent from data for fossil whales.

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Paleophysiology

The thermal physiology of dinosaurs has been debated for decades. Possible thermoregulatory strategies include:

1 high metabolic heat production (endothermy) with little variability in body temperature (homeothermy), as in extant birds and mammals;
2 low metabolic rates (ectothermy) with environmentally variable body temperatures (heterothermy), as in many living reptiles and amphibians;
3 mass homeothermy, where thermal inertia keeps body temperature higher and less variable than ambient temperature despite relatively low metabolic heat production;
4 behavioral homeothermy, where animals with low metabolic rates use behavioral traits (exercise, basking, shading, etc.) to maintain body temperatures within a narrower range than environmental temperatures.

Oxygen isotope data have been used to explore thermal physiology in two ways. Barrick & Showers (1994) noted that most endothermic homeotherms maintain temperatures within ±2°C across their entire bodies, whereas temperatures in the extremities of ectothermic heterotherms vary well beyond this range. Assuming that the $\delta^{18}O$ value of body water is constant across an individual, they argued that variations in bioapatite $\delta^{18}O$ values within an individual should reflect differences in body part temperatures. Their key working hypothesis was that an endotherm, with ≤4°C body temperature variability, should exhibit ≤1‰ variability in bone phosphate $\delta^{18}O$ values, whereas an ectothermic heterotherm should exhibit greater thermal and isotopic variability (Stoskopf et al. 2001). To date, they have examined large theropods (*Tyrannosaurus*, *Gigantosaurus*) and several ornithischians (small and large ceratopsians, a small hypsilophodont, and adult and juveniles of a large hadrosaur) (Barrick & Showers 1994, 1995, 1999; Barrick et al. 1996, 1998). Sample size limitations prevent rigorous statistical analysis, but most individuals exhibited within- and between-bone $\delta^{18}O$ variability near the limits of endothermic homeothermy. The preferred hypothesis of Barrick and colleagues is that many of the larger species of dinosaur were functionally homeothermic, but that they had lower metabolic rates than modern mammals or birds ("intermediate metabolism").

When dealing with ancient bone mineral, however, diagenetic homogenization is of great concern, as it would mimic the homeothermic pattern (Kolodny et al. 1996). Barrick & Showers (1994, 1995) measured an infrared index of crystallinity, which provided unambiguous evidence that fossil bones were recrystallized. Patterns of isotopic covariation among different phases (bioapatite phosphate, bioapatite carbonate, and diagenetic calcium carbonate) demonstrated that bioapatite carbonate $\delta^{13}C$ and $\delta^{18}O$ values were reset by recrystallization. Because $\delta^{18}O$ values of bioapatite phosphate and carbonate did not covary, Barrick & Showers (1994, 1995) argued that phosphate oxygen was not completely reset, though some degree of diagenetic

homogenization cannot be precluded. The strongest evidence for isotopic preservation was their demonstration that a small ectothermic lizard had high $\delta^{18}O$ variability, while ornithischians from the same deposits did not (Barrick et al. 1996). If apparently primary isotopic differences are preserved across the skeleton of a small reptile, it is *ad hoc* to propose that fluid flow and exchange have erased differences across much larger dinosaur skeletons. It is more reasonable to conclude that the dinosaurs had little primary $\delta^{18}O$ (and presumably body temperature) variability. Perhaps $\delta^{18}O$ differences across these skeletons were preserved, despite recrystallization, because:

1 the bones were affected by inorganic, rather than bacterially mediated recrystallization;
2 surrounding sediments had little phosphate to exchange with bones;
3 the scale of phosphate diffusion in pore fluids was small, so that phosphate in recrystallized bone had an extremely local source.

If these conjectures are correct, retention of bone phosphate $\delta^{18}O$ values is likely to be site specific and should be carefully monitored on a site-by-site basis.

Fricke & Rogers (2000) assessed thermoregulatory physiology in a different fashion, through isotopic analysis of tooth enamel phosphate from sympatric taxa across a climatic gradient. For both endotherms and ectotherms, they predicted that body water (and hence bioapatite) $\delta^{18}O$ values would partially track meteoric water $\delta^{18}O$ values and drop with increasing latitude (i.e., with decreasing mean annual temperature). Yet oxygen isotope fractionation between body water and bioapatite increases as temperature drops. Therefore in tooth mineral from ectotherms, the drop in body water $\delta^{18}O$ values in cold regions should be offset by increased water-to-mineral fractionation. In endotherms, with a constant body temperature, this offsetting should not occur, and bioapatite $\delta^{18}O$ values should more closely mirror changes in meteoric water $\delta^{18}O$ values. They discovered that Cretaceous theropod dinosaurs showed a greater shift in $\delta^{18}O$ values with latitude than ectothermic crocodiles, implying a greater degree of homeothermy in theropods. In the absence of a comparison to an undisputed endotherm, it is impossible to assess whether theropods show intermediate metabolic rates, as suggested by Barrick and colleagues, or high, mammal/bird-like metabolic rates.

