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Using stable isotope biogeochemistry to study marine mammal ecology

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ABSTRACT

Stable isotope analysis (SIA) has emerged as a common tool in ecology and has proven especially useful in the study of animal diet, habitat use, movement, and physiology. SIA has been vigorously applied to the study of marine mammals, because most species live in habitats or undergo large migrations/movements that make them difficult to observe. Our review supplies a complete list of published SIA contributions to marine mammal science and highlights informative case examples in four general research areas: (1) physiology and fractionation, (2) foraging ecology and habitat use, (3) ecotoxicology, and (4) historic ecology and paleoecology. We also provide a condensed background of isotopic nomenclature, highlight several physiological considerations important for accurate interpretation of isotopic data, and identify research areas ripe for future growth. Because it is impossible to conduct controlled laboratory experiments on most marine mammal species, future studies in marine mammal ecology must draw on isotopic data collected from other organisms and be cognizant of key assumptions often made in the application of SIA to the study of animal ecology. The review is designed to be accessible to all audiences, from students unfamiliar with SIA to those who have utilized it in published studies.

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Key words: marine mammals, stable isotopes, cetaceans, pinnipeds, trophic level, isotopic turnover.

Over the past decade the number of ecological studies using stable isotopes has grown exponentially and research focused on marine mammals is no exception (Fig. 1). Stable isotope values of carbon, nitrogen, hydrogen, and oxygen are now used routinely to study foraging ecology and trophic status, habitat use, migration, population connectivity, and physiology. Isotopes of other elements, such as sulfur, lead, and strontium, have also been used as sources of ecological information, though not as extensively (reviewed by Hobson 1999, Kelly 2000, Koch 2007). The stable isotope composition of an animal is primarily determined by the isotopic composition of the food, water, and gas that enter its body and from which it makes soft tissues and biological minerals. An animal's isotopic composition, however, is not exactly equal to the mass-weighted isotopic composition of these inputs, because the dissociation energies of molecules are often dependent on the relative mass of the elements from which they are made. The mass-dependent sorting of elements that occurs during many biochemical and physicochemical processes is called isotopic fractionation. Decades of laboratory and field research have revealed patterns produced by isotopic fractionation—both within animals and in their environments—that are useful in the study of ecology and animal physiology.

Our review explores four general categories of study that use stable isotope analysis (SIA) to investigate marine mammal ecology (Table 1). SIA is especially useful for examining diet and trophic level among and within individuals of species. Most marine mammals live in habitats that make them difficult to observe and are extraordinarily mobile and/or move great distances. Nearly half of the papers we found use SIA to study a combination of foraging ecology, habitat use, or migratory patterns. A second major category combines SIA with studies of contaminant concentrations to trace the sources and pathways of toxins such as organochlorides and heavy metals in food webs. A third group of papers addresses physiological issues such as isotopic turnover or the effects of diet, body condition, or reproductive status on isotopic fractionation. Finally, a growing number of studies adopt SIA to investigate marine mammal ecology on historic, archaeological, and paleoecological time scales. We use these major categories to organize our review and end by highlighting a few analytical considerations important for accurate interpretation of isotopic data, as well as a few research areas where we expect substantial advances in coming years.

Nomenclature

In the ecological literature stable isotope ratios are most often expressed as delta (δ) values, the normalized ratio of an unknown sample to an internationally accepted standard

$$\delta^{h}X = 1{,}000 \times [(R_{\text{sample}}/R_{\text{standard}}) - 1],$$

where X is the element, h is the mass of the heavy (and more rare) isotope, and R_{sample} and R_{standard} are the heavy-to-light isotope ratios (*e.g.*, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$) of the sample and standard, respectively (discussion based on Passey *et al*. 2005, Fry 2006, Sulzman 2007). The units are part per thousand (or per mil, ‰). Over a broad

Table 1. List of publications organized by major topic and subtopic, including species investigated, tissue analyzed, and isotope system used. Codes for tissue types: BAL (baleen), BC (bone collagen), BCAR (bone carbonate) BL (blood), BLB (blubber), F (fur), H (heart), L (liver), LG (lung), K (kidney), M (muscle), SED (sediment), SK (skin), SP (spleen), TD (tooth dentin), TE (tooth enamel), V (vibrissae). Note that we only include articles that were published prior to January 2009 except for author contributions that are in press or review.

Physiology fractionation	Species/Taxa	Tissues	δ	Diet-tissue fractionation	Isotopic turnover tissue growth	Nutritional stress
Hobson *et al.* 1996	*Pagophilus groenlandicus, Phoca hispida, Phoca vitulina*	BL/F/H/L/LGK/M/SK SP/V	C/N	X		
Hirons *et al.* 2001*b*	*P. vitulina, Eumetopias jubatus*	V	C/N		X	
Kurle 2002	*C. ursinus*	BL	C/N	X		
Kurle and Worthy 2002	*C. ursinus*	BR/BLB F/K/L/M	C/N		X	
Lesage *et al.* 2002	*P. groenlandicus, P. vitulina, Halichoerus grypus*	BL	C/N	X		
Greaves *et al.* 2004	*H. grypus*	V	C/N		X	
Zhao and Schell 2004	*P. vitulina*	V	C/N		X	
Zhao *et al.* 2006	*P. vitulina*	BL/F/N/V	C/N	X	X	X
Newsome *et al.* 2006	*C. ursinus, Zalophus californianus*	BC/TD	C/N		X	
Clementz *et al.* 2007	*Dugong dugon, Hydrodamalis gigas, Trichechus inunguis, Trichechus manatus*	BC/BCAR	C	X		
Stegall *et al.* 2008	*E. jubatus*	BL/V	C/N	X	X	
Newsome *et al.* in review	*Enhydra lutris*	C	C/N	X		

Continued

Table 1. (Continued)

Foraging ecology habtat use	Species/Taxa	Tissues	δ	Diet trophic lavel	Reproduction maternal care	Habitat use
McConnaughey and McRoy 1979	*P. vitulina*	BL/BLB L/M	C	X		
Rau *et al.* 1983	*Balaenoptera musculus*	M	C	X		
Schoeninger and DeNiro 1984	*Multitaxon compilation*	BC	C/N	X		
Schell *et al.* 1989	*Balaena mysticetus*	BAL	C/N	X		X
Ramsay and Hobson 1991	*P. hispida, Ursus maritimus*	BC/BLB/M	C	X		X
Rau *et al.* 1992	*A. gazella, Hydrurga leptonyx, Lobodon carcinophagus, Ommatophoca rossii*	M	C/N	X		
Ostrom *et al.* 1993	*Balaenoptera acutorostrata, B. musculus, Delphinus delphis, Delphinapterus leucas, Kogia breviceps, Lagenorhynchus albirostris, Megaptera novaeangliae, Mesoplodon bidens, Physeter macrocephalus*	M	C/N	X		
Abend and Smith 1995	*Globicephala melas*	BLB/M/SK/TD	N	X		
Borobia *et al.* 1995	*Balaenoptera physalus, Megaptera novaeangliae*	BLB	C	X		
Ames *et al.* 1996	*T. manatus*	BLB/K L/SK	C/N	X		
Best and Schell 1996	*Eubalaena australis*	BAL	C/N	X		X
Smith *et al.* 1996	*P. vitulina*	F	C/N			X
Abend and Smith 1997	*G. melas*	M/SK	C/N	X		

Hobson *et al.* 1997*b*	*C. ursinus, Eumetopias jubatus, P. vitulina*	M/F	C/N	X		X
Todd *et al.* 1997	*Megaptera novaeangliae*	BLB SK/M	C	X		
Burns *et al.* 1998	*Leptonychotes weddellii*	BL	C/N	X		
Hobson and Schell 1998	*B. mysticetus*	BAL	C/N	X		X
Hobson and Sease 1998	*C. ursinus, E. jubatus, Mirounga angustirostris*	TD	C/N	X	X	
Burton and Koch 1999	*C. ursinus, M. angustirostris, P. vitulina, Z. californianus*	BC	C/N	X		X
Walker and Macko 1999	*D. delphis, E. lutris, Kogia breviceps, Odobenus rosmarus, Orcinus orca, P. groenlandicus, T. manatus, Tursiops truncatus*	TD	C/N	X	X	X
Yoshii *et al.* 1999	*Phoca sibirica*	M	C/N	X		
Lawson and Hobson 2000	*P. groenlandicus*	M	C/N	X		
Clementz and Koch 2001	*C. ursinus, E. lutris, Globicephala macrorhynchus, M. angustirostris, P. vitulina, Phocoena phocoena, T. truncatus, Z. californianus*	TE	C/O	X		X
Holst *et al.* 2001	*P. hispida*	M	C/N	X		
Hooker *et al.* 2001	*Hyperoodon ampullatus*	SK	C/N	X		
Kurle and Worthy 2001	*C. ursinus*	SK	C/N	X		X

Continued

Table 1. (Continued)

Foraging ecology habtat use	Species/Taxa	Tissues	δ	Diet trophic lavel	Reproduction maternal care	Habitat use
Lesage *et al.* 2001	*Cystophora cristata, D. leucas, H. grypus, Phoca groenlandica*	BL/M	C/N	X		X
Davenport and Bax 2002	*Actrocephalus pusillus, Balaeonoptera acutorostrata, D. delphis, G. melas, Lissodelphis peronii, Mesoplodon grayi, Mesoplodon sp., Orcnius orca, Phocoena dioptrica, T. truncatus*	M	C/N	X		
Hoekstra *et al.* 2002	*B. mysticetus*	M	C/N/S	X		X
Kurle and Worthy 2002	*C. ursinus*	BLB/BR F/K/L/M	C/N	X		X
Das *et al.* 2003*b*	*Balaenoptera physalus, H. grypus, Lagenorhynchus acutus, Lagenorhynchus albirostris, Phoca vitulina, P. phocoena, P. macrocephalus*	M	C/N	X		X
Kazuhiro *et al.* 2003	*Peponocephala electra*			X	X	
Outridge *et al.* 2003	*O. rosmarus*	TC	Pb			X
Stewart *et al.* 2003	*O. rosmarus*	TD	Pb			X
Ruiz-Cooley *et al.* 2004	*P. macrocephalus*	SK	C/N	X		
Shao *et al.* 2004	*B. mysticetus*	BAL	C/N	X		X

Zhao *et al.* 2004	*H. leptonyx, Lepthonychotes weddellii, L. carcinophagus, Omatophoca rossii, P. vitulina*	BL	C/N	X		
Caraveo-Patino and Soto 2005	*Eschrichtius robustus*		C/N			
Hall-Aspland *et al.* 2005*a*	*H. leptonyx, L. carcinophagus*	BL/F	C/N	X		
Hall-Aspland *et al.* 2005*b*	*H. leptonyx*	V	C/N	X		
Hammill *et al.* 2005	*P. groenlandicus*	M	C/N	X		
Herman *et al.* 2005	*Orcinus orca*	SK	C/N	X		
Lee *et al.* 2005	*B. mysticetus*	M	C/N	X		X
Angerbjörn *et al.* 2006	*P. phocoena*	BC	C/N	X		X
Aurioles *et al.* 2006	*M. angustirostris*	F	C/N			X
Lewis *et al.* 2006	*Mirounga leonina*	V	C/N	X		
Lusseau and Wing 2006	*Tursiops spp.*	SK	C/N	X		X
Mitani *et al.* 2006	*B. acutorostrata*	BAL	C/N	X		X
Newsome *et al.* 2006	*C. ursinus, Z. californianus*	BC/TD	C/N	X	X	
Niño-Torres *et al.* 2006	*Delphinus capensis*	TD	C/N/S	X		X
Reich and Worthy 2006	*T. manatus*	SK	C/N	X		X
Segura *et al.* 2006	*T. truncatus*	SK	C/N	X		X

