

Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals

MARGARET J. SCHOENINGER* and MICHAEL J. DENIRO**

Department of Earth and Space Sciences, University of California, Los Angeles, California 90024

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Abstract—The stable nitrogen and carbon isotope ratios of bone collagen prepared from more than 100 animals representing 66 species of birds, fish, and mammals are presented. The $\delta^{15}\text{N}$ values of bone collagen from animals that fed exclusively in the marine environment are, on average, 9‰ more positive than those from animals that fed exclusively in the terrestrial environment; ranges for the two groups overlap by less than 1‰. Bone collagen $\delta^{15}\text{N}$ values also serve to separate marine fish from the small number of freshwater fish we analyzed. The bone collagen $\delta^{15}\text{N}$ values of birds and fish that spent part of their life cycles feeding in the marine environment and part in the freshwater environment are intermediate between those of animals that fed exclusively in one or the other system. Further, animals that fed at successive trophic levels in the marine and terrestrial environment are separated, on average, by a 3‰ difference in the $\delta^{15}\text{N}$ values of their bone collagen. Specifically, carnivorous and herbivorous terrestrial animals have mean $\delta^{15}\text{N}$ values for bone collagen of +8.0 and +5.3‰, respectively. Among marine animals, those that fed on fish have a mean $\delta^{15}\text{N}$ value for bone collagen of +16.5‰, whereas those that fed on invertebrates have a mean $\delta^{15}\text{N}$ value of +13.3‰. These results support previous suggestions of a 3‰ enrichment in $\delta^{15}\text{N}$ values at each successively higher trophic level. In contrast to the results for $\delta^{15}\text{N}$ values, the ranges of bone collagen $\delta^{13}\text{C}$ values from marine and terrestrial feeders overlap to a great extent. Additionally, bone collagen $\delta^{13}\text{C}$ values do not reflect the trophic levels at which the animals fed. These results indicate that bone collagen $\delta^{15}\text{N}$ values will be useful in determining relative dependence on marine and terrestrial food sources and in investigating trophic level relationships among different animal species within an ecosystem. This approach should be applicable to animals represented by prehistoric or fossilized bone in which collagen is preserved.

INTRODUCTION

PREVIOUS WORK suggests that the stable nitrogen and carbon isotope ratios of marine plants and animals are different from those of terrestrial plants and animals (DEUSER *et al.*, 1968; DEGENS *et al.*, 1968; WADA *et al.*, 1975; SWEENEY *et al.*, 1978; WADA, 1980; TAUBER, 1981; CHISHOLM *et al.*, 1982). If this is so, animals feeding on marine organisms should have isotopic compositions characteristically different from those feeding on terrestrial organisms because the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of an animal's tissues are determined by the corresponding isotope ratios of its diet (DENIRO and EPSTEIN, 1978, 1981; BENDER *et al.*, 1981; MACKO *et al.*, 1982). Furthermore, the isotopic composition of organisms feeding in either environment should also depend upon their position in the food chain. This follows from suggestions that there is a trophic level effect on the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of animals (MIYAKE and WADA, 1967; WADA and HATTORI, 1976; PANG and NRIAGU, 1977; MCCONNAUGHEY and MCROY, 1979; RAU *et al.*, in press). According to these workers, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values should become more positive as nitrogen and carbon are transferred along the continuum from plants, to herbivores, to primary carnivores, and finally to secondary carnivores. Laboratory experiments indicate that the average $\delta^{15}\text{N}$

and $\delta^{13}\text{C}$ values of animal tissues are, respectively, about 3‰ and 1‰ more positive than those of the animals' diets (DENIRO and EPSTEIN, 1978, 1981; BENDER *et al.*, 1981; MACKO *et al.*, 1982). These observations are consistent with the existence of the proposed trophic level effect for nitrogen isotopes and a small trophic level effect for carbon isotopes as well.

Nevertheless, the hypothesis that marine and terrestrial organisms have different $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios is not strongly supported by the available field data. Although the mean of published $\delta^{15}\text{N}$ values for land plants is 4‰ less positive than the mean for marine plants, the ranges overlap completely (Fig. 1). The same is true for the $\delta^{13}\text{C}$ values in plants. The range of values for terrestrial plants overlaps completely the range for marine plants (DEINES, 1980). In addition, the $\delta^{15}\text{N}$ values of terrestrial animals are overlapped by those for marine animals (Fig. 1). Likewise, in carbon there is considerable overlap between the distributions of $\delta^{13}\text{C}$ values of marine and terrestrial animals (DEGENS, 1969; SCHWARZ, 1969).

The existence of trophic level effects on the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of organisms is suggested, although not strongly supported, by the available field data. Marine and freshwater zooplankton appear to have $\delta^{15}\text{N}$ values that are, on average, 3‰ more positive than associated phytoplankton (MIYAKE and WADA, 1967; WADA and HATTORI, 1976; PANG and NRIAGU, 1977), but there are few data (Fig. 1). Results for animals that feed at higher trophic levels also suggest the existence of an enrichment in ^{15}N in successive trophic levels (HOERING, 1955; MIYAKE and WADA, 1967;

* Permanent address: Department of Cell Biology and Anatomy, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

** Also Archaeology Program.

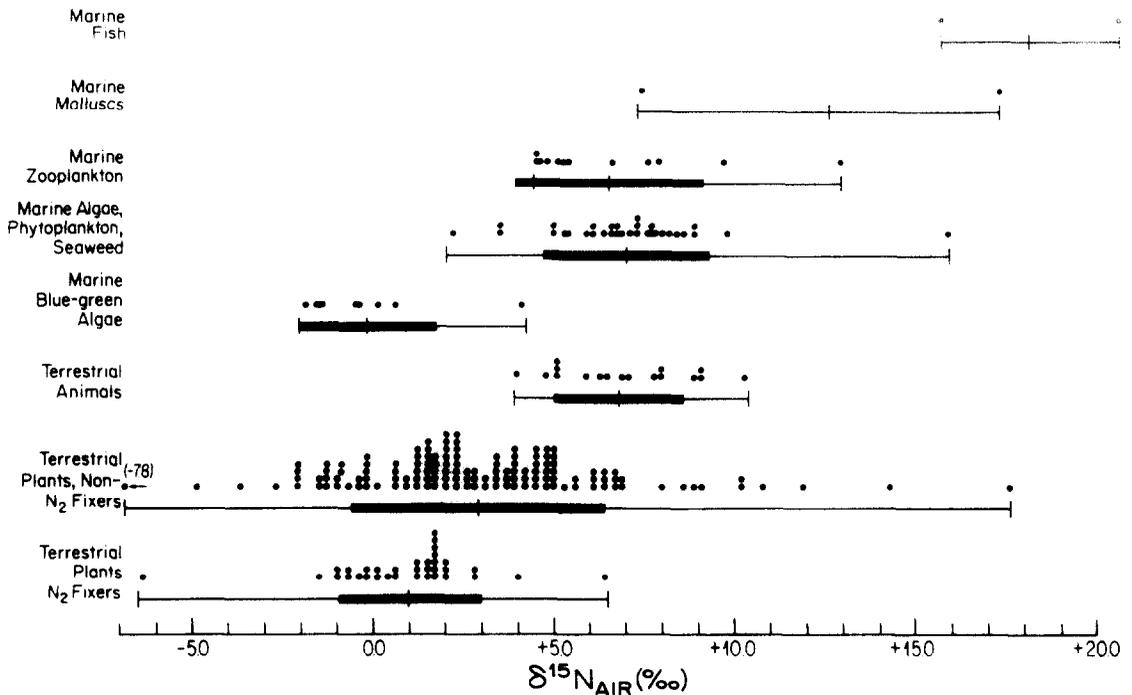


FIG. 1. Published $\delta^{15}\text{N}$ values of whole organic matter of plants and animals from the marine and terrestrial system. Only plants and animals collected in their natural habitats are included. Data are from BARDIN *et al.*, 1977; DELWICHE and STEYN, 1970; DELWICHE *et al.*, 1979; DENIRO and EPSTEIN, 1981; HOERING, 1955; MIYAKE and WADA, 1967; PANG and NRIAGU, 1977; PARWEL *et al.*, 1957; RENNIE *et al.*, 1976; STEELE and DANIEL, 1978; VIRGINIA and DELWICHE, 1982; WADA, 1980; WADA and HATTORI, 1976.

RAU, 1981), but there are even fewer data available for organisms at these higher levels (Fig. 1). In the case of carbon, VOGEL (cited in VAN DER MERWE, 1982) found no difference in $\delta^{13}\text{C}$ values of the same type of tissues from animals feeding at different trophic levels in a terrestrial environment, whereas FRY *et al.* (in press) and MCCONNAUGHEY and MCROY (1979) observed small increases in the ^{13}C content when they compared animals living at successively higher trophic levels in the open ocean. The $\delta^{13}\text{C}$ values of open ocean species from successive trophic levels presented by RAU *et al.* (in press) exhibit the same trend, whereas no consistent trend is obvious in their data for coastal water species.