Little work has been done on isotopes as a monitor of osmoregulation and water-use efficiency. In modern mammals and birds, differences in water-use efficiency and evaporative water loss lead to substantial among-species, within-species, and within-individual $\delta^{18}O$ and δD variability (Bocherens et al. 1996; Kohn et al. 1996; McKechnie et al. 2004). Differences in mean and variability in $\delta^{18}O$ value have been used to assess the habitat preferences of fossil vertebrates and will be discussed below. I am not aware, however, of any studies that have used isotopic methods to study questions about the physiology of water use in ancient vertebrates. Similarly, study of modern

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African herbivores has led to the conjecture that differences in urea concentrating mechanisms between obligate vs. non-obligate drinkers might contribute to differences in $\delta^{15}N$ values (Ambrose 1991). Yet this conjecture has never been verified experimentally, and alternate hypotheses exist to explain $\delta^{15}N$ differences among herbivores (Sealy et al. 1987). Again, I am aware of no attempts to use this approach to study the physiology of extinct vertebrates.

Despite evidence that nutritional stress, particularly protein deficient diets, might alter the $\delta^{15}N$ values of vertebrates, I am aware of no studies involving fossil vertebrates in which $\delta^{15}N$ values have been used to assess animal body condition, starvation, etc. Isotopic monitors of diet quality, especially the amount of protein in the diet, have been examined in concert with analyses of individual health and status (based on skeletal or dental metrics) and archaeological grave goods (e.g., Katzenberg et al. 1993; Ambrose et al. 2003). These studies shed light on the way that food availability and quality affect the health and demography of human populations, and how these impacts differ with gender, status and age.

Reproductive biology

Three aspects of reproductive biology have been studied using isotopic methods: nursing/weaning, lactation, and pregnancy. Only the first has been examined in fossil vertebrates. The diets of nursing mammals differ greatly from those of adults. As body water is ^{18}O-enriched relative to ingested water (Bryant & Froelich 1995; Kohn 1996), nursing young should have higher $\delta^{18}O$ values than adults. Very little information is available on calcium isotopes and nursing. A nursing porpoise and an adult porpoise had similar $\delta^{44}Ca$ values, suggesting that the maternal calcium pool used to synthesize milk is controlled by dietary calcium, not bone catabolism (Skulan et al. 1997). With respect to carbon and nitrogen, if lactating mothers catabolize their own tissues to produce milk, isotope values from nursing offspring should look like they are feeding at a trophic level higher than their mothers. For carbon isotopes, this prediction is complicated by the fact that milk is rich in lipids, and lipids are ^{13}C-depleted relative to proteins. Most nitrogen in milk occurs in protein, which is similar in $\delta^{15}N$ value to maternal body tissues, so nursing young should show a 3–5‰ trophic level enrichment relative to their mothers. This effect has been observed in a wide range of extant species (Fogel et al. 1989; Balasse et al. 2001; Jenkins et al. 2001; Polischuk et al. 2001). Thus, nursing offspring should have higher $\delta^{15}N$ values, but either lower or higher $\delta^{13}C$ values than their mothers, depending on the lipid content of the milk.

Nitrogen and carbon isotopes have been used to assess weaning age and the weaning process in prehistoric human populations (e.g., Fogel et al. 1989; Herring et al. 1998; Wright & Schwarcz 1999; Fuller et al. 2003). For example, isotopic and paleodemographic studies yielded the surprising result that

weaning age and birth rates did not change in North America following the introduction of intensive agricultural production (Fogel et al. 1997; Schurr & Powell 2005). In a non-human example, Balasse & Tresset (2002) showed that Neolithic cattle in France were weaned at an earlier age than many modern cattle, perhaps because herders were reserving a greater fraction of the milk for human consumption. Likewise, Newsome et al. (submitted) used $\delta^{15}N$ and $\delta^{13}C$ analysis to study the weaning age of the northern fur seal (*Callorhinus ursinus*), an eared seal that currently follows a strict weaning schedule (4 months) at its high-latitude rookery sites in the Bering Sea and elsewhere. In contrast, ancient northern fur seals from more temperate latitudes along the northeastern Pacific Rim weaned at >12 months, like nearly all other eared seals (Figure 5.3). In a deep-time study, Franz-Odendaal et al. (2003) used $\delta^{18}O$ values from tooth enamel to show that extinct