Continued

Table 1. (Continued)

Foraging ecology habtat use	Species/Taxa	Tissues	δ	Diet trophic lavel	Reproduction maternal care	Habitat use
Sinisalo *et al.* 2006	*P. hispida*	M	C/N	X		
Bode *et al.* 2007	*D. delphis*	M	C/N	X		
Caraveo-Patino *et al.* 2007	*E. robustus*	BAL	C/N	X		X
Cherel *et al.* 2007	*A. gazella, Arctocephalus tropicalis*	BL	C/N	X		X
Dehn *et al.* 2007	*Erignathus barbatus, O. rosmarus, Phoca fasciata, P. hispida, Phoca largha*	M	C/N	X		X
Hückstädt *et al.* 2007	*Otaria flavescens*	F/V	C/N	X		
Kurle and Gudmundson 2007	*E. jubatus*	BL	C/N	X		X
Marcoux *et al.* 2007	*P. macrocephalus*	SK	C/N	X		X
Mendes *et al.* 2007*a*	*P. macrocephalus*	TD	C/N	X	X	X
Mendes *et al.* 2007*b*	*P. macrocephalus*	TD	C/N	X		X
Tucker *et al.* 2007	*H. grypus*	SK	C/N	X		X
Cherel *et al.* 2008	*A. gazella, Arctocephalus tropicalis, M. leonina*	BL	C/N	X		X
de Stephanis *et al.* 2008	*G. melas*	SK	C/N	X		
Knoff *et al.* 2008	*T. truncatus*	TD	C/N	X	X	
Porras-Peters *et al.* 2008	*Z. californianus*	F	C/N	X		X
Sinisalo *et al.* 2008	*P. hispida*	BL/L/M	C/N	X		
Wolf *et al.* 2008	*Arctocephalus galapagoensis, Zalophus wollebaeki*	SK	C/N	X		X
Newsome *et al.* 2009*a*	*Orcinus orca*	TD	C/N	X	X	
Newsome *et al.* 2009*b*	*E. lutris*	V	C/N	X		

Ecotoxicology	Species/Taxa	Tissues	δ	Trophic transfer biomagnification	Population structure
Smith *et al.* 1990	*E. lutris*	TD	Pb	X (TM)	
Muir *et al.* 1995	*O. rosmarus, P. hispida*	M	C/N	X (OC)	
Jarman *et al.* 1996	*E. jubatus*	M	C/N	X (TM/OC)	
Jarman *et al.* 1997	*E. jubatus*	M	C/N	X (OC)	
Outridge *et al.* 1997	*D. leucas, O. rosmarus*	TD	Pb	X (TM)	
Atwell *et al.* 1998	*P. hispida, U. maritimus*	M	C/N	X (TM)	
Outridge and Stewart 1999	*O. rosmarus*	TD	Pb		X (TM)
Stern *et al.* 1999	*O. rosmarus*	TD	Pb		X (TM)
Das *et al.* 2000	*D. delphis, Stenella coeruleoalba*	M	C/N		X (TM)
Fisk *et al.* 2001	*P. hispida*	M	N	X (OC)	
Moisey *et al.* 2001	*P. hispida*	M	C/N	X (OC)	
Fisk *et al.* 2002*a*	*P. hispida*	M	C/N		X (OC)
Fisk *et al.* 2002*b*	*P. groenlandicus, P. hispida*	M	C/N	X (OC)	
Hobson *et al.* 2002	*P. hispida, U. maritimus*	M	C/N	X (OC)	
Outridge *et al.* 2002	*D. leucas, O. rosmarus*	TD	C/N	X (TM)	
Tittlemier *et al.* 2002	*P. hispida*	M	C/N	X (OC)	
Born *et al.* 2003	*B. acutorostrata*	M	C/N		X (TM)
Das *et al.* 2003*a*	*D. delphis, H. grypus, Lagenorhynchus acutus, Lagenorhynchus albirostris, P. phocoena, S. coeruleoalba*	M	C/N	X (TM)	

Continued

Table 1. (Continued)

Ecotoxicology	Species/Taxa	Tissues	δ	Trophic transfer biomagnification	Population structure
Van de Vijver *et al.* 2003	*Balaenoptera physalus, Cytophora cristata, H. grypus, Lagenorhynchus acutus, Lagenorhynchus albirostris, P. vitulina, P. phocoena, P. macrocephalus, S.coeruleoalba*	M	C/N	X (OC)	
Das *et al.* 2004*a*	*P. phocoena*	M	C/N	X (TM)	
Das *et al.* 2004*b*	*P. phocoena*	M	C/N		X (TM)
Dietz *et al.* 2004	*Monodon monoceros*	M	C/N	X (TM/OC)	
Hobson *et al.* 2004*a*	*B. acutorostrata*	BAL	C/N	X (TM)	
Tomy *et al.* 2004	*Delphinapterus leucas, Monodon monoceros*	L	C/N	X (OC)	
Borrell and Aguilar 2005	*D. delphis, S. coeruleoalba*	SK	C/N		X (OC)
Braune *et al.* 2005	Review				
Campbell *et al.* 2005	*P. hispida*	M	C/N	X (TM)	
Herman *et al.* 2005	*Orcinus orca*	SK	C/N		X (OC)
Routti *et al.* 2005	*H. grypus, P. hispida*	L	N	X (OC)	
Borrell *et al.* 2006	*T. truncatus*	SK	C/N		X (OC)
Caurant *et al.* 2006	*D. delphis, P. phocoena, S. coeruleoalba*	BC/TD	Pb	X (TM)	
Dehn *et al.* 2006*a*	*B. mysticetus, Delphinapterus leucas, E. robustus*	M	C/N	X (TM)	

Dehn *et al.* 2006*b*	*Delphinapterus leucas, E. barbatus, P. hispida, U. maritimus*	M	C/N	X (TM)		
Jardine *et al.* 2006	Review					
Brookens *et al.* 2007	*P. vitulina*	L	C/N	X (TM)		
Krahn *et al.* 2007	*B. acutorostrata, C. ursinus, E. robustus, E. jubatus, Orcinus orca*	SK/M	C/N		X (OC)	
McHugh *et al.* 2007	*Orcinus orca*	BLB	C/N	X (OC)		
Riget *et al.* 2007	*P. hispida*	M	C/N	X (TM)		
Van de Vijver *et al.* 2007	*P. phocoena*	M	C/N	X (OC)		
Butt *et al.* 2008	*P. hispida*	M	C/N	X (OC)		
Krahn *et al.* 2008	*Orcinus orca*	SK	C/N	X (OC)		
Historic ecology paleoecology	Species/Taxa	Tissues	δ	Historic	Archaeological	Paleo
Thewissen *et al.* 1996	*Ambulocetus natans, D. delphis, Indocetus sp., Nalacetus ratimitus, Orcinus orca, Pakicetus attocki, P. macrocephalus, Stenella sp., Sotalia fluviatilis, T. truncatus*	TE	O			X

Continued

Table 1. (Continued)

Historic ecology paleoecology	Species/Taxa	Tissues	δ	Historic	Archaeological	Paleo
Roe *et al.* 1998	*Ambulocetus, Andrewsiphius, Attockicetus, Delphinus, Gandakasia, Gaviacetus, Georgiacetus, Ichthyolestes, Indocetus, Inia, Lipotes, Nalacetus, Orcinus, Pakicetus, Phocoena, Physeter, Platanista, Remingtonocetus, Sotalia, Stenella, Tursiops*	BCAR/TD TE	C/O			X
Erskine *et al.* 1998	*M. leonina*	SED	N	X		
Katzenberg and Weber 1999	*Phoca siberica*	BC	C/N		X	
Walker *et al.* 1999	*T. truncatus*	TD	C/N	X		
Schell 2000	*B. mysticetus*	BAL	C	X		
Schell 2001	*B. mysticetus*	BAL	N	X		
Burton *et al.* 2001	*C. ursinus, P. vitulina*	BC	C/N		X	
Hirons *et al.* 2001*a*	*C. ursinus, E. jubatus, P. vitulina*	BC	C/N	X		
Burton *et al.* 2002	*C. ursinus, Phoca vitulina, Zalophus californianus*	BC	C/N		X	
Clementz *et al.* 2003	*Desmostylus*	TE	C/O			X
Coltrain *et al.* 2004	*B. mysticetus, E. barbatus, O. rosmarus, P. hispida*	BC	C/N		X	
Hobson *et al.* 2004*b*	*E. jubatus*	TD	C/N	X		
Liu *et al.* 2004	*A. gazella, L. weddellii, M. leonina*	BL/F	N		X	X

MacFadden *et al.* 2004	*Metaxytherium floridanum, T. manatus, Trichechus spp.*	TE	C/O			X
Liu *et al.* 2005	NA	SED	C	X	X	
Sun *et al.* 2005	NA	SED	Sr	X	X	
Clementz *et al.* 2006	*Babiacetus indicus, Basilosaurus isis, Dalanistes ahmedi, Durudon atrox, Eosiren libyca, Eotheroides sp., Halitherium taulannense, Himalayacetus subathuensis, Pakicetus inachus, Protosiren smithae, Rodhocetus kasrani*	TE	C/O			X
Moss *et al.* 2006	*C. ursinus*	BC	C/N		X	
Newsome *et al.* 2007*a*	*C. ursinus, P. vitulina*	BC	C/N		X	
Newsome *et al.* 2007*b*	*C. ursinus*	TD	C/N	X		
Thewissen *et al.* 2007	*Indohyus, Khirtharia*	TE	C/O			X
Corbett *et al.* 2008	*C. ursinus, E. lutris, E. jubatus, H. gigas, P. vitulina*	BC/BA	C/N/O		X	
Amiot *et al.* 2008	Multitaxon compilation	TE	O			X
Christensen and Richardson 2008	*P. phocoena*	BC	C/N	X		
Clementz *et al.* 2009	Dugongidae, Protosirenidae, Trichechidae	TE	C/O			X

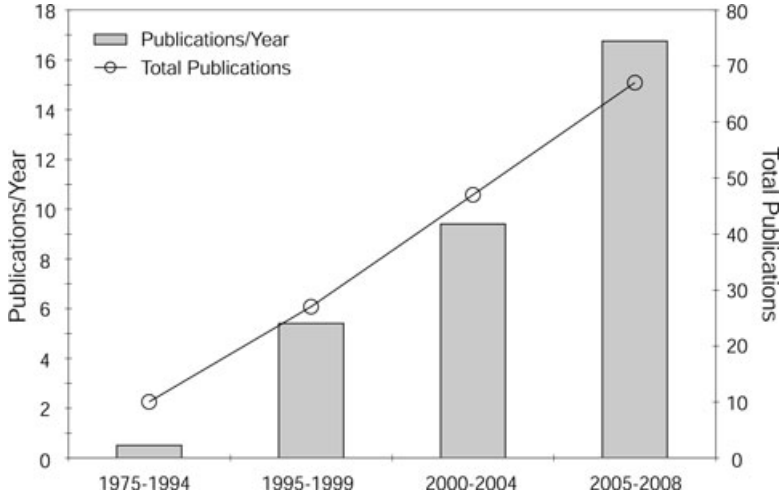


Figure 1. Publication rate (publications/year) and total number of publications utilizing stable isotopes to study marine mammal ecology. Articles used in this analysis are listed in Table 1. Note that we only include articles that were published prior to January 2009 except for author contributions that are in press or review.

range of values (~200‰), δ values covary linearly with the percent heavy isotope in the substance. The accepted standards are Vienna-Pee Dee Belemnite limestone (V-PDB) for carbon, V-PDB or Vienna-Standard Mean Ocean Water (V-SMOW) for oxygen, and atmospheric N_2 for nitrogen. By definition, the isotope value of these standards is 0‰.