In this paper, we report the results of an investigation into the effects of marine *versus* terrestrial feeding and of trophic level on the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of animals. Trophic levels within food webs overlap to a large extent (COHEN, 1978) and the most common approach has been to reconstruct feeding strategies of individuals within discrete food webs (MEARNS, 1982). In this project, on the other hand, we analyzed more than one hundred modern fish, birds, and mammals from multiple food webs to determine if their isotopic compositions would reflect effects of marine *versus* terrestrial feeding and of trophic level in a context broader than individual food webs.

We analyzed bone collagen because its isotopic composition reflects that of the animal's diet (DENIRO

and EPSTEIN, 1978, 1981; BENDER *et al.*, 1981), its chemistry is well defined, and its amino acid composition varies only slightly between species (VEIS, 1964). Thus, differences in the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of collagen from different animals should reflect differences in the isotopic composition of their diet and not differences in chemical composition. Additionally, the choice of bone collagen allows analysis of the remains of animals from prehistoric and earlier periods if the bones still contain collagen. Thus, the results of this investigation have important implications for biologists and ecologists interested in vertebrate feeding relationships, for soil chemists and biochemists interested in nitrogen and carbon cycling, and for prehistorians interested in estimating subsistence strategies of prehistoric humans.

MATERIALS AND METHODS

The animals included in this study and their diets are listed in Table 1. These animals represent a wide variety of marine and terrestrial vertebrates and were chosen so that more than one trophic level is represented in each system. Both terrestrial herbivores and terrestrial carnivores were selected, although as is true of their relative abundance in the wild, far fewer carnivores than herbivores were available for analysis. Although only one marine herbivore was sampled, the other marine animals we selected represent two broad trophic levels. The first group will be referred to as primary carnivores in the remainder of the paper. In the context of this project, these are vertebrates that feed on invertebrates including

Table 1. Diet, collection site, and bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for specimens analyzed in this study.

Group	Scientific and Common Names	Diet*	Site**	$\delta^{13}\text{C}(\text{‰})$	$\delta^{15}\text{N}(\text{‰})$	
Marine MAMMALS Order: Carnivora	423. <i>Zalophus californianus</i> Sea lion	fi	SC	-12.9	+17.6	
	424. <i>Zalophus californianus</i> Sea lion	fi	SC	-13.3	+17.2	
	178. <i>Zalophus californianus</i> Sea lion	fi	NC	-11.0	+19.3	
	179. <i>Zalophus californianus</i> Sea lion	fi	NC	-13.3	+23.0	
	428. <i>Phoca vitulina</i> Harbor seal	fi	SC	-11.7	+16.0	
	429. <i>Phoca vitulina</i> Harbor seal	fi	SC	-13.9	+19.3	
	430. <i>Phoca vitulina</i> Harbor seal	fi	—	-11.2	+17.5	
	133. <i>Pagophilus groenlandicus</i> Harp seal	fi	NWE	-15.2	+15.6	
	134. <i>Odobenus rosmarus</i> Walrus	mo	NWE	-14.1	+12.1	
	425. <i>Odobenus rosmarus</i> Walrus	mo	AK	-13.9	+12.5	
	426. <i>Odobenus rosmarus</i> Walrus	mo	AK	-13.6	+12.4	
	445. <i>Enhydra lutris</i> Sea otter	mo	NC	-10.0	+13.9	
	446. <i>Enhydra lutris</i> Sea otter	mo	NC	-9.7	+13.3	
	447. <i>Enhydra lutris</i> Sea otter	mo	NC	-9.6	+15.4	
	Order: Cetacea Suborder: Odontoceti	461. <i>Delphinapterus leucas</i> White whale	fi	CA	-13.9	+15.9
		462. <i>Delphinapterus leucas</i> White whale	fi	AK	-14.2	+17.8
		450. <i>Globicephala macrorhynchus</i> Short-finned pilot whale	fi	SC	-11.9	+16.9
		451. <i>Globicephala macrorhynchus</i> Short-finned pilot whale	fi	SC	-12.3	+16.3
		452. <i>Globicephala macrorhynchus</i> Short-finned pilot whale	fi	SC	-12.0	+15.8
		431. <i>Delphinus delphis</i> Common dolphin	fi	SC	-13.4	+15.5
432. <i>Delphinus delphis</i> Common dolphin		fi	SC	-14.9	+16.5	
433. <i>Delphinus delphis</i> Common dolphin		fi	SC	-13.3	+14.7	
434. <i>Tursiops truncatus</i> Bottle nose dolphin		fi	SC	-11.6	+17.2	
436. <i>Tursiops truncatus</i> Bottle nose dolphin		fi	SC	-13.3	+15.2	
437. <i>Lagenorhynchus obliquidens</i> Pacific white-sided dolphin		fi	SC	-12.7	+14.3	
439. <i>Lagenorhynchus obliquidens</i> Pacific white-sided dolphin		fi	SC	-12.1	+16.0	
440. <i>Lagenorhynchus obliquidens</i> Pacific white-sided dolphin		fi	SC	-11.9	+15.1	
441. <i>Phocoenoides dalli</i> Dall's porpoise		fi	SC	-12.6	+16.5	
442. <i>Phocoenoides dalli</i> Dall's porpoise		fi	SC	-13.5	+14.8	
444. <i>Phocoenoides dalli</i> Dall's porpoise		fi	SC	-12.6	+15.6	
443. <i>Phocoena phocoena</i> Harbor porpoise		fi	SC	-11.6	+16.6	

Table 1. Continued

Group	Scientific and Common Names	Diet*	Site**	$\delta^{13}\text{C}(‰)$	$\delta^{15}\text{N}(‰)$
Marine MAMMALS					
Order: Cetacea					
Suborder: Mysticeti					
	175. <i>Balaenoptera physalus</i> Fin whale	pl,ar,mo	NC	-15.4	+12.7
	457. <i>Balaenoptera acutorostrata</i> Minke whale	pl,ar,mo	SC	-15.4	+13.8
	458. <i>Balaenoptera acutorostrata</i> Minke whale	pl,ar,mo	SC	-12.6	+14.9
	459. <i>Balaenoptera musculus</i> Blue whale	pl,ar,mo	SC	-14.5	+11.7
	460. <i>Balaenoptera musculus</i> Blue whale	pl,ar,mo	SC	-12.6	+15.8
	453. <i>Balaena mysticetus</i> Bowhead whale	pl,ar,mo	AK	-16.1	+14.4
	455. <i>Balaena mysticetus</i> Bowhead whale	pl,ar,mo	AK	-16.4	+15.4
	456. <i>Balaena mysticetus</i> Bowhead whale	pl,ar,mo	AK	-15.3	+13.8
	448. <i>Eschrichtius robustus</i> Gray whale	pl,ar,mo	SC	-13.6	+14.0
	449. <i>Eschrichtius robustus</i> Gray whale	pl,ar,mo	SC	-13.0	+12.0
Marine FISH					
	159. <i>Scorpaenichthys marmoratus</i> Cabezon	mo,ar	SC	-10.0	+14.9
	160. <i>Katsuwonus pelamis</i> Tuna	fi	SC	-14.2	+11.4
	161. <i>Genyonemus lineatus</i> White croaker	mo,ar	SC	-12.1	+12.5
	163. <i>Menticirrhus undulatus</i> California corbina	mo,ar	SC	-11.9	+14.1
	164. <i>Citharichthys xanhostigma</i> Longfin sanddab	mo,ar	SC	-12.7	+14.8
	165. <i>Holocentrus suborbitalia</i> Squirrel fish	mo,ar	SC	-13.6	+14.4
	166. <i>Strongylura exilis</i> Needlefish	fi	SC	-11.1	+16.0
	167. <i>Porichthys notatus</i> Plainfin midshipman	mo,ar	SC	-14.4	+13.7
	168. <i>Hyperprosopon argenteum</i> Walleye surfperch	mo,ar	SC	-12.4	+14.7
	170. <i>Bagre panamensis</i> Catfish	fi	SC	-13.0	+11.1
Reef FISH					
	353. <i>Calamus sp.</i> Porgy	mo,ar	BA	-8.2	+6.6
	354. <i>Haemulon sp.</i> Grunt	pl,ar	BA	-4.9	+6.5
	356. <i>Albula vulpes</i> Bone fish	mo	BA	-4.5	+5.0
	355. <i>Sparisoma sp.</i> Parrot fish	al	BA	-7.8	+3.9
Marine BIRDS					
	157. <i>Ardea herodias</i> Great blue heron	fi	NC	-14.0	+15.7
	158. <i>Pelecanus occidentalis</i> Brown pelican	fi	SC	-13.6	+17.0
	174. <i>Pelecanus occidentalis</i> Brown pelican	fi	SC	-14.5	+17.9
	217. <i>Fulmarus sp.</i> Southern fulmer	mo,ar	FA	-19.6	+11.9
	218. <i>Fregata sp.</i> Frigate bird	fi,mo	YU	-12.1	+12.4
	216. <i>Aptenodytes sp.</i> King penguin	fi,mo	FA	-19.0	+13.0
	220. <i>Eudyptes sp.</i> Macaroni penguin	mo,ar	FA	-18.1	+9.4