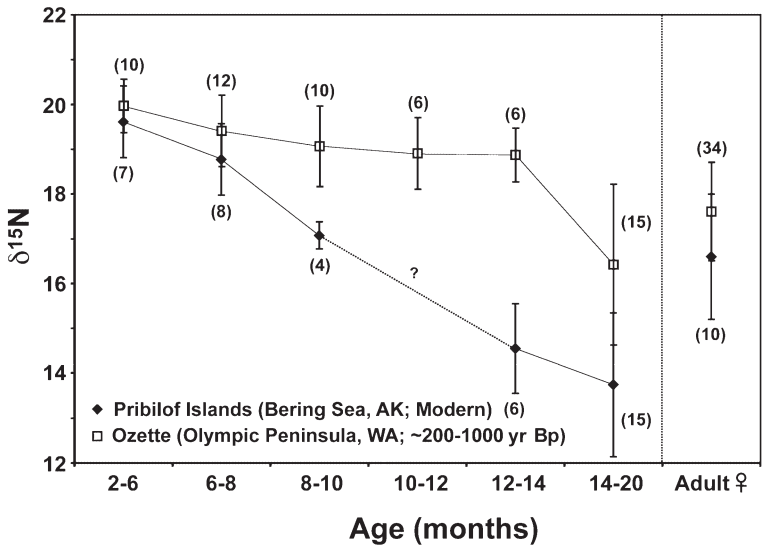

Figure 5.3 Nitrogen isotope evidence from bone collagen for a large change in weaning age for northern fur seals. $\delta^{15}N$ values (mean ± one standard deviation) are presented for animals in different age classes from a modern rookery in the Bering Sea (Pribilof Islands, filled symbol) and a late Holocene population from the Olympic Peninsula (Ozette, open symbol). Pribilof Island fur seals wean at 4 months; $\delta^{15}N$ values drop immediately (6–8 month age class) and are substantially lower for the 8–10 month old animals. In 12–14 month old animals, values are 5‰ lower than in unweaned pups, and are lower than values for adult females from the Pribilofs. Whereas $\delta^{15}N$ values are indistinguishable between Pribilof and Ozette populations for adult females and the youngest age class (2–6 month old animals), Ozette pups retain ^{15}N-enriched values for much longer than Pribilof pups. In Ozette seals, $\delta^{15}N$ values drop significantly only after 12–14 months, suggesting that this extinct population weaned at roughly this age. Numbers in parentheses indicate the number of specimens analyzed in each age class.

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sivatheres (a fossil giraffid) from the Pliocene weaned at the same ontogenetic age as modern giraffes.

The isotopic consequences of lactation and pregnancy are rich areas for further study. While not designed to study patterns in mothers, the early work on human nursing did not uncover an isotopic effect in lactating women (Fogel et al. 1989, 1997). In contrast, a study of wild horses from Shackleford Island, NC, by Koch (1997) showed that lactating females had lower $\delta^{15}N$ values than other adults (males, non-lactating females) and used mass balance calculations to argue that ^{15}N-depletion is the expected result of the nitrogen balance perturbations associated with lactation in this herbivore. Fuller et al. (2004) reported $\delta^{15}N$ and $\delta^{13}C$ variations among pregnant human females. They found no significant effects of pregnancy on $\delta^{13}C$ values, but that $\delta^{15}N$ values dropped from conception to birth, and that the magnitude of the drop correlated to the birth weight of the baby as well as the amount of weight gained by the mother. The physiological mechanisms underlying these patterns are unknown, but likely relate to a proportionally reduced loss of ^{15}N-depleted urinary nitrogen as pregnant females achieve positive nitrogen balance. If these patterns associated with pregnancy and lactation are common among mammals, they offer the potential to study inter-birth interval, neonatal survival rate, and other critical aspects of reproductive biology, at least for Pleistocene and Holocene mammals with good organic preservation.