Isotopic fractionation can be quantified different ways. Fractionation in reversible reactions that reach isotopic equilibrium is described using the fractionation factor (α). The fractionation factor describing the partitioning of isotopes between substances A and B is defined as

$$\alpha_{\text{A-B}} = (1{,}000 + \delta^b X_A)/(1{,}000 + \delta^b X_{\text{B}}).$$

Fractionation factors have values that are unwieldy and difficult to remember; furthermore they are not strictly applicable to many unidirectional or branching biological processes. Three more intuitive (but different and nearly mathematically equivalent) values are used to describe fractionation in ecological and geological literature. To make matters more confusing, they are defined differently in the two disciplines. Marine mammal ecology intersects both disciplines, so we lay out the alternatives in Table 2.

In trophic studies, fractionation is often described using the geochemical definition (called the trophic discrimination factor by Martínez del Rio *et al*. 2009)

$$\Delta^b X_{\text{A-B}} = \delta^b X_{\text{A}} - \delta^b X_{\text{B}}.$$

This equation denotes the difference in isotopic composition between a consumer (A) and its diet (B). Because consumers are typically enriched in the heavy isotope relative to diet, $\Delta_{\text{A-B}}$ values so defined are positive. When discussing the fractionation

Table 2. Different ways of quantifying the fractionation of isotopes between two substances (Sulzman 2007, Martínez del Rio *et al*. 2009).

Discipline	Term	Symbol	Formula
Geochemistry	*Equilibrium or kinetic fractionations. Typically A is the substance enriched in the heavier isotope.*		
	Difference/Offset	$\Delta_{A\text{-}B}$	$\delta^{h}X_{A} - \delta^{h}X_{B}$
	Enrichment	$\varepsilon_{A\text{-}B}$	$1{,}000(\alpha_{A\text{-}B} - 1)$
Ecology	*Nonequilibrium, branched or unidirectional reactions. Typically A is the reactant, B is typically the product.*		
	Discrimination	$\Delta_{A\text{-}B}$	$1{,}000(\alpha_{A\text{-}B} - 1)$
	Enrichment	$\varepsilon_{A\text{-}B}$	$1{,}000\ln \alpha_{A\text{-}B}$

between two tissues, for example skin and collagen, it is essential to indicate A and B using subscripts. $\Delta_{A\text{-}B}$ values are simple to calculate and intuitive. The downside is that they are approximations that vary depending on the region of the isotopic scale on which they are calculated, leading to detectable errors when A and B differ by more than 10‰. The alternative expressions in Table 2, formulated using α values, provide exact solutions.

Substrates for Isotopic Analysis

The bodies of marine mammals are built from tissues with different macromolecular and elemental compositions and different styles of growth and turnover (discussion based on review by Koch 2007). Soft tissues such as skin, muscle, hair, red blood cells, and plasma are most often used in studies of modern animals because they can be sampled during routine handling (or even remotely *via* darts) with minimal potential for animal mortality. These tissues contain different amounts of lipid and protein (often with different amino acid compositions), which may contribute to differences in isotopic composition, even when sampled from a single individual.

Mineralized tissues such as bone, tooth enamel, and tooth dentin are more commonly used in historical, archaeological or paleontological studies. These tissues are composites of mineral, protein, and lipid. The mineral is a highly substituted form of hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$) that we will call bioapatite. Bioapatite has a few weight percent carbonate substituting for OH and PO_4 and various cations (*e.g.*, Sr, Pb) substituting for Ca. Bone is composed of tiny bioapatite crystals intergrown with an organic matrix (chiefly made of the protein collagen) that is approximately 30% of its dry weight. Enamel is much less porous than bone. It contains <5 weight% organic matter (chiefly noncollagenous proteins) and has much larger crystals with fewer substitutions. The crystal size, organic content, and organic composition of tooth dentin resemble bone, whereas its porosity is intermediate between enamel and bone. These differences in crystal size and porosity lead to large differences in the ability of bioapatite from these tissues to retain isotopic values during burial and fossilization. In general, only tooth enamel bioapatite is highly retentive and useful for studies of paleontological materials (>10,000-yr-old), whereas bone and dentin are reliable in historical (<500-yr-old) specimens. Samples of intermediate age (10,000–500-yr-old) must be screened carefully.

Much more information can be obtained if isotopic analysis can be conducted at the level of individual organic molecules, rather than bulk tissue (see review by Evershed *et al.* 2007). Because the different amino or fatty acids in proteins or lipids have different biosynthetic pathways, they provide a finer probe of animal ecology and physiology. At the most basic level, by isolating and analyzing indispensable amino and fatty acids, which must be incorporated from the same compound in diet, we have very direct access to information on dietary sources. For dispensable amino and fatty acids, the extent to which they resemble "bulk" diet versus dietary protein or lipid may provide useful information on animal physiology and perhaps trophic level. This is a rich area that has received little attention in studies of marine mammal ecology, but has been applied to studies of other marine consumers (Popp *et al*. 2007). An added benefit of the compound-specific approach is that even fossils that have suffered breakdown of biological macromolecules may retain characteristic amino or fatty acids that can provide isotopic information (Fogel and Tuross 2003, Evershed *et al.* 2007).

Physiology and Fractionation

Accurate interpretation of isotopic differences within or among animal tissues is dependent on information on three sources of isotopic variation: (1) the isotopic composition of the potential inputs, (2) an understanding of the isotopic fractionations that occur between these sources and animal tissues, and (3) an understanding of how long it takes for the isotopic value of these sources to be reflected in a tissue (often referred to as isotopic turnover). Here, we focus on the latter two sources of variation: tissue-to-source isotopic fractionation and isotopic turnover rates. For tissue-to-source fractionations, we consider carbon and nitrogen, which are supplied by diet, separately from oxygen, which is largely supplied by ingested water. We lay out general patterns that might be expected from studies of other mammals and birds, but highlight whenever possible studies of marine mammals.

Tissue-to-Diet Nitrogen and Carbon Isotope Discrimination in Marine Mammals

A clear understanding of the tissue-to-diet isotope discrimination for a species is critical for interpreting ecological information from tissue isotope values. The magnitude of these fractionations can vary as a result of differences in metabolic routing of dietary components between tissues (*e.g.*, lipids, proteins, and carbohydrates), variation in an animal's growth rate and the nutritional quality of its diet, differences in the amino acid or lipid composition of tissues, and the interplay between these factors and temporal variation in the ecology and physiology of marine mammals. We discuss the impact of each of these factors on nitrogen and carbon isotope tissue-to-diet discrimination below.

The dominant source of nitrogen in marine mammals is dietary protein. An increase in $\delta^{15}N$ value with each trophic step has been recognized across taxonomic groups and food webs (typically +2‰–+5‰ for each increase in trophic level; Minagawa and Wada 1984, Kelly 2000, Vanderklift and Ponsard 2003). Trophic discrimination is thought to relate to excretion of urea and other nitrogenous wastes that are ^{15}N-depleted relative to body nitrogen pools. Isotopic fractionation of nitrogen occurs during deamination and transamination reactions flowing into and out of the TCA cycle and in the recycling of urea within the body (see review and modeling study by Balter *et al*. 2006). Dietary protein quantity and quality can

also influence the magnitude of isotopic fractionation (Robbins *et al*. 2005); both models and limited data suggest that $\Delta^{15}N_{tissue\text{-}diet}$ decreases with increasing dietary protein quality, but increases with increasing dietary protein quantity (Martínez del Rio *et al*. 2009). Based on differences in protein quantity, we might expect higher discriminations in carnivorous marine mammals (cetaceans, pinnipeds) than in herbivorous species (sirenians). Predictions related to differences in protein quantity *vs.* quality are more difficult to generate within these broad feeding categories.

$\Delta^{15}N_{tissue\text{-}diet}$ values for pinnipeds, the only group of marine mammals on which controlled feeding experiments have been conducted, are relatively consistent across taxa and are in the +3‰–+5‰ range commonly observed in studies of terrestrial carnivores (Table 3). Analyzing different tissues in captive phocids fed an isotopically homogenous diet, Hobson *et al.* (1996) found that $\Delta^{15}N$ values range from 1.7‰ for red blood cells to 3.1‰ for liver. Focusing on just blood and fur of captive phocids, Lesage *et al.* (2002) found a similar range in $\Delta^{15}N$ values. In contrast, Kurle (2002) found that $\Delta^{15}N$ values for various blood components in captive northern fur seals (*Callorhinus ursinus*) ranged from 4.1‰ to 5.2‰. Focusing on blood serum, Zhao *et al.* (2006) also found relatively large $\Delta^{15}N_{serum\text{-}diet}$ values for captive harbor seals (*Phoca vitulina*), ranging from 3.9‰ to 4.6‰. Recently, Newsome *et al*. (in review) found a mean $\Delta^{15}N_{vibrissae\text{-}diet}$ value of 3.5‰ for a wild population of California sea otters (*Enhydra lutris nereis*).

Whereas the nitrogen in an animal's diet is mainly sourced from the proteins it consumes, the carbon for an animal's tissues is supplied by dietary proteins, lipids, and carbohydrates, which may differ in their carbon isotope composition. In addition, carbon occurs in tissues composed of materials other than protein, such as bioapatite and lipids, which have a greater isotopic range than that observed for nitrogen from protein-rich tissues. In terrestrial mammals, the $\delta^{13}C$ value of bioapatite reflects that of bulk diet, whereas that of proteins and lipids is often biased toward the protein or lipid portion of the diet, respectively, as a result of dietary routing of these components. For most lipids, there is usually a balance between routing of dietary lipids to tissue and *de novo* synthesis of new lipids; bone cholesterol is the one lipid that strongly reflects bulk diet (Jim *et al*. 2003). For proteins, there is a similar balance between routing of amino acids—particularly indispensible amino acids that cannot be produced through *de novo* synthesis—and production of the R-groups of dispensable amino acids from bulk diet or carbohydrate and lipid carbon (Howland *et al*. 2003, Jim *et al*. 2006). For pinnipeds, cetaceans and otters, which consume protein-rich diets with variable amounts of fat, the $\delta^{13}C$ value of body protein should closely track that of bulk diet, but perhaps with different tissue-to-diet fractionations depending on dietary lipid content. Herbivorous sirenians would receive bulk dietary carbon from carbohydrates along with a smaller quantity of proteins from plants or protein-rich epizooans, which should, in turn, reflect plant-derived carbon.

Measured tissue-to-diet isotope discriminations for bioapatite, lipids and proteins are significantly different. For bioapatite, tissue-to-diet isotope fractions in terrestrial mammals differ between carnivores (+9‰) and herbivores (+12‰–+14‰) (reviewed in Koch 2007). The $\Delta^{13}C_{apatite\text{-}diet}$ value has been measured in manatees (*Trichechus manatus latirostrius*) on controlled diets and is +14‰ (MacFadden *et al*. 2004). While $\Delta^{13}C_{apatite\text{-}diet}$ values have not been determined experimentally for other marine mammals, field studies suggest they are similar to values for land carnivores (Clementz and Koch 2001, Clementz *et al.* 2007). In contrast, bulk consumer lipid is ^{13}C-depleted by 2‰–5‰ relative to bulk diet (DeNiro and Epstein

Table 3. Summary of diet-tissue discrimination factors observed for various species in controlled feeding experiments or inferred from wild populations. Numbers in parentheses indicate the mean $\Delta_{\text{tissue-diet}}$ value for carbon and nitrogen; see references for associated variance.