Table 1. Continued

Group	Scientific and Common Names	Diet*	Site**	$\delta^{13}\text{C}(\text{‰})$	$\delta^{15}\text{N}(\text{‰})$
Marine BIRDS	221. <i>Pygocelis</i> sp. Gentoo penguin	mo,ar	FA	-14.9	+10.9
	222. <i>Pygocelis</i> sp. Gentoo penguin	mo,ar	FA	-15.6	+10.8
	223. <i>Pygocelis</i> sp. Gentoo penguin	mo,ar	FA	-17.9	+10.3
Anadromous FISH	146. <i>Oncorhynchus nerka</i> Pink salmon	ar	AK	-17.2	+9.6
	147. <i>Oncorhynchus tshawytscha</i> King salmon	fi	AK	-19.3	+12.7
Aquatic Migratory BIRDS	172. <i>Podiceps nigricollis</i> Earred grebe	ar	SC	-26.3	+9.1
	171. <i>Aechmophorus occidentalis</i> Western grebe	fi	SC	-18.6	+14.2
	169. <i>Nycticorax nycticorax</i> Black crown night heron	fi	SC	-20.9	+10.1
Fresh-water FISH	144. <i>Ictalurus nebulosus</i> Catfish	de,fi	MI	-12.7	+8.6
	145. <i>Ictalurus furcatus</i> Catfish	de,fi	TN	-23.6	+6.8
	148. <i>Ictalurus furcatus</i> Catfish	de,fi	MX	-19.6	+8.3
	143. <i>Ictalurus nebulosus</i> Catfish	de,fi	FL	-23.7	+6.6
	149. <i>Salvelinus namaycush</i> Lake trout	fi	MI	-19.1	+9.5
Terrestrial BIRDS	219. <i>Chloephaga picta</i> Magellan goose	gr	FA	-22.5	+6.0
	360. <i>Meleagris gallopavo</i> Domestic turkey	gr	SC	-12.6	+4.9
	641. <i>Buteo lagopus</i> Rough legged hawk	ro	SC	-16.9	+6.8
	648. <i>Tyto alba</i> Barn owl	ro	SC	-21.2	+9.7
Terrestrial MAMMALS	150. <i>Dipodomys</i> sp. Kangaroo rat	ps	SC	-12.4	+8.4
	151. <i>Sciurus</i> sp. Squirrel	ps	SC	-19.9	+2.1
	156. <i>Citellus</i> sp. Squirrel	ps	SC	-19.0	+4.9
	153. <i>Neotoma lepida</i> Wood rat	ps	SC	-11.9	+2.4
	155. <i>Neotoma lepida</i> Wood rat	ps	SC	-16.9	+9.2
	154. <i>Lepus</i> sp. Rabbit	ps	SC	-22.0	+1.9
	699. <i>Lama</i> sp. Llama	ps	PE	-20.6	+5.8
	700. <i>Lama</i> sp. Llama	ps	PE	-20.5	+6.0
	701. <i>Lama</i> sp. Llama	ps	PE	-20.5	+6.5
	702. <i>Lama</i> sp. Llama	ps	PE	-20.2	+6.6
	703. <i>Lama</i> sp. Llama	ps	PE	-20.5	+4.1
	251. <i>Ovis aries</i> Sheep	ps	SY	-20.5	+4.7
	463. <i>Ovis aries</i> Lamb	ps	SC	-20.5	+5.4
	252. <i>Equus</i> sp. Horse	ps	SY	-21.2	+3.4

Table 1. Continued

Group	Scientific and Common Names	Diet*	Site**	$\delta^{13}\text{C}(‰)$	$\delta^{15}\text{N}(‰)$
Terrestrial MAMMALS					
	253. <i>Capra sp.</i> Goat	ps	SY	-20.3	+6.9
	283. <i>Capra sp.</i> Goat	ps	CY	-17.9	+7.3
	254. <i>Bos taurus</i> Cow	ps	SY	-20.6	+3.4
	263. <i>Sus scrofa</i> Pig	ps	—	-14.0	+5.0
	477. <i>Odocoileus hemionus</i> Deer	ps	FL	-22.0	+5.8
	627. <i>Leo tigris</i> Tiger	ma	IN	-18.0	+8.1
	631. <i>Alopex lagopus</i> Arctic fox	ma	AK	-20.6	+5.9
	633. <i>Felis concolor</i> Mountain lion	ma	SC	-17.6	+7.6
	636. <i>Lynx canadensis</i> Bobcat	ma,bi	SC	-15.8	+10.0

*Diets are abbreviated as follows:

al, algae; ar, arthropods; bi, birds; de, detritus; fi, fish; gr, grain; ma, mammals; mo, molluscs; pl, plankton; ps, plants; ro, rodents.

Taken from: BARNHART 1936; MURPHY, 1936; PALMER, 1962; RANDALL, 1968; FITCH, 1969; BAILEY *et al.*, 1970.; FITCH and LAVENBERG, 1975; WALKER, 1975; THRESHER, 1980; SCHREIBER, 1982.

**Sites are abbreviated as follows:

AK, Alaska; BA, Bahamas; CA, Canada; CY, Cyprus; FA, Falkland Islands; FL, Florida; IN, India; MI, Michigan; MX, Mexico; NC, Northern California; NWE, Northwestern Europe; PE, Peru; SC, Southern California; SY, Syria; TN, Tennessee; YU, Yucatan Peninsula.

plankton, arthropods, and molluscs. The animals in the higher broadly defined trophic level will be referred to as secondary carnivores. These are vertebrates that feed on other vertebrates.

Bone samples were cleaned mechanically before collagen was extracted. In the case of very fresh bone, dermestid beetles were used to remove most of the flesh; remaining traces were removed using dissecting scissors and scalpel. We took care to analyze only those museum specimens that had been cleaned in the same fashion. The bones were dried, then powdered to less than 0.71 mm. Collagen was prepared from the bone powder as described previously (DENIRO and EPSTEIN, 1981). One modification greatly simplified collagen preparation. All chemical treatments were performed in a funnel fitted with a coarse fritted-glass filter and attached to a tube which had an affixed stopcock. All chemical treatments involved in collagen preparation were done using this apparatus in conjunction with a filter flask. In this manner, sample transfers were avoided and the risk of losing portions of samples was eliminated.

Collagen samples were combusted using a modified version of the STUMP and FRAZER (1973) method (NORTHELT *et al.*, 1981). The resulting CO_2 and N_2 were separated and purified in a vacuum system by cryogenic distillation prior to determination of their isotope ratios by mass spectrometry. The isotope ratios are reported in the δ notation, where

$$\delta^{13}\text{C} = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right] \times 1000‰$$

$$\delta^{15}\text{N} = \left[\frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} - 1 \right] \times 1000‰.$$

The standard for $\delta^{13}\text{C}$ measurements is the Pee Dee belemnite (PDB) carbonate, while that for $\delta^{15}\text{N}$ measurements is atmospheric (AIR) nitrogen.

The means and standard deviations (1 S.D. values) for 27 analyses of a thiourea standard were $-23.1 \pm 0.3‰$ for $\delta^{13}\text{C}$

values, and $-1.1 \pm 0.2‰$ for $\delta^{15}\text{N}$ values. Analysis of collagen samples prepared from two aliquots of bone powder from each of twelve samples from this and other studies (SCHOENINGER *et al.*, 1983; DENIRO and SCHOENINGER, 1983) produced means and standard deviations (1 S.D. values) of the differences between each pair of $0.1 \pm 0.2‰$ for $\delta^{13}\text{C}$ values and $0.2 \pm 0.3‰$ for $\delta^{15}\text{N}$ values.