Habitat preference

For terrestrial animals, isotopic differences among taxa at a site or at different sites are sometimes interpreted as evidence for habitat partitioning or habitat preferences. The case is clearest where habitat is essentially congruent with diet. For example, many late Miocene to Recent fossil sites contain species with diets sourced from both C3 and C4 food webs. If we assume that these sites are not time-averaged, such localities must sample a habitat mosaic, with C3 feeders focusing on woodland/forest habitats, and C4 feeders focusing on grasslands. Cerling et al. (1999) discovered that from 5 to 1 Ma, most lineages of African and south Asian proboscideans (e.g., *Loxodonta*, *Elephas*, *Anancus*, *Stegotetrabelodon*, etc.) foraged in C4 grasslands; the only exceptions were C3-feeding deinotheres. Yet the two surviving modern genera (*Elephas* in Asia, *Loxodonta* in Africa), though opportunistic feeders, show a strong preference for C3 vegetation in forests and woodlands. The cause for this shift in diet and habitat is unclear, but it may relate to increased harassment by human hunters on grasslands. Among C3 feeders, extremely low $\delta^{13}C$ values have been viewed as evidence for foraging in dense forests below a closed canopy (Koch et al. 1998; Kohn et al. 2005; Palombo et al. 2005). Bocherens et al. (1996) showed that modern and fossil hippopotamus have lower $\delta^{18}O$ values than co-occurring terrestrial vertebrates, and speculated that this may reflect

a reduced evaporative water flux due to daytime immersion or consumption of aquatic vegetation. MacFadden (1998) used this approach to test (and falsify) the hypothesis that the Miocene rhinoceros, *Teleoceras*, was aquatic.

Isotopic methods are excellent monitors of habitat preferences in aquatic vertebrates. For example, in fully marine settings, carbon isotope analysis revealed that earlier in the Holocene, northern fur seals foraged offshore, whereas harbor seals foraged close to shore, as these species do today (Burton et al. 2001). Similarity in tooth enamel $\delta^{18}O$ values in Jurassic pycnodont teleosts and sharks (*Asteracanthus*) from sites located at different paleodepths led Lécuyer et al. (2003) to conclude that these taxa lived in warm surface waters. Likewise, Billon-Bruyat et al. (2005) used bioapatite phosphate $\delta^{18}O$ values to estimate temperatures in order to illuminate the ecological and habitat preferences of fish, turtles, and crocodillians from Late Jurassic lithographic limestone deposits in Europe. They reconstructed plesiochelyid turtles as inhabitants of marine environments, making these the first known marine turtles, pre-dating chelonid sea turtles by ten million years.

For fossils from marginal marine deposits, it can be difficult to determine if co-occurring taxa are autochthonous marine species or if they represent a mixed assemblage of terrestrial, freshwater, estuarine, and marine species. Clementz & Koch (2001) analyzed tooth enamel from modern mammals across a gradient from terrestrial to open marine ecosystems. They found that $\delta^{13}C$ and $\delta^{18}O$ values differed among mammals from freshwater, estuarine, kelp, nearshore and offshore marine environments, but that they could not discriminate between terrestrial and some marine systems. The intra-population variance in $\delta^{18}O$ values was substantially lower in aquatic mammals than in terrestrial mammals, however, offering an independent means of discriminating among animals from all these habitats. Clementz et al. (2003b) used these isotopic proxies (as well as $^{87}Sr/^{86}Sr$ ratios) to demonstrate that Miocene desmostylians on the eastern Pacific margin were fully aquatic mammals foraging in estuaries and open marine systems. Tooth enamel $\delta^{13}C$ and $\delta^{18}O$ values suggested that the first sirenians (manatees and dugongs) consumed seagrass in shallow marine settings and estuaries (MacFadden et al. 2004a; Clementz et al. in press). Later lineages diversified to consume macroalgae in open marine water, and then freshwater and terrestrial plants. The earliest known archaeocetes (primitive toothed whales) were also fully aquatic, but in contrast to sirenians, they fed in freshwater ecosystems, and only later invaded marine systems (Roe et al. 1998; Clementz et al. 2006) (Figure 5.4). Finally, Patterson (1999) measured $\delta^{13}C$ and $\delta^{18}O$ values from Jurassic teleost otoliths in Europe and was able to distinguish marine from estuarine taxa.

Migration

Any isotope system that varies spatially has the potential to provide information on animal movement or the movement of animals by other processes