Citation	Species	$\Delta^{13}C_{\text{tissue-diet}}$ discrimination	$\Delta^{15}N_{\text{tissue-diet}}$ discrimination	Lipid extracted
Hobson *et al.* 1996	*P. groenlandicus*	RBC (+1.7)	RBC (+1.7)	Y
	P. vitulina	Fur (+2.8)	Fur (+3.0)	N
	P. hispida	Liver (+0.6)	Liver (+3.1)	N
		Muscle (1.3)	Muscle (2.4)	Y
		Nails (+2.8)	Nails (+2.3)	N
		Skin (+2.8)	Skin (+2.3)	N
		Vibrissae (+3.2)	Vibrissae (+2.8)	N
	Prey			Y
Kurle 2002	*C. ursinus*	RBC (+1.3)	RBC (+4.1)	N
		Plasma (+1.0)	Plasma (+5.2)	N
		Serum (+0.6)	Serum (+5.2)	N
	Prey			Y
Lesage *et al.* 2002	*H. grypus*	RBC (+1.5)	Blood-RBC (+1.7)	N
	P. groenlandicus	Serum (+0.8)	Blood-Serum (+3.1)	N
	P. vitulina	Fur (+2.3)	Fur (+2.3)	N
	Prey			N
Zhao *et al.* 2006	*P. vitulina*	Serum (−0.6–1.7)	Serum (+3.9–+4.6)	N
	Prey			Y
Newsome *et al.* in review	*E. lutris*	Vibrissae (+2.2)	Vibrissae (+3.5)	N
	Prey			N
Clementz *et al.* 2007	*D. dugon*	Bioapatite (+11–+14)		NA
	H. gigas			
	T. inunguis			
	T. manatus			
	Prey			N

1978, Tieszen *et al*. 1983, Howland *et al*. 2003) and controlled feeding studies of captive pinnipeds show that trophic $\Delta^{13}C$ values for consumer proteins range from +0.5‰ to +2.0‰ for most tissues (Table 3), except those composed of keratin (*e.g.*, fur, vibrissae), which range from +2‰ to +3‰. The only study of a wild marine mammal population found that mean $\Delta^{13}C_{vibrissae\text{-}diet}$ values of California sea otters was 2.2‰ (Newsome *et al*., in review), within the range found for captive pinnipeds (Table 3). Unfortunately, there are no controlled studies in which collagen has been measured, so most workers assume a value of +5‰, as seen in other mammals and birds.

Along with preferential routing of dietary components into different tissues, nutritional status and growth rate have been shown to affect tissue-to-diet isotope fractionation, particularly trophic ^{15}N enrichment (Vanderklift and Ponsard 2003, Robbins *et al*. 2005). With the exception of sirenians, all marine mammals are carnivores that consume prey with a high nitrogen concentration; lipid-extracted marine mammal prey typically have atomic C/N ratios of 3–4. Because urea $\delta^{15}N$ values can be up to 10‰ lower than serum (see review by Balter *et al*. 2006), theoretical considerations and empirical data suggest that a higher fractional loss of nitrogen as urea—which typically correlates positively with both the rate of protein intake and the rate of urea loss—will lead to higher body $\delta^{15}N$ values (reviewed and modeled by Martínez del Rio and Wolf 2005 and Martínez del Rio *et al*. 2009). Zhao *et al.* (2006) found that captive harbor seals fed a protein-rich diet of pollock had slightly higher $\Delta^{15}N$ values (4.6‰ *vs.* 3.9‰, Table 2) than animals that consumed a relatively protein-poor diet of herring. While subtle, this pattern agrees with findings on other taxa that show nitrogen isotope fractionation can be influenced by protein quantity. These findings suggest that trophic $\Delta^{15}N$ values for sirenians—herbivores that consume low protein food—might be lower than the range seen in carnivorous marine mammal species.

Different amino acids in a single tissue can vary in $\delta^{13}C$ and $\delta^{15}N$ values by more than 15‰ (*e.g.*, Hare *et al*. 1991). As different proteins contain distinct proportions of amino acids, differences in the protein composition among tissue types can yield dissimilar isotopic compositions irrespective of changes in diet. For example, Kurle (2002) found differences in the ^{15}N-enrichment of various tissues relative to the diet of captive northern fur seals that were fed a strict diet of known isotopic composition. Red blood cells had $\delta^{15}N$ values approximately 4.1‰ higher than diet, whereas plasma and serum were enriched by approximately 5.2‰ relative to diet. This discrepancy in trophic discrimination among tissue types was interpreted as a consequence of differences in amino acid composition between these tissues. Stegall *et al*. (2008) found that vibrissae $\delta^{13}C$ values were approximately 2‰ higher than serum from Steller sea lion (*Eumetopias jubatus*) pups and juveniles but found no significant differences in $\delta^{15}N$ values between these tissues. Again, the observed differences in $\delta^{13}C$ values likely result from differences in the amino acid composition of blood serum *vs.* vibrissae keratins. It has been long recognized that another commonly analyzed tissue, bone collagen, has a distinctive amino acid composition that produces larger than normal diet-tissue $\delta^{13}C$ fractionation. While "soft" tissues such as muscle, liver, and skin are ^{13}C-enriched by only 1‰–2‰ relative to diet, bone collagen typically has $\delta^{13}C$ values that are 4‰–5‰ higher than diet (Koch 2007). Accurate interpretation of intertissue isotopic differences requires careful consideration of such tissue-dependent discrimination patterns.

Many marine mammals experience seasonal cycles in food intake and energy demands that may impact the physiological processes that govern isotopic fractionation

during metabolism and tissue synthesis. For example, many pinniped and mysticetes are capital breeders, storing vast amounts of fat to provide energy during reproduction and nursing. Some of these animals also undertake long migrations during which food intake may be limited. Because blubber is primarily composed of ^{13}C-depleted lipids, it has a significantly lower $\delta^{13}C$ value than a piscivorous (pinniped) or planktonic (mysticete) diet. An animal that relies on blubber stores to maintain metabolism will be "consuming" a food source with a lower $\delta^{13}C$ value than its regular diet and have a $\Delta^{13}C_{tissue\text{-}diet}$ value that is lower than when it is not relying on fat.

Such factors may influence $\Delta^{15}N_{tissue\text{-}diet}$ values as well. Catabolism of protein from lean tissues (*e.g.*, muscle) during periods of nutritional stress may cause $\delta^{15}N$ values to rise as the animal continues to shed waste that is ^{14}N-enriched relative to the body. Furthermore, the nitrogen source for any additional protein deposition is body tissue, which is already ^{15}N-enriched relative to dietary sources. A number of laboratory and a few field experiments have explored the utility of stable isotopes as proxies of nutritional stress (*e.g.*, Hobson *et al.* 1993, Polischuck *et al*. 2001, Cherel *et al*. 2005). For experiments in which no exogenous protein was supplied to subjects, significant bulk tissue or whole body ^{15}N-enrichments of 0.5‰–2.5‰ were observed. In a wild population, Cherel *et al.* (2005) found significant ^{15}N-enrichments in the plasma, red blood cells, and feathers of fasting penguins, which rely exclusively on endogenous protein when breeding and molting. Finally, in a longitudinal study tracking pregnant women, in those with severe morning sickness who entered negative nitrogen balance, hair $\delta^{15}N$ values rose by 0.4‰–1.2‰ (Fuller *et al*. 2004, 2005). Overall, such effects would lead to increased $\Delta^{15}N_{tissue\text{-}diet}$ values for animals in nutritional stress.

Isotopic consequences of growth, pregnancy, and lactation have received little study. We might expect that growing, pregnant, or nursing animals might lose relatively less body nitrogen as urinary waste and therefore have lower $\Delta^{15}N$ values. While not designed to study such patterns in mothers, early work on human nursing did not detect an isotopic effect in lactating women (Fogel *et al*. 1997). In contrast, a study of wild horses showed that lactating females had lower $\delta^{15}N$ values than other adults (males, nonlactating females) and used mass balance calculations to argue that this ^{15}N-depletion is the expected result of the nitrogen balance perturbations associated with lactation (Koch 1997). Further support for this trend was reported in Kurle (2002), where blood $\delta^{15}N$ values of a single lactating northern fur seal were approximately 1‰ lower than those for nulliparous females. Fuller *et al*. (2004) reported $\delta^{15}N$ variations among pregnant human females. They found 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. If these phenomena occur in marine mammals, they would reduce $\Delta^{15}N_{tissue\text{-}diet}$ values for growing or pregnant females. Expectations for lactating females are more complex and may depend on whether animals feed or fast while lactating (*i.e.*, income vs. capital breeders).

Oxygen Isotope Fractionation in Marine Mammals

The $\delta^{18}O$ value of a biomineral depends on the temperature at which it forms and the ^{18}O value of the body fluid from which it precipitates (discussion below based on Clementz and Koch 2001 and Koch 2007). For mammals there is a constant offset between the ^{18}O value of body water and phosphate ($\sim$+18‰), and between

the phosphate and carbonate components of bioapatite (~+8‰), close to values predicted for isotopic equilibrium at typical body temperatures.

Physiology affects the ^{18}O value of body water by altering the fluxes of oxygen into and out of the body, as well as fractionations associated with transport and/or transformation of oxygen-bearing compounds. Ingested water is a major flux of oxygen into marine mammals and includes preformed water in food, seawater consumed incidentally when eating, and water taken by active drinking (mariposia). The proportion of water gained from these sources varies widely among marine mammals (Ortiz 2001), yet as these processes do not strongly fractionate oxygen, these fluxes should all have ^{18}O values close to that of seawater (0‰ V-SMOW). Metabolic water generated by oxidation of food dry matter may contribute to marine mammal body water. This water may be ^{18}O-enriched relative to ingested water, as atmospheric O_2 is much heavier than ingested water (~+21‰ V-SMOW). Finally, there is evidence in cetaceans for a substantial flux of water across the skin (Hui 1981, Andersen and Nielsen 1983); it is unlikely that this process greatly fractionates oxygen isotopes, though the issue has not been studied. Fluxes of oxygen out of the body include respired carbon dioxide (which strongly fractionates), water and organic matter in waste, and water lost during exhalation.

Overall, the ^{18}O value of marine mammal body water is similar to that of environmental water, as their bioapatite phosphate and carbonate form in near isotopic equilibrium with environmental water. Clementz and Koch (2001) noted that there is a systematic difference in apatite ^{18}O values between pinnipeds and cetaceans. Pinnipeds have values expected for equilibrium with seawater at body temperature, whereas cetacean values are about 2‰ higher. They speculated on potential causes for this difference, but were unable to explain the difference. Clementz and Koch (2001) also noted that bioapatite ^{18}O values from aquatic mammal teeth showed little within-population variability, presumably because body water ^{18}O values vary little within an individual during its lifetime or among individuals in populations.