RESULTS

Collagen $\delta^{15}\text{N}$ values and $\delta^{13}\text{C}$ values are listed in Table 1. The $\delta^{15}\text{N}$ values and $\delta^{13}\text{C}$ values of bone collagen from all the animals we sampled are plotted in Fig. 2 and Fig. 4 respectively. In these figures, the animals are grouped into the following categories: terrestrial mammals and terrestrial birds (both groups having fed exclusively in the terrestrial system); freshwater fish (lake or river dwellers that fed on detritus when small and on other fish when large); aquatic migratory birds (birds that spent part of the year along the marine coast and the rest of the year on inland lakes); anadromous fish (fish that spent part of their life cycle in the ocean and part in freshwater streams); marine fish, marine birds, and marine mammals (all having fed exclusively in the marine system); and reef fish (from shallow water flats off a Bahamian island). Means and standard deviations (1 S.D. values) and ranges of the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for these different groups of animals are presented in Table 2. These statistics are presented in order to illustrate the variation within each group. No statistical test for difference

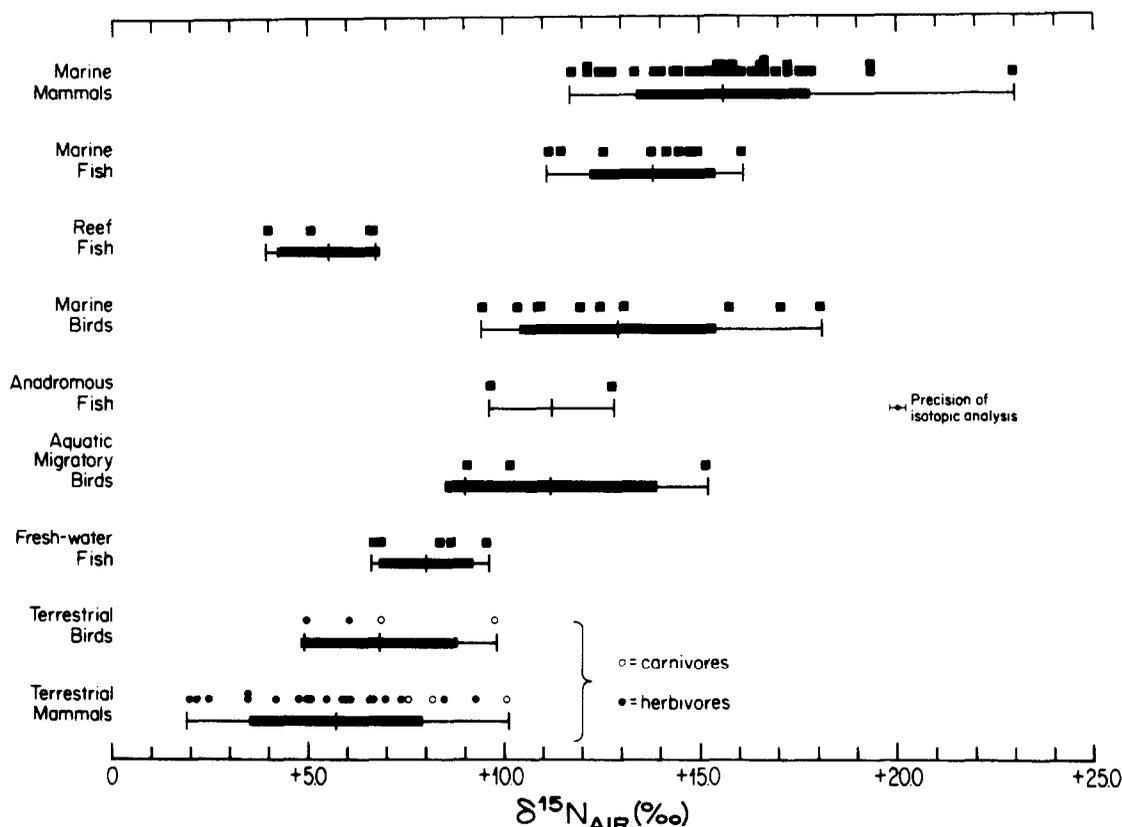


FIG. 2. Bone collagen $\delta^{15}\text{N}$ values. Each point represents a sample from one individual. All animals were collected in their natural habitats. For the indicated groups, the means are given by the central vertical bars, the standard deviations by the heavy horizontal bars, and the ranges by the narrow horizontal bars. The trophic levels occupied by individuals are indicated only for terrestrial animals.

between means was performed because neither the samples that we analyzed nor the populations from which they were drawn can be assumed to be normally distributed. For example, the population of all terrestrial mammals is most likely skewed because there are more species of herbivores than of carnivores. In addition, our samples do not represent randomly drawn samples from all possible populations of any group that is included. Such a sampling strategy was beyond the scope of this project.

The results shown in Fig. 2 and in Tables 1 and 2 indicate that there is a discrete difference between the $\delta^{15}\text{N}$ values of bone collagen from animals feeding in the terrestrial system and those feeding in the marine system. For 41 marine mammals, the lowest $\delta^{15}\text{N}$ value is $+11.7\text{‰}$. Marine fish ($n = 10$) have $\delta^{15}\text{N}$ values that range from $+11.4$ to $+16.0\text{‰}$, and marine birds ($n = 10$) have $\delta^{15}\text{N}$ values that range from $+9.4$ to $+17.9\text{‰}$. In sum, the $\delta^{15}\text{N}$ values of animals feeding exclusively in the marine environment range from $+9.4$ to $+23.0\text{‰}$ with a mean $\delta^{15}\text{N}$ value of $+14.8 \pm 2.5\text{‰}$ ($n = 61$). Among the terrestrial animals (including mammals and birds), on the other hand, the highest $\delta^{15}\text{N}$ value is $+10.0\text{‰}$ and the mean is $+5.9 \pm 2.2\text{‰}$ ($n = 27$). There is only a 1‰ overlap between the ranges for those feeding in the marine and in the terrestrial system; and the mean for marine animals is

almost 9‰ more positive than the mean for terrestrial animals. If only mammals are considered, the range of $\delta^{15}\text{N}$ values for terrestrial mammals is separated from the range for marine mammals by about 2‰ and the means are separated by almost 10‰ . Although we recognize that the mean $\delta^{15}\text{N}$ value for any category is affected by the numbers and types of animals that were selected to make up the category, these differences in means and virtual lack of overlap in ranges of $\delta^{15}\text{N}$ values in such a large variety of animals indicate that a real difference exists in the $\delta^{15}\text{N}$ values of bone collagen from marine and terrestrial vertebrates. The one exception to this statement is provided by the reef fish from the Bahamas (Fig. 2). The mean $\delta^{15}\text{N}$ value for these four fish is $+5.5 \pm 1.3\text{‰}$, which is very close to the mean for terrestrial animals. The basis for the low $\delta^{15}\text{N}$ values in these fish will be discussed below.

Three groups of animals we studied do not fit into the marine/terrestrial classification. The first of these groups includes five freshwater fish, whose mean bone collagen $\delta^{15}\text{N}$ value is $+8.0 \pm 1.2\text{‰}$. These fish are carnivorous throughout most of their life cycles (G. SMITH, pers. commun.), and it is interesting to note that their bone collagen $\delta^{15}\text{N}$ values are the same as those for the six terrestrial carnivores ($\delta^{15}\text{N} = +8.0 \pm 1.6\text{‰}$). The other two groups of animals that do not fit into the marine/terrestrial classification are the

Table 2. Means, standard deviations (± 1 S.D. value), and ranges of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bone collagen from various groupings of animals discussed in the text.

	$\delta^{15}\text{N}$ (‰)		$\delta^{13}\text{C}$ (‰)	
	$\bar{X} \pm \text{S.D.}$	Range	$\bar{X} \pm \text{S.D.}$	Range
Terrestrial MAMMALS (n=23)	+5.7 \pm 2.2	+1.9, +10.0	-18.8 \pm 2.9	-22.0, -11.9
Terrestrial BIRDS (n=4)	+6.8 \pm 2.0	+4.9, +9.7	-18.3 \pm 4.5	-22.5, -12.6
Terrestrial BIRDS and MAMMALS (n=27)	+5.9 \pm 2.2	+1.9, +10.0	-18.8 \pm 3.1	-22.5, -11.9
herbivores	+5.3 \pm 1.9	+1.9, +9.2	-18.9 \pm 2.3	-22.0, -11.9
carnivores	+8.0 \pm 1.6	+5.9, +10.0	-18.3 \pm 2.1	-21.3, -15.8
Freshwater FISH (n=5)	+8.0 \pm 1.2	+6.6, +9.5	-19.7 \pm 4.5	-23.7, -12.7
Aquatic migratory BIRDS (n=3)	+11.1 \pm 2.7	+9.1, +14.2	-21.9 \pm 3.9	-26.3, -18.6
Anadromous FISH (n=2)	+11.1	+9.6, +12.7	-18.2	-19.3, -17.2
Reef FISH (n=4)	+5.5 \pm 1.3	+3.9, +6.6	-6.3 \pm 1.9	-8.2, -4.5
Marine FISH (n=10)	+13.8 \pm 1.6	+11.4, +16.0	-12.5 \pm 1.4	-14.4, -10.0
invertebrate eaters (n=6)	+14.1 \pm 0.9	+12.5, +14.9	-12.2 \pm 1.4	-14.4, -10.0
fish eaters (n=2)	+13.7	+11.4, +16.0	-12.6	-14.2, -11.1
Marine BIRDS (n=10)	+12.9 \pm 2.9	+9.4, +17.9	-15.9 \pm 2.5	-19.6, -12.1
invertebrate eaters (n=4)	+10.3 \pm 0.7	+9.4, +10.9	-16.6 \pm 1.6	-14.9, -18.1
fish eaters (n=6)	+14.6 \pm 2.5	+11.9, +17.9	-15.5 \pm 3.1	-19.6, -12.1
Marine MAMMALS (n=41)	+15.6 \pm 2.2	+11.7, +22.9	-13.1 \pm 1.6	-16.4, -9.6
invertebrate eaters (n=16)	+13.6 \pm 1.3	+11.7, +15.8	-13.5 \pm 2.2	-16.4, -9.6
fish eaters (n=25)	+16.7 \pm 1.8	+14.3, +23.0	-12.8 \pm 1.1	-15.2, -11.0
Marine FISH, BIRDS and MAMMALS (n=61)	+14.8 \pm 2.5	+9.4, +23.0	-13.5 \pm 2.1	-19.6, -9.6
invertebrate eaters	+13.3 \pm 1.7	+9.4, +15.8	-13.7 \pm 2.4	-16.4, -9.6
fish eaters	+16.5 \pm 1.9	+11.4, +23.0	-12.9 \pm 1.2	-19.6, -11.0