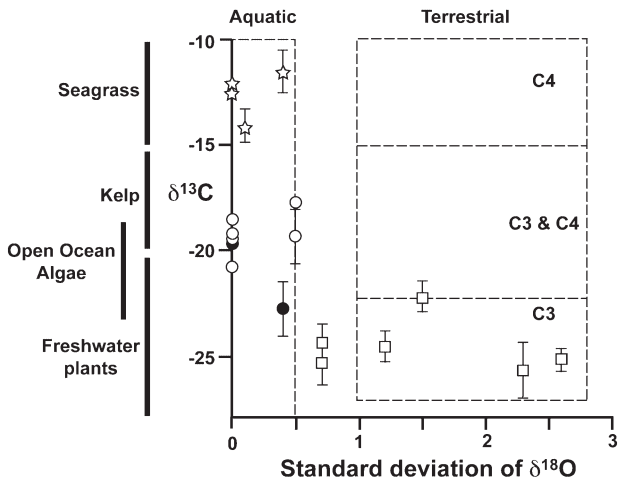

Figure 5.4 Isotopic evidence from tooth enamel carbonate for the paleoecology of Eocene sirenians (sea cows, stars), achaeocetes (archaic toothed whales, circles), and land mammals (squares) from Pakistan, northern Africa, and France. Each symbol represents data from a different species. Among archaeocetes the least derived group, the pakicetids, are marked with filled symbols. Mean $\delta^{13}C$ values (±1 standard deviation) have been converted to dietary isotope values by applying appropriate diet-to-apatite fractionations for carnivores and herbivores. The *x* axis reports the standard deviation for $\delta^{18}O$ values for fossil populations. For symbols plotting along the *y* axis, only single specimens were available, hence population $\delta^{18}O$ standard deviations could not be calculated. Finally, the fields for aquatic versus terrestrial adaptations (on the *x* axis), and the different types of terrestrial and aquatic ecosystems (along the *y* axis) are indicated by boxes and bars. As expected, land animals have highly variable $\delta^{18}O$ values, but $\delta^{13}C$ values indicating consumption of C3 land plants. The low $\delta^{18}O$ standard deviation of Eocene sea cows indicates they were fully aquatic, but very high $\delta^{13}C$ values indicate consumption of sea grass. Pakicetids have invariant $\delta^{18}O$ values, indicating fully aquatic lifestyes, as well as low mean $\delta^{18}O$ values (not plotted) and low $\delta^{13}C$ values, consistent with freshwater habitats. Finally, more derived cetaceans have mean $\delta^{13}C$ values and both mean (not plotted) and variance values for $\delta^{18}O$ that are consistent with life in marine habitats.

(natural and human predators, fluvial transport). There is a vast and growing literature in wildlife biology using isotope variations to study animal migration (Hobson 1999). The key to a successful study is to develop an isotopic map of the region over which animals or animal products might move. Because isotope values are so variable in soils, hydrologic systems, and plants, isotopic maps are often constructed using animals that have a small geographic range (e.g., rodents, rabbits, domestic pigs, etc.) (Sillen et al. 1998; Hoppe et al. 1999; Price et al. 2002; Budd et al. 2004; Hodell et al. 2004). A second key to success is the use of multiple isotopic or elemental tracers. Here, I will focus on studies of migration that use bulk tissue analysis, rather than studies based on isotopic time series from accreted tissues.

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Isotopic research on human movement patterns, or the identification of the proportion of individuals in a skeletal population who are not local, is increasingly common (e.g., Price et al. 1994, 2000; Teschler-Nicola et al. 1999; Ezzo & Price 2002; Budd et al. 2004; White et al. 2004; Knudson et al. 2005; Wright, 2005). Interesting results include the recognition that an immigrant to an oasis along the Nile ca. 1750 yr BP had leprosy, perhaps indicating his exile (Dupras & Schwarcz 2001), that Anglo-Saxon (Scandinavian) immigrants to England from 1600 to 1400 yr BP included both sexes and all ages classes, not just a male military elite (Montgomery et al. 2005), and that first-generation slaves from tropical Africa were present in a late 18th/early19th century burial ground in Cape Town, South Africa (Cox et al. 2001). Isotopic and other data have revealed the place of origin and lifetime movements of the Alpine iceman (Müller et al. 2003).

Studies of migration in other ancient terrestrial and marine vertebrates are much more rare. Hoppe et al. (1999) and Hoppe & Koch (2006) compared $^{87}Sr/^{86}Sr$ ratios, and $\delta^{13}C$ and $\delta^{18}O$ values in mastodons, mammoths, and co-occurring fauna from Pleistocene sites in Florida. They discovered that mastodons in northern Florida were making considerable migrations to the north to feed in forests on sediments sourced from the Appalachians, whereas mammoths across Florida were grazers on sediments derived from platform carbonates in peninsular Florida (Figure 5.5). The scale of movement was hundreds of miles, but not thousands. Hoppe (2004) used the same approach to study mammoth herd structure and movement, as well as the hypothesis that Paleoindians hunted mammoth family groups in the late Pleistocene. The lack of substantial isotopic differences in a suite of dinosaur eggshells led Cojan et al. (2003) to the conclusion that the dinosaurs that laid these eggs did not migrate over substantial distances prior to nesting. Finally, using pinnipeds that do not undertake large-scale migration, Burton & Koch (1999) showed that $\delta^{13}C$ and $\delta^{15}N$ differences at the base of northeast Pacific food webs cascade up to label top marine carnivores. Burton et al. (2001) and Newsome et al. (submitted) verified that this same map applied in the Holocene, then used it to document that northern fur seals from along the California coast were not seasonal migrants from the Bering Sea (site of the current dominant rookery), but instead were sourced by mid-latitude rookeries.