Isotopic Turnover

Isotopic turnover rates can vary within or among individuals as a function of body size, growth rate, and protein turnover. A simple single-component box model shows that the rate of isotopic turnover is approximately equal to the net rate of influx of new material divided by the size of the pool of the element in the tissue. Because of the large daily fluxes of oxygen into and out of mammals, turnover times are rapid, on the scale of a week to a month, and are well established from the literature on isotope dilution and measurement of metabolic rate (Nagy and Costa 1980, Ortiz 2001). For carbon and nitrogen in tissues, the rate of elemental incorporation is approximately proportional to body mass (m_b) to the 3/4 power (Martinez del Rio and Wolf 2005, Martinez del Rio *et al*. 2009), whereas the mass of animal tissues usually scales isometrically with m_b. Thus, isotopic turnover of metabolically active tissues is proportional to $m_b^{-1/4}$ (*i.e.*, $m_b^{3/4}/m_b$). This prediction has only been empirically tested on a single tissue (red blood cells) from a few small bird species (Carleton and Martínez del Rio 2005).

In addition to overall body size, both the growth of new tissue and the amount of tissue replacement due to catabolic turnover play fundamental roles in determining isotopic turnover rates. In short, the isotopic turnover rate equals the sum of the growth rate and the allometric effect of body size on catabolic turnover ($m_b^{-1/4}$). Most marine mammals undergo determinate growth, so for adults that are not

nutritionally stressed, the growth term is zero; thus isotopic turnover rates should scale allometrically with $m_b^{-1/4}$. Like most endotherms, marine mammals only experience exponential growth during the first year of life and thus the growth of new tissue need only be considered for this ontogenetic stage. During this phase, mass-specific growth rate also scales with $m_b^{-1/4}$ because maximal growth rate (in units of mass per unit time) scales with $m_b^{3/4}$ (Martinez del Rio and Wolf 2005, Martinez del Rio *et al*. 2009). Therefore the contributions of catabolic turnover and growth on isotopic incorporation both scale allometrically with $m_b^{-1/4}$ for very young animals.

In addition to factors related to body size and growth rate, isotopic turnover rates vary among tissue types. Carleton and Martínez del Rio (2005) hypothesized that protein turnover is the primary determinant of isotopic turnover rate for the most commonly used tissues in isotopic ecology, especially since samples are typically lipid-extracted prior to analysis. While this prediction has not been tested by simultaneously measuring protein turnover and isotopic turnover in the same organism, there are data from the laboratory and field studies that suggest a close link between these processes. The first is the observation that splanchnic organs (*e.g.*, liver) and plasma proteins, which have relatively high rates of protein turnover, also have higher isotopic turnover rates than structural elements (*e.g.*, collagen, striated muscle). Second, several studies have shown that protein intake, or the amount of dietary nitrogen is positively correlated with isotopic turnover rates. Because pinnipeds, cetaceans, and sea otters consume high quality, nitrogen-rich carnivorous diets, protein intake rate is not likely to be an important source of variation in isotopic turnover. Diet quality could be an important factor for sirenians, which consume nitrogen-poor sea grass and algae.

A relatively new contribution to the discussion of isotopic turnover is the concern that multiple isotope pools may exist within an organism and each of these pools may have different turnover rates. Ayliffe *et al*. (2004) were the first to discuss this issue when interpreting carbon isotope turnover in tail hair and breath CO_2 from domestic horses. They were able to isolate three carbon pools with distinct turnover rates ranging from fast ($t1/2 \sim 0.2$–0.5 d) to slow ($t1/2 \sim 50$–140 d). Cerling *et al*. (2007) refined this approach further by presenting the "reaction-progress variable" as a method for determining whether isotopic turnover was best expressed using a single exponential function or by using multiple linear functions, an approach that has been effectively used in geochemical studies. Martínez del Rio and Anderson-Sprecher (2008) and Carleton *et al*. (2008) have evaluated the necessity of this approach by quantifying the uncertainty inherent in estimates of isotope retention by multicompartment models and by testing whether multicompartment models are more effective than single-compartment models. They argued that the appropriate model may depend upon the type of tissue. The significance of the these findings has yet to be determined for isotopic incorporation studies for marine mammals; turnover rates are determined by diet-switching experiments, which are difficult to perform on marine mammals, so few studies have produced data on isotopic turnover for metabolically active tissues (Table 1, Zhao *et al.* 2006, Newsome *et al.* 2006, Orr *et al.* 2009). Future switching experiments on marine mammals should be designed to develop allometric relationships between body size and isotopic turnover in tissues such as muscle and blood components, as well as to test whether single- or multicompartment models are appropriate.

While isotopic turnover rates are important for the interpretation of tissues that undergo catabolic replacement, other tissues are metabolically inert and do not

experience continual exchange once synthesized. For such tissues, there will still be an isotopic turnover time for the pool from which the tissue is synthesized. Four types of metabolically inert and continually growing tissues have proven useful in studies of marine mammal ecology: (1) fur or vibrissae (keratin), (2) baleen (keratin and bioapatite), (3) tooth dentin (collagen and bioapatite), and (4) tooth enamel (bioapatite). When interpreting data from fur, vibrissae, and baleen, consideration of tissue growth rate is a much more important issue than isotopic turnover. For teeth, the critical factor is the time of tissue formation. Tooth enamel, even on permanent dentition, forms early in life, and for many cetaceans and pinnipeds enamel on many teeth begins to form prior to weaning (Perrin and Myrick 1980, Modig *et al.* 1997, Stewart *et al.* 1998). Tooth dentin, in contrast, may deposit within the crown and root of a tooth for decades. Annual lamellae are pronounced in many species, providing material for the construction of ontogenetic time series of isotope values.

Diet and Foraging Ecology

The majority of papers in our literature review used isotopes to characterize diet (chiefly the trophic level of prey consumed). Here we explore several case studies where isotopic data have provided crucial constraints on the diets of free-ranging marine mammals. We then turn to the use of isotopic data to study mother-to-pup nutrient transfer and weaning age.

Diet and Trophic Level

The most common and earliest use of stable isotope biochemistry to study marine mammal ecology focused on the characterization of diet and trophic level (Hobson and Welch 1992). To highlight this approach we present data from an Alaskan Arctic food web (Fig. 2, Schell *et al.* 1998, Hoekstra *et al.* 2002, Dehn *et al.* 2007) that shows a general increase in both ^{13}C and ^{15}N values with increasing trophic level. Multivariate-spaces have been used for decades to trace the flow of energy and resources within and between marine and terrestrial ecological communities. This approach has also been used in conjunction with ecotoxicological analysis (see below). Furthermore, the application of tissue-specific trophic fractionation factors to consumer isotope values allows for a qualitative estimate of diet or in some cases may yield quantitative results through the use of isotope mixing models (see below). Early papers often compare isotope values among sympatric or closely related species (*e.g.*, Rau *et al.* 1992, Ostrom *et al.* 1993, Walker and Macko 1999), analyze a suite of tissue types, and typically do not include data for common prey, which are sometimes difficult to obtain from open-ocean habitats. More recent studies typically focus on a single species and use a single tissue to provide foraging information for a specific period of time, dependent on the isotopic turnover rate of the tissue, often inferred from controlled feeding experiments on other taxa (birds or terrestrial mammals). In some cases, accretionary tissues (*e.g.*, Hobson and Sease 1998, Niño-Torres *et al.* 2006, Newsome *et al.* 2007*b*, 2009*a*), continuously growing but metabolically inert tissue (*e.g.*, Schell *et al.* 1989, Lewis *et al.* 2006, Newsome *et al*. 2009*b*), or a suite of tissues assumed to have different isotopic incorporation rates (*e.g.*, Sinisalo *et al.* 2008) have been analyzed to construct a longitudinal record of dietary or trophic level variation.

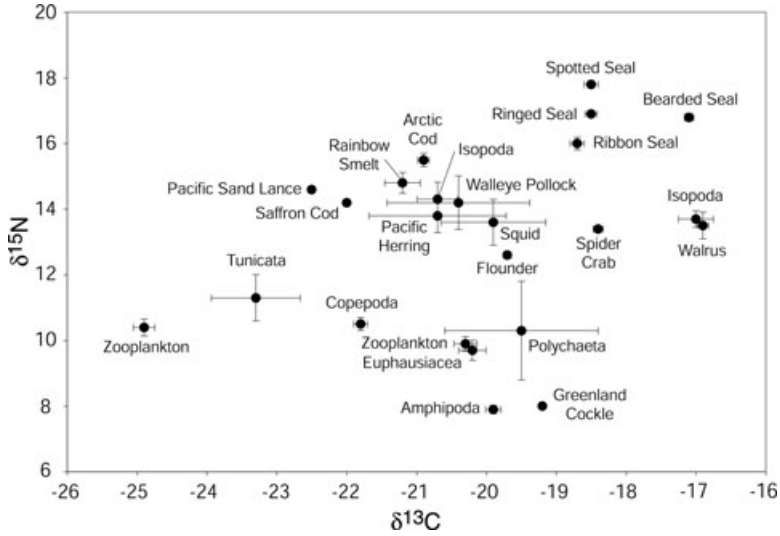


Figure 2. Bivariate isotopic space of an Alaskan arctic food web showing a general increase in mean $\delta^{13}C$ and $\delta^{15}N$ values with increasing trophic level from the lower left to the upper right corner the figure; errors bars are standard error. All samples were collected from Barrow, Alaska, the Bering Strait, or eastern Chukchi Sea. Refer to original sources for exact collection location, sample sizes, and scientific names of species. Data from Schell *et al.* (1998), Hoekstra *et al.* (2002), Dehn *et al.* (2007).

Nursing and Weaning

Mother-to-offspring transfer of nutrients during pregnancy and nursing has been the focus of several recent isotopic studies (Jenkins *et al.* 2001, Polischuck *et al.* 2001, Newsome *et al.* 2006, Stegall *et al.* 2008, York *et al*. 2008). Isotopic methods are particularly useful in evaluating mother-to-offspring nutrient transfer because lactating mothers catabolize their tissues to produce milk; nursing offspring are consuming their mother's tissues and thus are feeding a trophic level higher than their mothers. For carbon isotopes, this prediction is complicated by the fact that milk can have a high concentration of ^{13}C-depleted lipid. An animal that produces milk with a high-lipid content, such as an otariid with milk that is 15–50 weight% lipid (Costa 2002), feeds its young a food source with a relatively low $\delta^{13}C$ value. There are no pronounced differences in $\delta^{15}N$ value between lipids and associated proteins, so the consumption of lipid-rich milk would not affect ^{15}N-enrichment. Thus, nursing offspring should have $\delta^{15}N$ values 3–5‰ higher and $\delta^{13}C$ values either lower or similar to their mothers, depending on milk lipid content.

Isotopic studies of nursing and recently weaned marine mammals have used samples from ontogenetic series of bones and/or annuli in dentin from sectioned teeth. For pinnipeds, analysis of dental annuli in Steller sea lions and California sea lions (*Zalophus californianus*) shows that nursing young have higher $\delta^{15}N$ values (2‰–3‰) and lower $\delta^{13}C$ values (1‰–2‰) than adult females (Hobson and Sease 1998, Newsome *et al.* 2006, York *et al*. 2008). York *et al*. (2008) used isotopic and growth line data from canines to argue that weaning age increased and growth rate decreased in Steller sea lions from the 1960s to the 1980s, perhaps due to a reduction in

available resources. Ontogenetic series of modern northern fur seal bones from the Pribilof Islands (southeastern Bering Sea) show that preweaned and recently weaned pups (aged 2–6 mo) have $\delta^{15}N$ values that are approximately 5‰ higher than juveniles aged 12–20 mo (Newsome *et al.* 2006). Furthermore, adult female $\delta^{15}N$ values are 2‰–3‰ lower than young pups (aged 2–6 mo), but significantly higher than those of juveniles. The $\delta^{13}C$ values of the ontogenetic series show no trend with age. In contrast, this technique is not reliable for assessing maternal strategies in phocids (Hobson and Sease 1998), because they typically wean their young at a very young age (1–2 mo). Most of the dentin formed in their first year of life represents independent foraging for prey, not ^{15}N-enriched dentin deposited during the nursing period.