aquatic migratory birds and the anadromous fish. The bone collagen $\delta^{15}\text{N}$ values for both groups overlap the marine and freshwater distributions, reflecting their use of food sources in both environments.

In addition to the discrete distributions of bone collagen $\delta^{15}\text{N}$ values for animals feeding exclusively on marine or on terrestrial food sources, there is evidence for a trophic level effect in each environment. As shown in Fig. 2, carnivorous terrestrial animals (2 birds, 4 mammals) have a mean bone collagen $\delta^{15}\text{N}$ value of $+8.0 \pm 1.6\text{‰}$, while herbivorous terrestrial animals (2 birds, 9 mammals) have a mean $\delta^{15}\text{N}$ value of $+5.3 \pm 1.9\text{‰}$. Within the marine system a 3‰ difference between broadly defined trophic levels is also observed in our samples, as shown in Fig. 3. Among marine birds, those that feed on invertebrates (molluscs and arthropods) have a mean $\delta^{15}\text{N}$ value of $+10.3 \pm 0.7\text{‰}$ ($n = 4$) compared with values of $+14.6 \pm 2.5\text{‰}$ ($n = 6$) for those that include fish in their diet. The same difference is observed within marine mammals. Those marine mammals (baleen whales, sea otters, and walrus) that feed on molluscs, arthropods, and plankton have a mean bone collagen $\delta^{15}\text{N}$ value of $+13.6 \pm 1.3\text{‰}$ ($n = 16$), whereas those (toothed whales, seals, and sea lions) that include fish in their diet have a mean $\delta^{15}\text{N}$ value of $+16.7 \pm 1.8\text{‰}$ ($n = 25$). When all marine animals are considered, fish eaters ($n = 30$; 2 fish, 3

birds, 25 mammals) have a mean $\delta^{15}\text{N}$ value of $+16.5 \pm 1.9\text{‰}$, whereas those animals that do not feed on fish ($n = 26$; 6 fish, 4 birds, 16 mammals) have a mean $\delta^{15}\text{N}$ value of $+13.3 \pm 1.7\text{‰}$. In all of the examples cited above, a difference of 3‰ is observed between broadly defined trophic levels.

Results shown in Fig. 4 and in Tables 1 and 2 indicate that bone collagen $\delta^{13}\text{C}$ values also separate animals that fed exclusively in the ocean from those that fed only on terrestrial foods, although the ranges are not as discrete as they are for $\delta^{15}\text{N}$ values. For example, although the mean bone collagen $\delta^{13}\text{C}$ value for marine feeders ($n = 61$) is 5.5‰ less negative than that for terrestrial feeders ($n = 27$), there is an 8‰ overlap in the ranges of $\delta^{13}\text{C}$ values for the two groups. Similar overlaps in the ranges of bone collagen $\delta^{13}\text{C}$ values are observed when terrestrial mammals or birds are compared with marine animals or birds, although in each case the average $\delta^{13}\text{C}$ value for the marine group is less negative than that for the terrestrial group.

Bone collagen $\delta^{13}\text{C}$ values for freshwater fish, for the two groups of animals that fed in both the marine and freshwater systems (aquatic migratory birds and anadromous fish) and for marine birds, have ranges that are virtually indistinguishable from those of terrestrial animals. For those animals that live at least part time in freshwater the reason may be uptake of

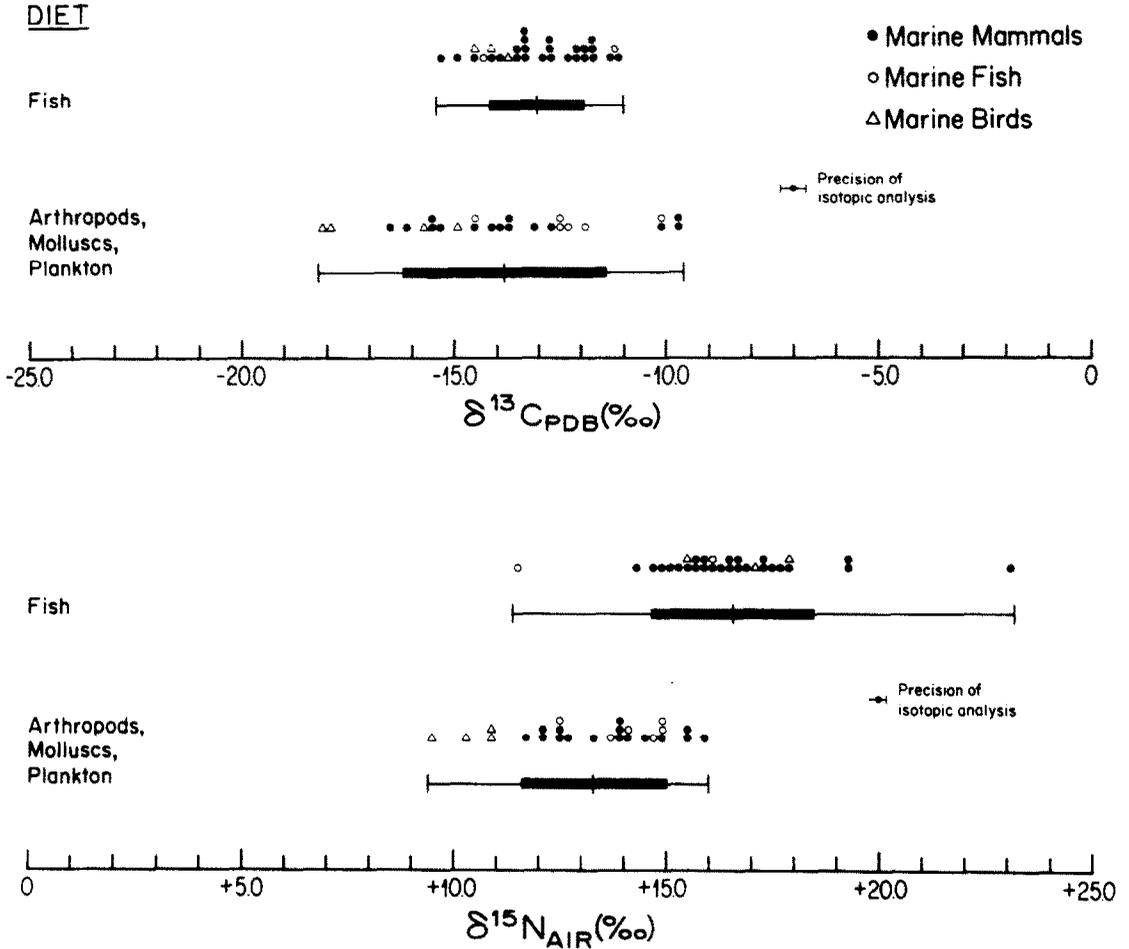


FIG. 3. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of bone collagen from marine animals plotted according to their feeding preferences. The means are given by the central vertical bars, the standard deviations by the heavy horizontal bars, and the ranges by the narrow horizontal bars. Those animals feeding on invertebrates (plankton, molluscs, and arthropods) are separated from those feeding on fish.

terrestrial carbon by freshwater organisms (RAU, 1980; SCHELL, 1983). Since very little terrestrial carbon is transferred above the microbial level in the marine system (SCHELL, 1983), however, input of terrestrial carbon probably does not account for the lack of difference in $\delta^{13}\text{C}$ values that we observed between marine birds and terrestrial animals.