Isotopic time series from accreted tissues

Samples collected in sequence from accreted tissues preserve a time series that can be used to explore aspects of animal biology that vary through ontogeny or with the seasons. These samples are typically collected by micromilling or laser ablation. A first step is to understand the pace and phasing of tissue accretion. Assuming that annual cycles in the $\delta^{18}O$ value of meteoric water are transmitted to body tissues via ingested water, annual cycles have been identified in fossil tusks and ever-growing teeth (Koch et al. 1989;

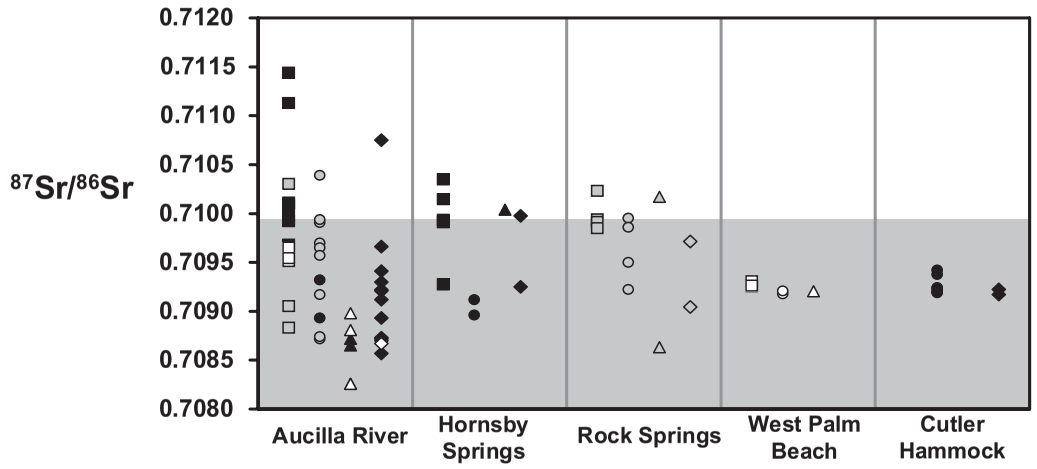

Figure 5.5 Strontium isotope values for bulk tooth enamel for Pleistocene mammals from Florida. Data are presented for mastodons (*Mammut*, squares), mammoths (*Mammuthus*, circles), tapirs (*Tapirus*, triangles), and deer (*Odocoileus*, diamonds). Solid symbols are post-glacial individuals (15,000 to 10,000 yr BP), open symbols are from the Last Glacial Maximum or earlier (15,000 to 70,000 yr BP), and gray-filled symbols are of indeterminate age (Rancholabrean). Aucilla River sites are on the Florida panhandle, Rock Springs and Hornsby Springs are in north-central Florida, and West Palm Beach and Cutler Hammock are in southern Florida. The gray shaded region indicates the range of values seen in different types of environmental samples from Florida, with values on the Plio-Pleistocene platform carbonates in southern and eastern coastal Florida similar to the modern ocean value of 0.7092. Higher isotope values in animals require inputs of strontium from sediments sourced by the Appalachians, to the north in Georgia. All taxa in southern Florida foraged on recent marine geological substrates. At northern sites (especially those along the Aucilla River), some individuals (especially mastodons) have $^{87}Sr/^{86}Sr$ ratios indicating that they foraged part of the year in the Appalachians or their foothills, several hundred miles from the sites where the animals died.