This technique has proven to be effective for investigating maternal strategies in large odontocetes, such as sperm whales (Mendes *et al.* 2007*b*) and killer whales (Newsome *et al.* 2009*a*). The approach has also been applied to small odontocetes that have relatively small teeth. In such cases, individual growth layers must be combined to generate enough dentin for isotopic analysis (Knoff *et al.* 2008). Alternatively, a single tooth from different individuals of various ages can be homogenized and analyzed (Niño-Torres *et al.* 2006) to create a population level compilation of ontogenetic patterns in isotope values. Despite these limitations, ontogenetic dietary shifts associated with weaning have been observed in teeth of bottlenose dolphins (*Tursiops truncatus*, Knoff *et al.* 2008) from the southeast United States and long-beaked common dolphins (*Delphinus capensis*, Niño-Torres *et al.* 2006) from the Gulf of California.

To further highlight the isotopic trends associated with nursing and weaning, we present data from three species that employ different maternal strategies (Fig. 3). The data represent a time series of serially sampled dentinal growth layers from California sea lion, killer whale, and sperm whale teeth. Relatively high $\delta^{15}N$ values in the first year of life for each profile denote a period when the individuals were dependent on their mother's milk. Intermediate $\delta^{15}N$ values in the second (California sea lion, Fig. 3A) and sometimes third annulus of some individuals (killer whale, Fig. 3B; sperm whale, Fig. 3C) represent a period when young animals consume a mixture of milk and solid prey. Once animals are fully weaned, $\delta^{15}N$ values stabilize and remain relatively constant from year to year. If $\delta^{15}N$ values for both the second and third year are higher than average values from later years, then weaning was likely gradual. In addition to offering insight into maternal strategies, these data also offer information on age-related shifts in diet and within-individual isotopic variation, which can be compared to among-individual variation when evaluating individual dietary specialization and temporal variation in niche width (*e.g.*, Lewis *et al.* 2006, Cherel *et al.* 2007, Newsome *et al.* 2009*b*).

While isotopic data can yield unique information on species that are difficult or near impossible to observe in the wild, uncertainty about the rates of isotopic turnover in tissues, especially tissues with relatively slow rates such as bone collagen, complicate assessment of absolute weaning age. For example, in the study of the ontogenetic series from northern fur seals (Newsome *et al.* 2006), the $\delta^{15}N$ ontogenetic series was used to determine the amount of time it takes for bone collagen turnover to dilute the nursing signal. Modern northern fur seals are abruptly weaned at 4 mo. If northern fur seals begin to ingest solid food shortly after weaning and if recently weaned animals consume similar prey types as 1- and 2-yr-old juveniles, it takes approximately 8 mo for the $\delta^{15}N$ signal of weaning to be completely diluted by bone collagen turnover. Bone collagen $\delta^{15}N$ values of these seals do not fully reflect those of their fish and cephalopod prey until animals are approximately 12-mo-old.

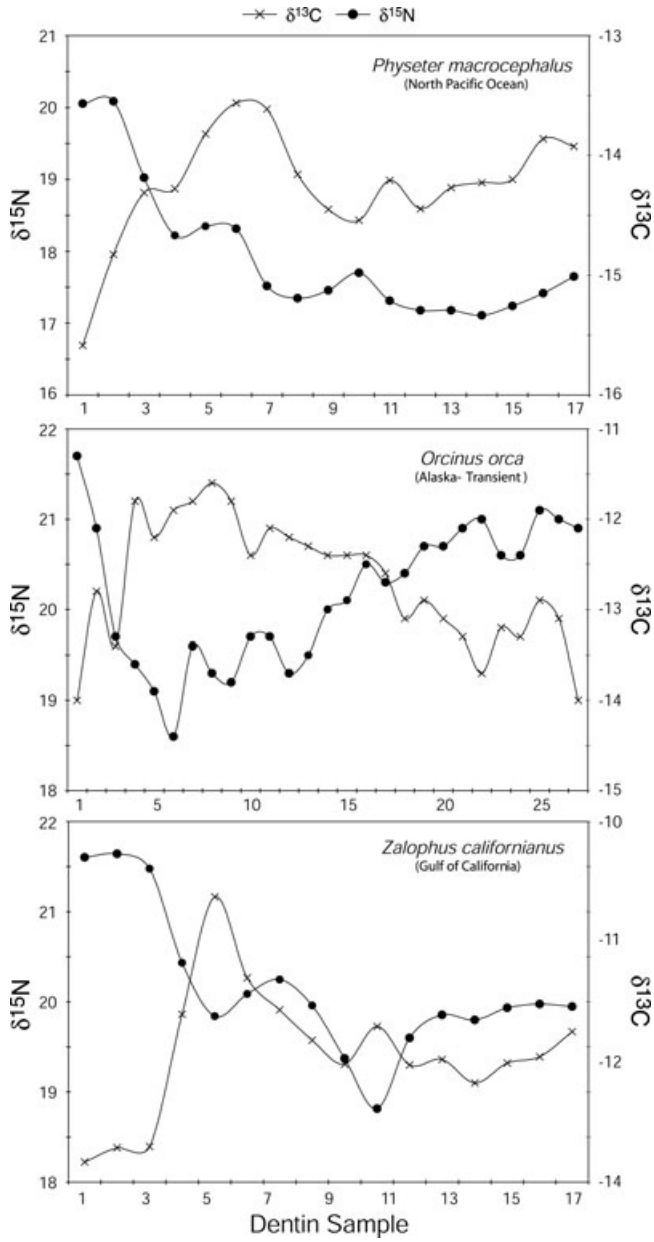


Figure 3. Ontogenetic tooth dentin $\delta^{15}N$ and $\delta^{13}C$ profiles derived from growth layers (annuli) in individual marine mammal teeth. Approximate collection location of each specimen is indicated. Note that the first several annuli typically have higher $\delta^{15}N$ and lower $\delta^{13}C$ values than subsequent growth layers, likely resulting from the consumption of milk during the extended nursing period in these species. Data for *Orcinus orca* are from Newsome *et al.* (2009*a*); profiles for *P. macrocephalus* and *Z. californianus* are from Newsome *et al*., unpublished data.

For retrospective studies that use bone collagen to examine the timing and rate of weaning (abrupt *vs.* gradual), quantitative comparisons within and among species are possible if isotopic turnover rates and errors associated with age determination are carefully considered (Newsome *et al.* 2007*a*).

Habitat Use

The isotopic composition of consumers in marine systems is ultimately set by the isotopic composition of the food and water the animal ingests. These inputs can show strong spatial isotopic gradients, consequently isotopic data can be used to study habitat preference (*i.e.*, pelagic *vs.* benthic, nearshore *vs.* offshore *vs.* estuarine), movement among habitats, and migration patterns at an ocean basin scale. Here, we briefly discuss the factors that create isotopic gradients in marine systems. We focus on carbon and nitrogen isotopes, and only briefly mention oxygen isotopes, which have primarily been used in paleontological studies. We then provide examples within two regions, the eastern North Pacific Ocean and Bering Sea.

Through decades of experiments and field collections, oceanographers have come to understand the physicochemical and biological factors that are responsible for the gradients in primary producer carbon isotope values. At the most general level, higher $\delta^{13}C$ values are associated with rapid growth and lower values are associated with slow growth (Goericke and Fry 1994, Popp *et al*. 1998). Within oceanic basins, therefore, primary producer (and particulate organic matter or POM) $\delta^{13}C$ values track productivity, with higher values found in productive nearshore regions, such as upwelling zones, in comparison to less productive offshore regions. Because of the preferential uptake of ^{12}C by plants during photosynthesis, nutrient-driven blooms in upwelling zones increase the $\delta^{13}C$ of aqueous CO_2 by a few per mil as they draw down its concentration. Low aqueous $[CO_2]$ can itself lead to lower isotopic fractionation during photosynthesis (and therefore higher plankton or macroalgae $\delta^{13}C$ values). In offshore regions, especially in temperate and equatorial regions where the water column is strongly stratified, low nutrient levels lead to low growth rates, so these factors are less important and $\delta^{13}C$ values are lower. The gradient in $\delta^{13}C$ values between primary producers in nearshore *vs.* offshore pelagic ecosystems has other, additive causes, including the effects of phytoplankton size and taxonomic differences on isotopic fractionation (Bidigare *et al*. 1997, Pancost *et al*. 1997, Rau *et al*. 2001). Finally, macroscopic marine plants, such as kelp and sea grass, have substantially higher $\delta^{13}C$ values than phytoplankton. Using data compiled from the literature, Clementz and Koch (2001) showed that major marine and marginal marine habitat types (open ocean, nearshore, sea grass, kelp forests) have distinct $\delta^{13}C$ values.

The $\delta^{13}C$ values of primary producers and POM also vary predictably among ocean basins. High-latitude pelagic ecosystems typically have much lower $\delta^{13}C$ values than lower latitude ecosystems. In colder regions, aqueous $[CO_2]$ is high due to seasonally low photosynthetic rates, vertical mixing of a water column that is not strongly thermally stratified, and the greater solubility of CO_2. Under high aqueous $[CO_2]$, the fractionation associated with photosynthetic CO_2 uptake is strongly expressed, leading to low $\delta^{13}C$ values. The converse applies in the warm, well lit, stratified waters of temperate and equatorial latitudes. Finally, taxon-specific biological variables and local conditions must be important, because meridional gradients in POM $\delta^{13}C$ values are different in the southern *vs.* northern oceans (Goericke and Fry 1994).

The $\delta^{15}N$ values of plankton at the base of marine food webs (and particulate organic nitrogen or PON) also show spatial gradients (discussion based on Montoya

2007). N_2 fixation by cyanobacteria, which is important in oligotrophic regions such as the North Pacific Subtropical Gyre or the Sargasso Sea, generates organic matter with low $\delta^{15}N$ values (−2–0‰). In most regions, however, marine production is fueled by nitrate. The $\delta^{15}N$ values of phytoplankton in these regions reflects two factors: (1) the $\delta^{15}N$ values of sources of nitrate to the photic zone, especially the upwelling of nitrate-rich deep water, and (2) whether or not nitrate uptake by phytoplankton approaches 100%. Where nitrate uptake is complete (the situation in most regions), the annually integrated $\delta^{15}N$ value of primary production must equal the $\delta^{15}N$ value of inputs. The vast subsurface nitrate pool that mixes into the photic zone averages approximately +5‰. However, below highly productive regions, pelagic deep water can become suboxic to anoxic. In the absence of adequate O_2, bacteria turn to nitrate to respire organic matter (denitrification), which preferentially removes ^{14}N-enriched nitrate and leaves the residual nitrate strongly ^{15}N-enriched (+15‰–+20‰). Geographic differences in upwelling intensity and the extent of subsurface denitrification create large-scale spatial differences in the $\delta^{15}N$ value of phytoplankton. Finally, if uptake of nitrate is incomplete, then marine organic matter can have lower $\delta^{15}N$ values, because phytoplankton preferentially assimilate ^{14}N-enriched nitrate.