The four reef fish from the Bahamas have bone collagen $\delta^{13}\text{C}$ values that are less negative than those in any other animals included in this study (Fig. 4). The average $\delta^{13}\text{C}$ value of this group is almost 7‰ less negative than the average for marine mammals (see Table 2) and the two ranges are separated by 1‰. As mentioned above, these fish had unique bone collagen $\delta^{15}\text{N}$ values as well. The reasons for their $\delta^{13}\text{C}$ values will be discussed below.

Our results exhibit essentially no trophic level effect on bone collagen $\delta^{13}\text{C}$ values (Figs. 3 and 4). In each case in which animals at successive trophic levels can be compared (terrestrial herbivores and carnivores; primary and secondary carnivores among marine fish, marine birds, or marine mammals), there is a small

(<1‰) increase in bone collagen $\delta^{13}\text{C}$ values going up the food chain, but the ranges for the two trophic levels overlap almost completely.

DISCUSSION AND CONCLUSIONS

Nitrogen isotopic composition of bone collagen

Our results indicate that the $\delta^{15}\text{N}$ values of bone collagen from most marine animals are more positive than those for terrestrial animals. Additionally, we observed that within the different orders of animals we studied (birds, fish, and mammals), the $\delta^{15}\text{N}$ values of bone collagen reflect particular aspects of the feeding regimes.

For example, for the 10 marine birds we studied, the $\delta^{15}\text{N}$ values ranged from +9.4 to +17.9‰. Macaroni and Gentoo penguins, which feed on molluscs and arthropods (MURPHY, 1936), have the lowest values (< +11‰). Brown pelicans and a great blue heron, which feed almost exclusively on fish (PALMER, 1962; SCHREIBER, 1982) have the highest values (> +15‰).

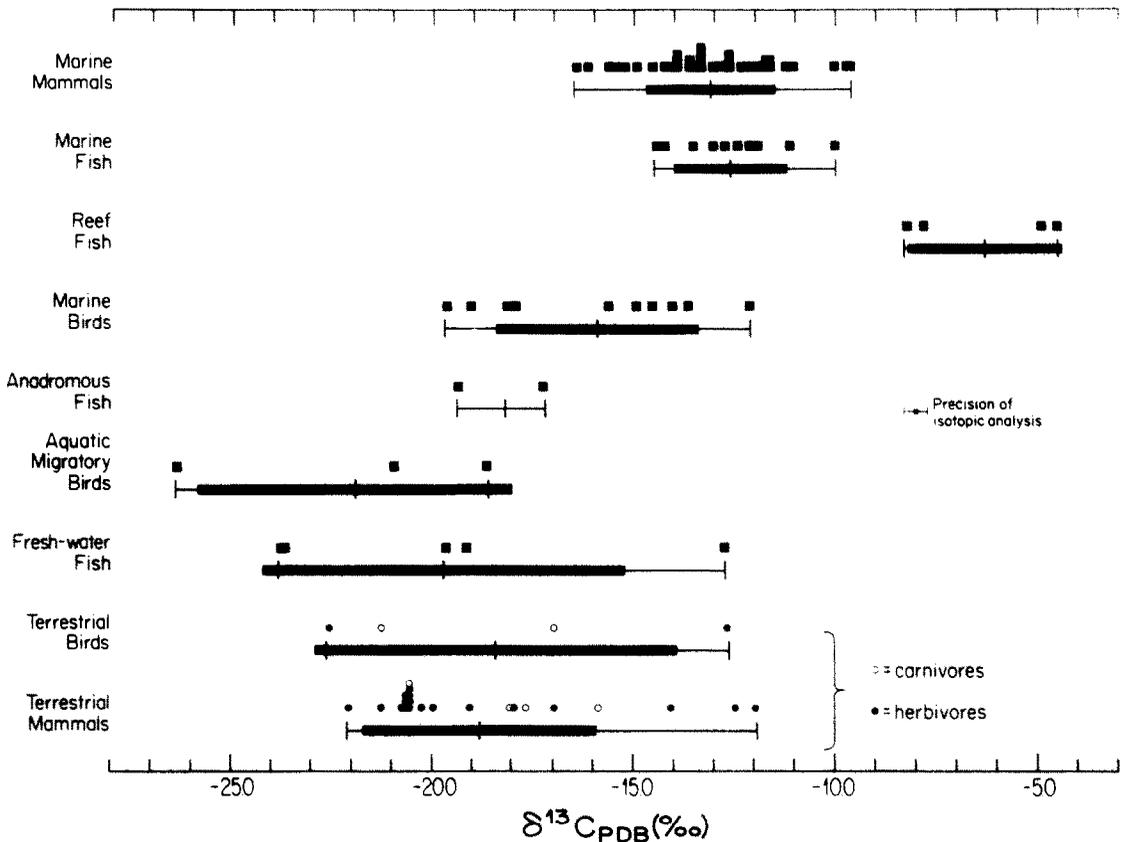


FIG. 4. Bone collagen $\delta^{13}\text{C}$ values. Each point represents a sample from one individual. For the indicated groups, the means are given by the central vertical bars, the standard deviations by the heavy bars, and the ranges by the narrow horizontal bars. The trophic levels occupied by individuals are indicated only for terrestrial animals.

Between these two extremes fall the $\delta^{15}\text{N}$ values for the southern fulmar, frigate bird, and king penguin. These three species feed on both fish and invertebrates (PALMER, 1962).

Further, bone collagen $\delta^{15}\text{N}$ values of the aquatic migratory birds and the anadromous fish appear to be influenced both by the mixed freshwater/marine diets these animals consume and by the position in the food chain each species occupies. Over the course of a year, the migratory birds we studied eat both marine and freshwater fish and invertebrates since they winter along the coast of southern California and then disperse inland for the rest of the year. The $\delta^{15}\text{N}$ values of bone collagen for the 3 species we studied range from +9.1 to +14.2‰, spanning the ranges for pure freshwater and pure marine feeders. The eared grebe, whose diet is composed largely of aquatic and land insects (PALMER, 1962), has the lowest value. The Western grebe, whose diet is composed largely of fish (>80%) (PALMER, 1962), has the highest value. The black crown night heron, whose diet is about half fish and half arthropods (PALMER, 1962), falls in between the two extremes.

The two anadromous fish we studied are a king salmon and a pink salmon. The king salmon has a four to five year life cycle of which three years are spent in the ocean. They feed on arthropods when

small and later they feed on other fish (G. SMITH, pers. commun.). The relatively high $\delta^{15}\text{N}$ values (+12.7‰) reflect this emphasis on marine foods. Pink salmon, on the other hand, have a two to three year life cycle of which only one year is spent in the ocean. They feed on freshwater insects when small and include marine arthropods in their diets when large (G. SMITH, pers. commun.). The lower $\delta^{15}\text{N}$ value (+9.6‰) reflects the shorter time in the ocean and their lower trophic level when compared with the king salmon.

In sum, the distribution of bone collagen $\delta^{15}\text{N}$ values among marine birds, aquatic migratory birds, and anadromous fish reflects each animal's dependence on marine and freshwater foods and also the position of each species in the food chain. The latter serves as further evidence for a trophic level effect on nitrogen isotope ratios of bone collagen.

There is one group of animals whose bone collagen $\delta^{15}\text{N}$ values are not consistent with their dependence on marine food. This is the set of four reef fish from the Bahamas. Their $\delta^{15}\text{N}$ values vary from +3.9 to +6.5‰, which span the middle of the range for terrestrial mammals and are lower than those for other marine fish. We believe the explanation for the low $\delta^{15}\text{N}$ values for these fish lies in the large amount of nitrogen fixation by blue-green algae (STEWART, 1978)

that occurs in coral reefs relative to that which occurs in other parts of the ocean. CAPONE and CARPENTER (1982) reported that the rate of nitrogen fixation in reef systems is 25 g/m²-year compared to 0.1 g/m²-year for the upper 100 m. of the rest of the oceans. Blue-green algae and zooplankton feeding on them have lower $\delta^{15}\text{N}$ values than phytoplankton which do not fix nitrogen and their associated zooplankton (WADA and HATTORI, 1976; WADA, 1980). If the bulk of nitrogen brought into a reef system is that which is fixed by blue-green algae, the relatively low $\delta^{15}\text{N}$ values would be carried up the food chain, thus accounting for the low $\delta^{15}\text{N}$ values observed for reef fish. A detailed study of the ^{15}N distribution in blue-green algae, in the other primary producers, and in the consumers within a reef ecosystem will be required to confirm the explanation we advance here.