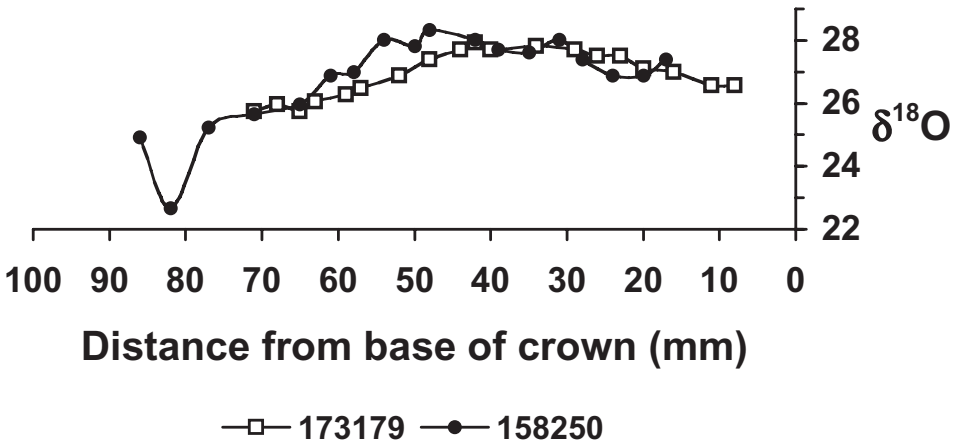

Figure 5.6 Oxygen isotope values for microsamples milled from the canines of two saber-toothed cats (*Smilodon fatalis*) from Rancho La Brea, California (Feranec 2004b). Approximately 1 year of growth is recorded in 80 mm of growth, indicating that the entire tooth crown formed in 18 months.

Stuart-Williams & Schwarcz 1997; Fricke et al. 1998b), enamel on tooth crowns (Bryant et al. 1996; Fricke & O'Neil 1996; Fricke et al. 1998a; Kohn et al. 1998; Sharp & Cerling 1998; Wiedemann et al. 1999; Franz-Odendaal et al. 2003; Botha et al. 2005), the long, blade like teeth of carnivorous mammals and reptiles (Feranec 2004b; Straight et al. 2004) (Figure 5.6), dental batteries in animals with continuously replaced teeth (Thomas & Carlson 2004), and bones with incremental growth features (MacFadden et al. 2004b; Tütken et al. 2004). These oscillations offer a seasonal chronometer against which to correlate other events recorded in the biogeochemistry and mineralogy of teeth and bones.

This approach has shown that incremental features in proboscidean tusks, dinosaur long bones, and shark vertebral centra are indeed annual, allowing estimates of growth rate and, in the case of proboscideans, season-of-death (Koch et al. 1989; MacFadden et al. 2004b; Tütken et al. 2004). Analyses of sheep tooth crowns revealed that there were two lambing seasons on the southwestern coast of South Africa ca. 2000 to 1000 yr BP (Balasse et al. 2003). Isotopic oscillations in time series from animals will be damped relative to environmental cycles due to reservoir effects in the body, bone turnover, and prolonged maturation of tooth enamel. This damping impacts paleoclimatic research (e.g., Dettman et al. 2001; Higgins & MacFadden 2004), but is of less concern if oscillations are used as a chronometer.

Oscillations in otolith $\delta^{18}O$ values, driven by changes in growth temperature and/or the $\delta^{18}O$ value of meteoric water, demarcate annual growth increments in both marine and continental settings (e.g., Patterson 1998; Weidman & Millner 2000). Most studies of isotopic time series from fossil otoliths have

explored paleoclimatic, rather than paleobiologic, questions (e.g., Patterson 1998; Ivany et al. 2000; Wurster & Patterson 2001). An exception is the study by Wurster & Patterson (2003), which examined shifts in metabolic rate implied by changes in $\delta^{13}C$ values from core to rim in Holocene otoliths.

Migration is particularly amenable to analysis via isotopic time series. Koch et al. (1992) examined $^{87}Sr/^{86}Sr$ variations in the vertebral centra of modern and Neogene salmonids to assess their potential as a monitor of freshwater to marine migration. Bone turnover reduced the signal of migration in modern salmonids, and diagenesis completely erased it in fossil bone. Still, the approach can be successful if applied to unaltered fossil otoliths and is currently being developed to study the natal rivers of modern salmonids (Ingram & Weber 1999; Kennedy et al. 2000). An example is the study of $^{87}Sr/^{86}Sr$ ratios and $\delta^{13}C$ and $\delta^{18}O$ values from aragonitic otoliths of the teleost *Vorhisia vulpes* from late Cretaceous estuarine deposits in South Dakota, USA (Carpenter et al. 2003), which showed that fish spawned in brackish water, then migrated in their first year to open marine waters in the Western Interior Seaway. They remained at sea for 3 years before returning to estuaries to spawn and die. An example involving terrestrial vertebrates again comes from the work of Balasse et al. (2002), who measured the $^{87}Sr/^{86}Sr$ ratios and $\delta^{13}C$ and $\delta^{18}O$ values of the teeth of domestic sheep and cows on the southwestern coast of South Africa. They found regular oscillations in $\delta^{13}C$ values, indicating seasonal dietary change, but $^{87}Sr/^{86}Sr$ ratios offered no support for the hypothesis that these herders moved their animals seasonally between the coastal zone and inland regions. Schweissing & Grupe (2003) offered a different approach to time series construction. They examined multiple teeth from the same individual and constructed a time series of $^{87}Sr/^{86}Sr$ ratios by bulk sampling entire tooth crowns, and then estimating and modeling the time of crown formation. They identified at least three different sources for individuals in a late Roman site (ca. 1650–1550 yr BP) in Germany, confirming a hypothesis of population admixture as a result of Roman population policy.

Seasonal or ontogenetic dietary shifts are also amenable to study with isotopic time series, though few studies have been conducted to date. Koch et al. (1995) demonstrated the feasibility of sampling time series from molar roots and uncovered large changes in the diets of some modern elephants through $\delta^{13}C$ analysis. As discussed previously, Balasse et al. (2002) observed subtle $\delta^{13}C$ shifts in Holocene domestic sheep from the southwestern coast of South Africa. Sharp & Cerling (1998) collected $\delta^{13}C$ profiles from tooth enamel from several Pleistocene horses, and detected seasonal and unidirectional shifts in diet. In contrast, in the most comprehensive study of dietary time series, Fox & Fisher (2004) detected no seasonal shifts in the $\delta^{13}C$ values of tusk enamel from Miocene gomphotheres. Similarly, $\delta^{13}C$ data from individual therapsids showed small regular variations, but not in phase with variations in $\delta^{18}O$ values (Botha et al. 2005). Finally, Thomas & Carlson (2004) analyzed an ontogenetic series of teeth from a Cretaceous hadrosaur. They found moderately large (2–4‰) variations in $\delta^{13}C$ values, roughly in

phase with $\delta^{18}O$ variations. However, the very high absolute $\delta^{13}C$ values, which were well outside the range for C3 plants, as well as clear evidence that diagenetically-altered apatite was ^{13}C-enriched, led them to be cautious in interpreting their data as solely reflecting diet.

Conclusions

The explosion of work on modern vertebrates has made life much easier for paleobiologists. New experimental data and extensive field observations increasingly offer a solid foundation for interpreting data from fossil vertebrates. Technological advances have made it possible to analyze large numbers of samples for paleobiological studies, they have opened up new isotope systems and/or new substrates (e.g., calcium in biominerals, oxygen in protein, hydrogen in individual molecules, laser sampling of biomineral oxygen). Many of these new developments have yet to be deployed to answer critical questions in palaebiology. The new methods that rely on organic tissues have not yet been applied in any systematic fashion to the study of Quaternary vertebrates and there is a potential to apply them to deep-time questions in fossils showing exceptional preservation. Studies using biominerals have largely focused on Cenozoic vertebrates, but there are enough successful studies of Mesozoic vertebrates to warrant further exploration. Finally, while paleodiet has received the lion's share of past research, future studies will hopefully fill out the paleobiology of vertebrates and explore physiology, reproduction, migration and habitat use.

A post-script on workshops and literature resources

Progress on the isotope paleobiology of vertebrates has been promoted by workshops that produced books and special issues of journals. The Advanced Seminars on Paleodietary Research provide a venue for exploration of major theoretical and analytical questions concerning chemical and isotopic approaches to hominid paleobiology. Each seminar has had a slightly different focus, emphasizing the most pressing questions facing workers. Experts from outside archaeology and paleoanthropology, including botanists, geochemists, soil scientists, paleontologists, physiologists, and calcified tissue biochemists, have contributed vital, multidisciplinary context. There have been six seminars and associated publications: 1986, Santa Fe, USA (Price 1989); 1989, Cape Town, South Africa (Sillen & Armelagos 1991); 1991, Bad Homburg, Germany (Lambert & Grupe 1993); 1993, Banff, Canada (Ambrose & Katzenberg 2000); 1997, Valbonne, France (Bocherens & Van Klinken 1999); and 2001, Santa Cruz, USA (Koch & Burton 2003). Taphonomy and diagenesis are also pressing concerns for biogeochemical study of vertebrates. The International Workshops on Bone Diagenesis have been the forum for

exploring these issues. There have been four workshops and associated publications: 1988, Oxford, UK (Schwarcz et al. 1989); 1993, Oxford, UK (Hedges & Van Klinken 1995); 1996, Paris, France (Bocherens & Denys 1997–1998), and 2000, Albarracín, Spain (Fernández-Jalvo et al. 2002). Important volumes were produced following the Hal Krueger Memorial Symposium (Ambrose & Krigbaum 2003a) and the symposium on Incremental Growth in Vertebrate Skeletal Tissues (MacFadden 2004).

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