Some of our results suggest that bone collagen $\delta^{15}\text{N}$ values may also be affected by metabolic processes. The most positive $\delta^{15}\text{N}$ values among terrestrial herbivores were observed for a kangaroo rat ($\delta^{15}\text{N} = +8.4\%$) and a wood rat ($\delta^{15}\text{N} = +9.2\%$). The kangaroo rat seldom, if ever, drinks water, being able to synthesize water from its dry food. Because of this, kangaroo rats have restricted or delayed nitrogen excretion (SCHMIDT-NIELSEN, 1964; VAUGHN, 1972). Some other rats are able to exist on dry food without water, and are likely to have nitrogen excretion similar to that of the kangaroo rat. This ability varies between populations and species and appears to depend on a particular animal's environment (VAUGHN, 1972). It is possible that these unique metabolic adaptations to xerophytic environments may have some effect on the nitrogen isotope fractionation which occurs during amino acid synthesis (GAEBLER *et al.*, 1966). Perhaps amino acid synthesis in non-drinking desert-adapted animals involves isotopic fractionations different from those occurring in other animals, so that more $\delta^{15}\text{N}$ is incorporated into collagen. It would be interesting to know in this regard if the second wood rat we analyzed, whose bone collagen $\delta^{15}\text{N}$ was $+2.4\%$, came from a less xerophytic environment than the wood rat whose $\delta^{15}\text{N}$ value was $+9.2\%$. Unfortunately this information is not available.

A summary of our results on the $\delta^{15}\text{N}$ values of bone collagen in animals at different trophic levels in the marine and terrestrial systems is shown in Fig. 5. In a simplistic representation of the food chain, we have plotted the means and standard deviations of published values for marine and terrestrial plants at the bottom of the graph. As can be seen from the figure, the standard deviations of the marine and the terrestrial plants overlap. This overlap does not occur between primary carnivores in the two systems. It appears that the small difference in mean $\delta^{15}\text{N}$ values between marine and terrestrial plants is increased as nitrogen moves up the food chains in the two environments. This magnification may be due to selective feeding by animals. In addition, there is at least one more trophic level in the marine system than in the

terrestrial system. In combination, these phenomena appear to account for the observed 9‰ difference in mean $\delta^{15}\text{N}$ values between marine and terrestrial animals even though the means of marine and terrestrial plants are separated by only 4‰. The data shown in Fig. 5 also are consistent with the existence of a 3‰ enrichment in $\delta^{15}\text{N}$ values as nitrogen is transferred between trophic levels. The slopes of the lines drawn through the data for the terrestrial and marine systems indicate enrichments of 2.7‰ and 3.3‰ per trophic level respectively.

Carbon isotopic composition of bone collagen

The $\delta^{13}\text{C}$ values of bone collagen from marine animals are less negative than those from terrestrial animals that we studied. The mean $\delta^{13}\text{C}$ values we observed (Table 2) are close to the values that CHISHOLM *et al.* (1982) predicted should be observed for pure marine feeders (-13%) and pure terrestrial feeders (-20%). The one exception to this observation, the marine birds, will be discussed below.

The ranges of $\delta^{13}\text{C}$ values we obtained for marine or terrestrial feeders, however, are larger than have been reported previously. Whereas previous results indicate that bone collagen $\delta^{13}\text{C}$ values for human populations whose diets were primarily marine or primarily terrestrial do not overlap (TAUBER, 1981; CHISHOLM *et al.*, 1982; SCHOENINGER *et al.*, 1983), the range of $\delta^{13}\text{C}$ values we obtained for terrestrial mammals overlapped the range of $\delta^{13}\text{C}$ values in marine mammals by about 4.5‰ (Table 2). This observation possibly can be explained by considering the diets of the terrestrial animals whose bone collagen $\delta^{13}\text{C}$ values are in the zone of overlap (-16.5 to -12.0%). These include a domestic turkey, a kangaroo rat, a wood rat, a pig, and a lynx. The herbivores may have fed on significant amounts of C_4 plants, since all feed on grass seeds (WALKER, 1975) and many grasses are C_4 plants. The lynx may have fed on herbivores whose diets were composed largely of C_4 plants. The $\delta^{13}\text{C}$ values of C_4 plants are less negative than those of C_3 plants (SMITH and EPSTEIN, 1971; BENDER, 1971). Eating substantial amounts of C_4 plants (in the case of herbivores) or animals that have eaten large amounts of C_4 plants (in the case of carnivores) would produce bone collagen $\delta^{13}\text{C}$ values in the range we observed for the animals in question (DENIRO and EPSTEIN, 1978).

There are two observations which suggest that there may be some metabolic difference in carbon isotope fractionation between birds and mammals. First, birds that fed exclusively on marine foods have bone collagen $\delta^{13}\text{C}$ values that are more negative than those in fish and mammals that also fed on marine foods (Fig. 4). Second, the aquatic migratory birds that fed in both the marine and terrestrial system have bone collagen $\delta^{13}\text{C}$ values that are more negative than those in terrestrial mammals (Fig. 4). Based solely on their feeding regime, we expected that they would have had collagen $\delta^{13}\text{C}$ values intermediate between those for terrestrial

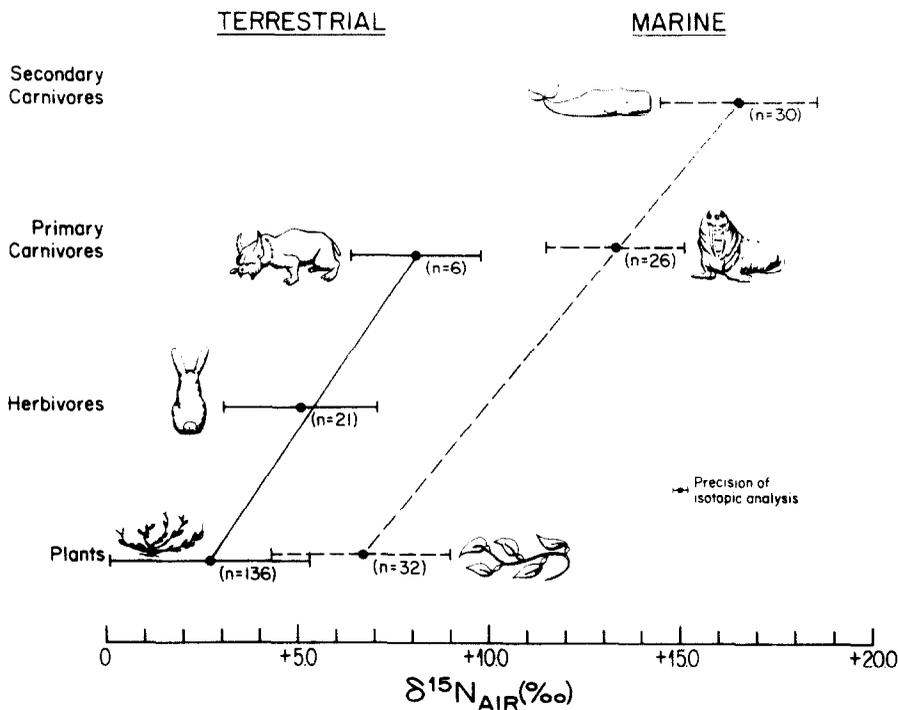


FIG. 5. Means and standard deviations of $\delta^{15}\text{N}$ values of plants (published values) and of bone collagen in animals (this study) in the terrestrial and marine environments. The means are indicated by the central points and the standard deviation by the horizontal bars. Plants at the base of the food chains in both systems are shown at the bottom of the graph. The animals are divided into broad trophic levels. The lines are drawn to connect the mean $\delta^{15}\text{N}$ values for the organisms at the top and bottom of the food chains in each environment. Plants which fix atmospheric nitrogen and animals feeding on them are not included.

animals and marine animals. The number of samples is too small, however, to take these two observations as any more than a suggestion that birds may process carbon isotopes differently than mammals do. Additionally, other explanations may account for some aspects of these observations. For example, the effect of temperature on the $\delta^{13}\text{C}$ values of plankton (RAU *et al.*, 1982) may explain the observation that the six marine birds with most negative $\delta^{13}\text{C}$ values were collected in the Falkland Islands.

The extreme ^{13}C enrichment observed in the four reef fish (Fig. 4) probably reflects feeding in an area where seagrasses and coral form a significant part of the base of the food chain (D. MILLER, pers. commun.; MEYER *et al.*, 1983). A similar enrichment in tissues of fish from coral reefs and seagrass meadows compared with offshore fish has been reported (FRY *et al.*, 1982 and in press). The basis for this difference is probably related to the enrichment of ^{13}C in shallow water reef building corals (LAND *et al.*, 1975; SWART, 1983) and in seagrasses (BENEDICT *et al.*, 1980).

A summary of our results on the $\delta^{13}\text{C}$ values of bone collagen in animals at different trophic levels in the marine and terrestrial systems is shown in Fig. 6. In contrast to the situation for $\delta^{15}\text{N}$ values (Fig. 5), $\delta^{13}\text{C}$ values of bone collagen do not appear to reflect, to any useful degree, the trophic levels at which animals fed in either environment.

Diet estimation based on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of bone collagen

Based on the results of this study, several types of diet estimation using isotope ratios of bone collagen appear to be feasible.

The $\delta^{15}\text{N}$ values of bone collagen can be used to distinguish some animals that fed exclusively on marine foods from others that fed exclusively on terrestrial foods. In our sample, animals with bone collagen $\delta^{15}\text{N}$ values less than +9‰ fed only on terrestrial foods; those with bone collagen $\delta^{15}\text{N}$ values greater than +15‰ fed only on marine foods. In cases where the bone collagen $\delta^{15}\text{N}$ values fall between +9 and +15‰, other information on living habits must be used in conjunction with the isotope data in order to identify food sources. Two mammals and one bird out of 25 in our sample of terrestrial animals have bone collagen $\delta^{15}\text{N}$ values in this range and, thus, could not have been identified as animals who fed strictly on terrestrial foods. Of these three, two are carnivores and one is an animal that lived in a xerophytic environment. Morphological analysis would clarify two of the three cases since carnivory in mammals is often reflected by the shape of the posterior teeth in the mouth. Among the marine animals in our sample, almost 50% had $\delta^{15}\text{N}$ values between +9 and +16‰ and thus could not be identified as having fed on marine foods based

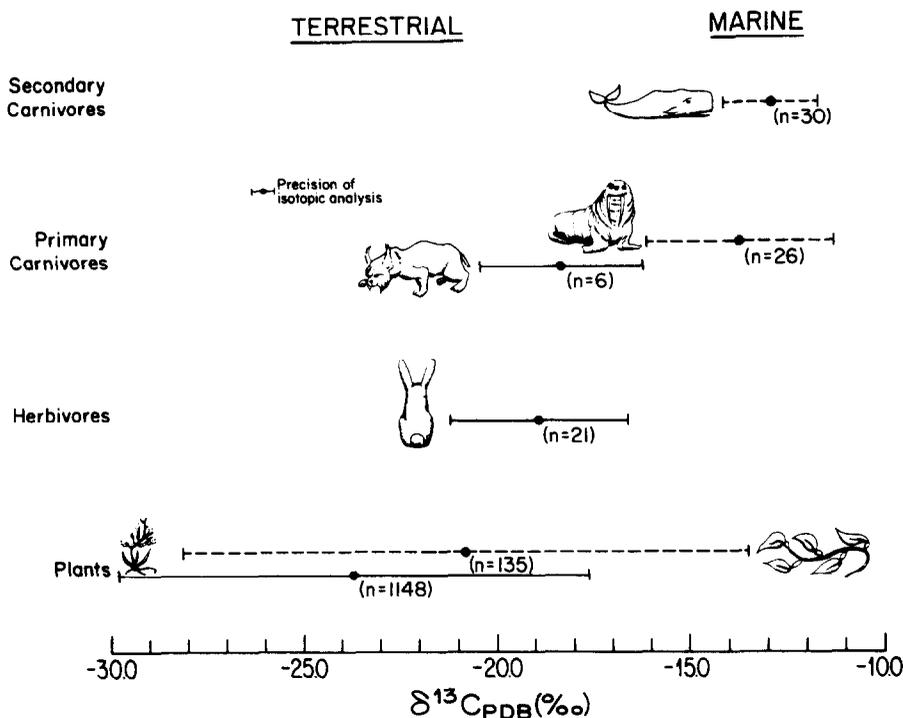


FIG. 6. Means and standard deviations of $\delta^{13}C$ values of plants (DEINES, 1980) and of bone collagen in animals (this study) in the terrestrial and marine environments. The means are indicated by the central points and the standard deviation by the horizontal bars. Plants at the base of the food chains in both systems are shown at the bottom of the graph. The animals are divided into broad trophic levels, as in Fig. 5. The four reef fish are not included for reasons explained in the text.

solely on bone collagen $\delta^{15}N$ values. Among mammals and fish this would not pose a problem. Since all known mammals and fish that live in the ocean also feed in the ocean, it would be obvious that low $\delta^{15}N$ values in animals identified as marine by morphological criteria are influenced by their trophic levels. Among birds, overall body form could be used in conjunction with bone collagen $\delta^{15}N$ values in order to distinguish between carnivorous birds (*e.g.*, hawks) that feed on terrestrial animals and marine birds (*e.g.*, penguins) that feed on marine invertebrates. In cases in which the animals are known to use both marine and terrestrial food sources, it should be possible to use $\delta^{15}N$ values of bone collagen to estimate general proportions (although not exact percentages) of dependence on marine and terrestrial foods.

The $\delta^{13}C$ values of bone collagen can be used for estimating dependence on marine and terrestrial foods only when there is no possibility of feeding on C_4 plants. In areas where C_3 and C_4 plants are utilized as food sources, those animals feeding on a mixture of marine and terrestrial foods could not be distinguished from those feeding exclusively on terrestrial foods. In geographical areas or within animal populations for which C_4 plants are known to be unimportant (VOGEL, 1978; VAN DER MERWE, 1982), $\delta^{13}C$ values of bone collagen are useful in estimating dependence on marine versus terrestrial food sources (TAUBER, 1981; CHISHOLM *et al.*, 1982; SCHOENINGER *et al.*, 1983).

In the case of vertebrates living in tropical areas surrounded by reef systems, an exceptional combination of bone collagen $\delta^{13}C$ values (greater than -9‰) and bone collagen $\delta^{15}N$ values (less than $+7\text{‰}$) should serve as a signature of total dependence on marine foods. As far as is now known, this combination of low $\delta^{15}N$ values and high $\delta^{13}C$ values is unique among animals. Vertebrates feeding solely in the terrestrial system and those feeding in both the marine and terrestrial systems in these areas must be analyzed before further predictions can be made.

A vertebrate's position in a trophic system can be predicted, within limits, for animals feeding only in the marine system or only in the terrestrial system based on bone collagen $\delta^{15}N$ values. Within the marine system, animals in our sample with bone collagen $\delta^{15}N$ values less than $+13\text{‰}$ fed on invertebrates whereas those with $\delta^{15}N$ values greater than $+16.5\text{‰}$ fed on other vertebrates. More terrestrial animals should be analyzed before a similar prediction can be made for the terrestrial system. It would be difficult, however, to distinguish between vertebrates feeding on both marine and terrestrial foods and those feeding only on marine invertebrates based solely on $\delta^{15}N$ values of bone collagen. Other lines of evidence would have to be used if such a situation were a possibility. Our observation that $\delta^{13}C$ values of bone collagen can not be used in predicting feeding positions in trophic systems may be a result of our sampling strategy. We

analyzed individuals from multiple food webs, whereas previous workers reporting a trophic level effect in carbon analyzed individuals from single food webs (McCONNAUGHEY and McROY, 1979; RAU *et al.*, in press).

The types of dietary analysis discussed above for modern animals should also be useful for reconstructing the diets of vertebrates from prehistoric and earlier periods. In such cases, however, postmortem alteration of bone collagen is a possibility that must be considered. Methods for ascertaining whether or not such alteration has occurred must be developed and applied before $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values of bone collagen of prehistoric or paleontologic age can be accepted as representing the values that were present when the animal was alive. With this caveat in mind, we have shown that bone collagen $\delta^{15}\text{N}$ values are useful in estimating marine and terrestrial components of diet in prehistoric humans (SCHOENINGER *et al.*, 1983).

A final comment is in order. The sampling strategy we employed in this study was designed to obtain large numbers of species of animals feeding in the marine and terrestrial environments. Accordingly, we do not have results that allow us to determine with great certainty the variability of bone collagen $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values among different individuals of a species that fed on the same diet. Nevertheless, analysis of the 22 cases in which we have at least two individuals of the same species indicates that the average range of values among individuals of the same species is $1.8 \pm 1.2\%$ for $\delta^{15}\text{N}$ values and $1.9 \pm 2.4\%$ for $\delta^{13}\text{C}$ values. These ranges are about the same as those we obtained from determination of collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from the bones of 15 mink raised on the same diet (DENIRO and SCHOENINGER, 1983). This amount of variability among individuals is small relative to the differences we observed for marine *versus* terrestrial feeding, but is of the same magnitude as the differences between $\delta^{15}\text{N}$ values of organisms feeding on successive trophic levels. Thus, it is likely that in order to differentiate trophic levels based on $\delta^{15}\text{N}$ values of bone collagen, it will be necessary to analyze several individuals from each species being studied. On the other hand, this amount of variability in bone collagen $\delta^{15}\text{N}$ values is small enough so that it should be possible to identify marine *versus* terrestrial feeding preferences in individual animals.

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