Environmental factors that might affect the $\delta^{18}O$ value of ambient water for marine mammals are few. Meteoric water $\delta^{18}O$ values vary spatially and temporally, with higher values in warm regions or seasons, lower values in colder regions or seasons, and total amplitude of variation of nearly 40‰. Ocean surface water, in contrast, shows only minor variations in $\delta^{18}O$ value. Values are slightly higher (+1–+2‰) in regions affected by evaporation. In areas receiving heavy rainfall or that are affected by runoff of strongly ^{18}O-depleted freshwater (principally at high latitudes), marine $\delta^{18}O$ values can be lower (−3‰–−5‰) (LeGrande and Schmidt 2006). Overall, the subtle variations in marine $\delta^{18}O$ values are positively correlated to salinity and negatively correlated with latitude. Species that make occasional or regular use of brackish or fresh water habitats may encounter waters with $\delta^{18}O$ values substantially lower than seawater.

Case Study: Northeast Pacific Ocean

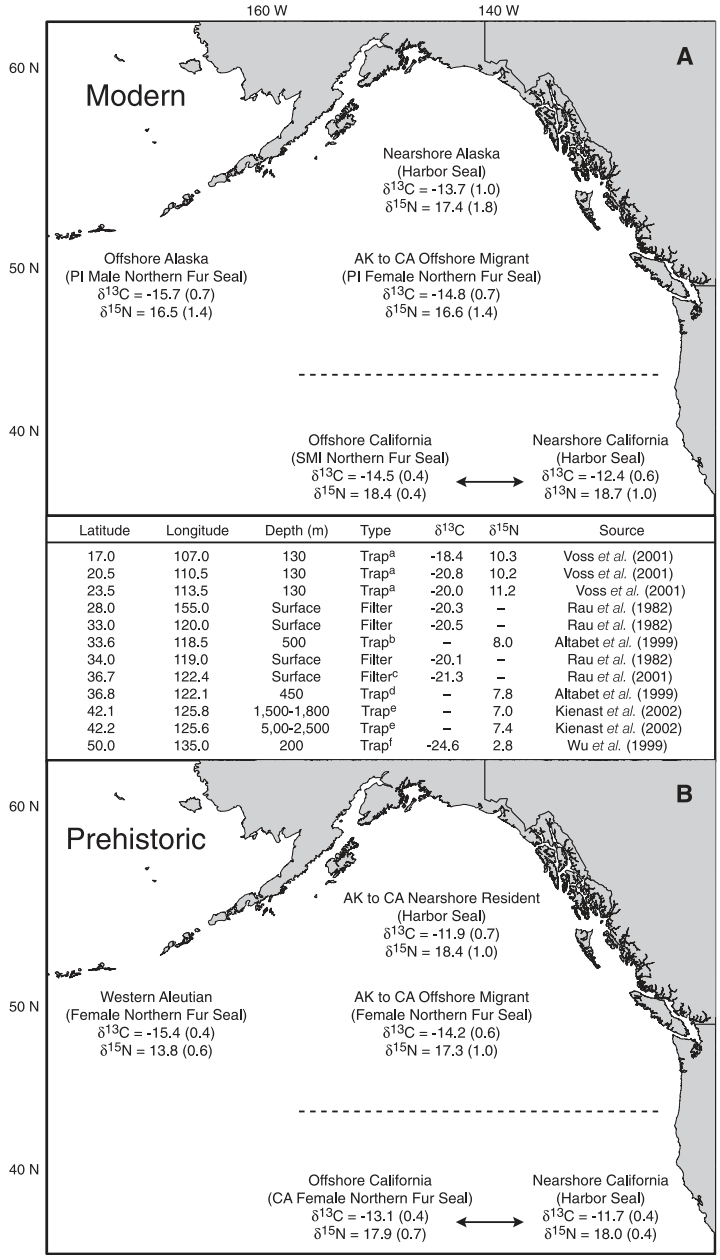
To illustrate how patterns in isotope values can be used to study marine mammal ecology at a regional scale, we offer a short description of carbon and nitrogen isotope gradients in the eastern North Pacific Ocean and Bering Sea. The geographical patterns in phytoplankton and primary consumer (*i.e.*, zooplankton) isotope values have been established in the region through oceanographic study, and it is home to a diverse group of marine mammals, some of which have recently been the focus of studies utilizing stable isotopes.

There is a 2‰–3‰ decrease in food web $\delta^{13}C$ and $\delta^{15}N$ values from temperate (approximately 30°–35°N) to high-latitude (~50°N) northeast Pacific pelagic ecosystems (Fig. 3; Saino and Hattori 1987, Goericke and Fry 1994, Altabet *et al.* 1999, Rau *et al.* 2001, Kienast *et al.* 2002). Higher temperatures and extensive upwelling lead to higher phytoplankton growth rates (and higher $\delta^{13}C$ values) in the California Current (CC) relative to the Gulf of Alaska. Higher productivity in coastal systems along the entire eastern Pacific and southern Bering Sea lead to higher nearshore ecosystem $\delta^{13}C$ values when compared to offshore systems. Off the central and northern California coast, phytoplankton growth rates (and $\delta^{13}C$ values) are also higher in nearshore environments affected by seasonal upwelling when compared

to offshore habitats. Similar onshore-offshore differences have been documented in the Bering Sea. Zooplankton and euphausiid $\delta^{13}C$ values decrease from east to west by approximately 2‰ across the continental shelf-slope break in the southeastern Bering Sea, and are even lower to the north, in the Arctic Ocean and Beaufort Sea (Schell *et al.* 1998).

Nitrogen isotope values are also higher at temperate latitudes in the northeastern Pacific because intermediate waters in the CC are sourced from the eastern tropical Pacific Ocean, where there is substantial denitrification at depth (Altabet *et al.* 1999; Voss *et al*. 1996, 2001). This ^{15}N-enriched nitrate is carried northward at depth *via* the California Undercurrent and is an important source of nitrogen to surface waters in the CC. A second pattern emerges when comparing sediments from the Gulf of California (assumed to reflect the $\delta^{15}N$ value of sinking PON) to those from the CC. Sediment $\delta^{15}N$ values are approximately 2‰ higher in the Gulf of California (Altabet *et al.* 1999), most likely due to the influence of local denitrification and to the Gulf's closer proximity to the ^{15}N-enriched waters of eastern tropical Pacific Ocean. Last, primary producer and consumer $\delta^{15}N$ values decrease by approximately 3‰ from east to west in the southeastern Bering Sea across the shelf-slope break (Schell *et al.* 1998), most likely due to differences in the extent of vertical mixing and incomplete utilization of nitrate in the western Bering Sea.

The regional gradients outlined above have been used extensively to characterize marine mammal movement patterns for a variety of species. Schell's (1989) pioneering work showed that the large $\delta^{13}C$ and $\delta^{15}N$ gradients in high-latitude food webs could be exploited to study seasonal migration of bowhead whales (*Balaena mysticetus*) between the Bering and Beaufort Seas. This study was followed by a series of papers that used baleen plates as continuous recorders of ecological information (Hobson and Schell 1998; Schell 2000, 2001; Lee *et al.* 2005). Hobson *et al.* (1997*b*) suggested that differences in $\delta^{13}C$ values between harbor seals and Steller sea lions from Washington and Alaska were likely due to meridional and onshore *vs.* offshore differences in preferred foraging habitat between the two species. Burton and Koch (1999) and Burton *et al*. (2001) compared bone collagen $\delta^{13}C$ and $\delta^{15}N$ values among four species of sympatric pinnipeds in the northeast Pacific and found that at a single latitude, nearshore foragers (*e.g.*, harbor seals) have higher $\delta^{13}C$ values than species that forage offshore at the continental shelf-slope break (*e.g.*, northern fur seals) (Fig. 4). Intraspecific comparisons also showed that high latitude populations in Alaskan waters have lower $\delta^{13}C$ and $\delta^{15}N$ values than temperate latitude populations from California, whereas animals that migrate between Alaska and California (*e.g.*, adult female northern fur seals from Alaskan rookeries) have intermediate values. Furthermore, male northern elephant seals (*Mirounga angustirostris*) from Point Año Nuevo, California, have $\delta^{13}C$ and $\delta^{15}N$ values similar to higher latitude harbor seals, confirming that they foraged nearshore at high latitudes (a fact supported by tracking data (Le Boeuf *et al*. 2000), whereas females from this rookery have values more similar to animals foraging offshore at middle latitudes. Aurioles *et al.* (2006) showed that northern elephant seal pups from breeding colonies off the Pacific coast of Baja California have lower hair $\delta^{13}C$ and $\delta^{15}N$ values than pups from central California, and suggested that adult females from Mexico forage, on average, at lower latitudes than their northern counterparts. Last, spatial gradients in food web values have also been used to investigate prehistoric pinniped ecology, as discussed in detail in the *Historic Ecology and Paleoecology* section below.



Latitude	Longitude	Depth (m)	Type	$\delta^{13}C$	$\delta^{15}N$	Source
17.0	107.0	130	Trap[a]	-18.4	10.3	Voss *et al.* (2001)
20.5	110.5	130	Trap[a]	-20.8	10.2	Voss *et al.* (2001)
23.5	113.5	130	Trap[a]	-20.0	11.2	Voss *et al.* (2001)
28.0	155.0	Surface	Filter	-20.3	–	Rau *et al.* (1982)
33.0	120.0	Surface	Filter	-20.5	–	Rau *et al.* (1982)
33.6	118.5	500	Trap[b]	–	8.0	Altabet *et al.* (1999)
34.0	119.0	Surface	Filter	-20.1	–	Rau *et al.* (1982)
36.7	122.4	Surface	Filter[c]	-21.3	–	Rau *et al.* (2001)
36.8	122.1	450	Trap[d]	–	7.8	Altabet *et al.* (1999)
42.1	125.8	1,500-1,800	Trap[e]	–	7.0	Kienast *et al.* (2002)
42.2	125.6	5,00-2,500	Trap[e]	–	7.4	Kienast *et al.* (2002)
50.0	135.0	200	Trap[f]	-24.6	2.8	Wu *et al.* (1999)

Figure 4. Mean $\delta^{13}C$ and $\delta^{15}N$ values (SD) of modern pinniped bone collagen and particulate organic matter (POM) sourced from the northeast Pacific Ocean. For pinniped data, PI and SMI refer to Pribilof Islands, Alaska, and San Miguel Island, California, respectively. Superscripts adjacent to POM collection types are as follows: (a) traps were deployed for 33–38 h, data from offshore sites with highest sedimentation rates; (b) weighted average for biweekly trip samples spanning 6 mo; (c) weighted average for data spanning 4 yr; (d) weighted average for biweekly trap samples spanning 3.25 yr; (e) average for multiple traps left open for 6 mo; (f) weighted average for 19 (N) or 20 (C) traps spanning 1 yr. Pinniped data from Burton and Koch (1999).

Tracking Contaminants

Identification of the sources, pathways, and degree of biomagnification of different organic contaminants and trace metals in food webs is essential to assess current and future impacts of anthropogenic activities on marine mammal health and population viability. Studies of this type focus on the relationship of trace metals or organic pollutants with biological factors such as diet, age, sex, nutritional status, and movement patterns. For air-breathing species in marine (or aquatic) food webs, the primary route of contaminant exposure is diet, so SIA is a natural extension to ecotoxicological research that can help constrain the impacts of these biological factors. This rapidly expanding area of research was recently reviewed by Jardine *et al.* (2006), who outlined several sources of uncertainty that require careful consideration when applying SIA to ecotoxicological studies. In light of these efforts, here we provide a brief summary of this approach and then highlight a few examples that fall into two general types of applications: studies that investigate the trophic transfer or biomagnification of contaminants and those that use contaminant profiles to characterize marine mammal population structure and niche variation (Table 1).

Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), organochloride pesticides (*e.g.*, DDT and its derivatives), perflourinated organochemicals (FOCs) and heavy metals (*e.g.*, Hg, Pb) are just a few types of hazardous contaminants that have been found in marine mammal tissues. These compounds are products (or byproducts) of industrial and agricultural applications. They are especially persistent because biological processes for the most part lack the capability to excrete such molecules and heavy metals or to transform them into less hazardous compounds. Studies of top marine consumers can also provide information on the relative concentration of contaminants at lower trophic levels. Some of these compounds are subject to biomagnification as they move up food chains and can be described using log transformed plots of contaminant concentration *vs.* $\delta^{15}N$ value.

The isotopic and contaminant analysis of marine mammal tissues has been applied in a wide range of marine environments, from assumed pristine arctic ecosystems to areas immediately adjacent to intensive industrial and/or agricultural activities. Geographical variability in marine mammal tissue contaminant concentrations is not only due to spatial variation in the types and concentrations of contaminant source(s), but is also assumed to result from interspecific and interpopulational differences in behavior. Temporal and/or seasonal shifts in marine mammal contaminant concentrations are other important, but less intensively studied, factors in determining exposure risk, especially in light of the high degree of mobility and strongly seasonal reproductive cycles that characterize many species.

Trophic Transfer and Biomagnification

There is no evidence for the biomagnification of Ag, Zn, or Cu with increasing trophic level (as assessed isotopically), and Cd decreased with increasing trophic level in several small cetacean species in the northeast Atlantic Ocean (Das *et al.* 2003*a*, Dehn *et al.* 2006*a*). Conclusions on Hg biomagnification are mixed. Dehn *et al.* (2006*b*) found little evidence for Hg biomagnification, whereas data from Atwell *et al.* (1998) suggest a significant, positive log relationship between [Hg] and $\delta^{15}N$ values in arctic food webs. Theory and empirical studies show that [Pb] is lowest in

top trophic level consumers (Michaels and Flegal 1990), because Pb is biodepleted relative to its biogeochemical analogue calcium. The combination of [Pb] and stable Pb isotopes ($^{207}Pb/^{206}Pb$) have been especially useful in documenting historical shifts in the source(s) and sometimes concentrations of Pb between preindustrial and modern times (Smith *et al.* 1990, Outridge *et al.* 1997, Caurant *et al.* 2006).

Most of the studies on organochemical contaminants evaluate exposure at the community or ecosystem level and present data from multiple trophic levels that often include one or more marine mammal species. Significant, positive correlations among PCB, DDT, and FOC concentrations and trophic level, as derived from $\delta^{15}N$ values, are strong evidence for bioaccumulation of these compounds in marine food webs (Jarman *et al.* 1996, Fisk *et al.* 2001, Hobson *et al.* 2002, Tomy *et al.* 2004). Coupled contaminant and $\delta^{13}C$ analysis also suggest differences in FOC contaminant loads among marine mammal species that occupy nearshore versus offshore habitats (Van de Vijver *et al.* 2003).

Population Structure and Niche Variation

The blending of contaminant and SIA also yields information on population structure and niche variation at the individual, population, or species level. At first, contaminant concentrations alone were used in this capacity (see review by Aguilar 1987). More recently, however, researchers have begun to integrate toxicological and isotopic proxies. In essence, geographic variability in natural elements (*i.e.*, food web isotope values) or anthropogenic compounds (*i.e.*, contaminants) provides independent but complimentary chemical tracers that can have signatures unique to the region(s) in which an organism forages. This strategy has been applied to small cetacean populations in the southwestern Mediterranean Sea, the northeast Atlantic Ocean, and Black Sea (Das *et al.* 2000, 2004*b*; Borrell and Aguilar 2005; Borrell *et al.* 2006); ringed seal populations in the Canadian Arctic (Fisk *et al.* 2002*a*); minke whales in the North Atlantic (Born *et al.* 2003); and killer whale ecotypes in the North Pacific Ocean (Herman *et al.* 2005; Krahn *et al.* 2007, 2008).

As with most ecological applications of stable isotope analysis, diet and habitat preferences are the primary pieces of information acquired through study of population structure and niche separation. The success of this approach depends on the presence of sufficiently large and distinct isotopic differences between different prey types or foraging locations (refer to the section on *Habitat Use*) that can be exploited to assess the level of communication and ecological overlap between populations. This also requires large sample sizes from each population to ensure adequate representation and estimation of the range of isotopic values typical for each population or species. Also, researchers must take care to select tissues with relatively slow turnover rates and long integration times (*e.g.*, skin) to ensure that short-term records of diet change do not erroneously inflate the range in isotopic values and thus complicate discrimination of different populations. Furthermore, *a priori* knowledge of the populations of interest through previous field observations or genetic studies is needed to ensure appropriate sampling of individuals. For example, Krahn *et al*. (2007) relied heavily on long-term field studies and mtDNA haplotype identification to separate killer whale specimens into three North Pacific ecotypes: transient, resident, and offshore. Without this information, random sampling of individuals would have been insufficient to guarantee adequate representation of each population in the pool of specimens sampled and, therefore, isotopic and contaminant differences among

individuals would have been difficult to interpret in a meaningful way. When combined with contaminant concentrations and other lines of ecological information (*e.g.*, fatty acid profiles), stable isotope analyses of marine mammal tissues can be a powerful tool for gaining insight into the structure and diet variation of separate populations. Given the significant role these projects can play in regards to justifying the protection of unique marine mammal populations or species, effort must be made to ensure that all populations of interest are identified and adequately sampled over the course of these studies.

Historical Ecology and Paleoecology

In considering applications of SIA to historical ecology and paleoecology, we examine studies on three temporal scales: the last few centuries, the last 10,000 yr, and deep time (millions of years). Studies spanning the last few centuries or millennia typically involve extant or recently extinct populations or species. They are used to illuminate the full range of a species' response to environmental change or anthropogenic perturbation. Deep time studies typically involve extinct species. They explore the paleoecology of particular groups, as well as the evolutionary ecology of the transition from land to water in cetaceans and sirenians.

The Last Few Centuries

The simplest historical studies are those that assess whether the behavior of a species documented during a period of direct observation is characteristic for the species on a longer time scale. For example, Walker *et al*. (1999) studied the diets of bottlenose dolphins (*T. truncatus)* in the western North Atlantic in the 1980s, searching for a contrast between coastal and offshore ecotypes. Coastal foragers had higher $\delta^{15}N$ and $\delta^{13}C$ values than offshore foragers. They argued that coastal dolphins fed on piscivorous fish from benthic food webs, whereas offshore dolphins fed on smaller pelagic squid and fish. Walker *et al*. (1999) then examined coastal dolphin specimens spanning the previous century. These animals had similar isotopic values in the 1880s, 1920s, and 1980s, leading Walker *et al*. (1999) to conclude that coastal bottlenose dolphin diets had changed little over the last century—an idea supported by scant published records of gut contents from the prior century.

Isotopic methods may be used to test the "killer whale" hypothesis, which explains the collapse of marine mammal populations in the north Pacific in the latter half of the 20th century as a result of prey switching by killer whales (*Orcinus orca*) (Springer *et al*. 2003, 2008). The hypothesis posits that industrial whaling in the mid 20th century reduced the biomass of great whale prey for killer whales. Killer whales were forced to switch to predation first on pinnipeds (Steller sea lions, harbor seals, northern fur seals), and then on sea otters (*E. lutris*), leading to the sequential collapse of marine mammal prey populations. It is unlikely that SIA of killer whales could detect a switch from a pinniped diet to one that included but was not entirely based on sea otters, as might have occurred 1980s and 1990s (Williams *et al*. 2004). However, a shift from a baleen whale diet to one rich in pinnipeds in the 1950s or 1960s should be testable. A promising way to evaluate this hypothesis is through isotopic analysis of tooth dentin growth layers from modern and historic transient whales archived in museum collections. Killer whale teeth provide a longitudinal, near

annual resolution record of foraging information at the individual level (Newsome *et al.* 2009*a*).

In other cases, isotopic records from marine mammals have been used as proxies to study changes in the biosphere over the last few centuries. For example, Smith *et al.* (1990) compared the stable Pb isotope ratios of contemporary sea otters from the Aleutians to those of preindustrial otters (as measured from the teeth of fossils from middens). While [Pb] did not differ between modern and preindustrial otters, isotopic composition did, demonstrating that otters today receive Pb from industrial sources.

Another major set of studies has revolved around the claim by Schell (2000) that the $\delta^{13}C$ value of North Pacific and Bering Sea food webs has decreased since the 1960s. Schell (2000) argued that this decrease signaled a drop in photosynthetic rate and therefore a drop in primary production in the region, perhaps explaining the collapse of the marine mammal populations discussed above. The time series in Schell (2000) was constructed using data from 37 bowhead whale baleen plates. The plates have annual growth bands that can be counted to produce a chronology and sampled subannually for SIA. These within-individual time series exhibit strong $\delta^{13}C$ cycles, which Schell *et al*. (1989) and Schell (2000) related to seasonal migration between the Bering Sea (high values) and Chukchi Sea (low values). Using just the high values for a given year, Schell (2000) compiled an isotopic time series for the Bering Sea.

The study raised questions on two grounds. First, the shifts Schell (2000) detected may relate more to changes in whale migration or diet than to any shift in $\delta^{13}C$ values of Bering Sea phytoplankton. Second, as noted by Cullen *et al*. (2001), phytoplankton $\delta^{13}C$ values should have dropped over the last 60 yr due to the rise in atmospheric CO_2, because fossil fuel combustion pumps ^{13}C-depleted carbon into global ecosystems, and because high aqueous [CO_2] leads to increased photosynthetic fractionation. The concern about the "reality" of the drop in North Pacific $\delta^{13}C$ values has been addressed through study of additional time series from other species, including pinnipeds and sea birds (Hirons *et al*. 2001*a*, Hobson *et al*. 2004*b*). The most controlled study in temporal, spatial, and taxonomic terms is Newsome *et al*. (2007*b*). The authors sampled dentin from the third dental annulus of male northern fur seals from a single rookery on Saint Paul Island in the Pribilofs, with intensive sampling (~5 samples/yr) from 1948 to 2000, as well as a few scattered samples from the early 20th century. Mean annual $\delta^{13}C$ values declined by approximately 1.1‰ from 1948 to 2000 (Fig. 5A), while long-term mean annual $\delta^{15}N$ values did not change significantly (Fig. 5B). The relatively small but significant long-term drop in $\delta^{13}C$ values can be entirely explained by the anthropogenic changes in surface ocean carbon reservoirs noted by Cullen *et al*. (2001) and need not entail a decline in primary productivity as posited by Schell (2000, 2001). Finally, both $\delta^{13}C$ and $\delta^{15}N$ time series showed low amplitude oscillations with a frequency of 20–25 yr that may be related to shifts in climatic and/or oceanographic conditions resulting from the Pacific Decadal Oscillation.

The Holocene

The Pleistocene epoch, beginning approximately 1.8 mya, was marked by many dramatic climatic shifts, the waxing and waning of massive continental ice sheets, and large (~120 m), rapid fluctuations in sea level. The changes must have had

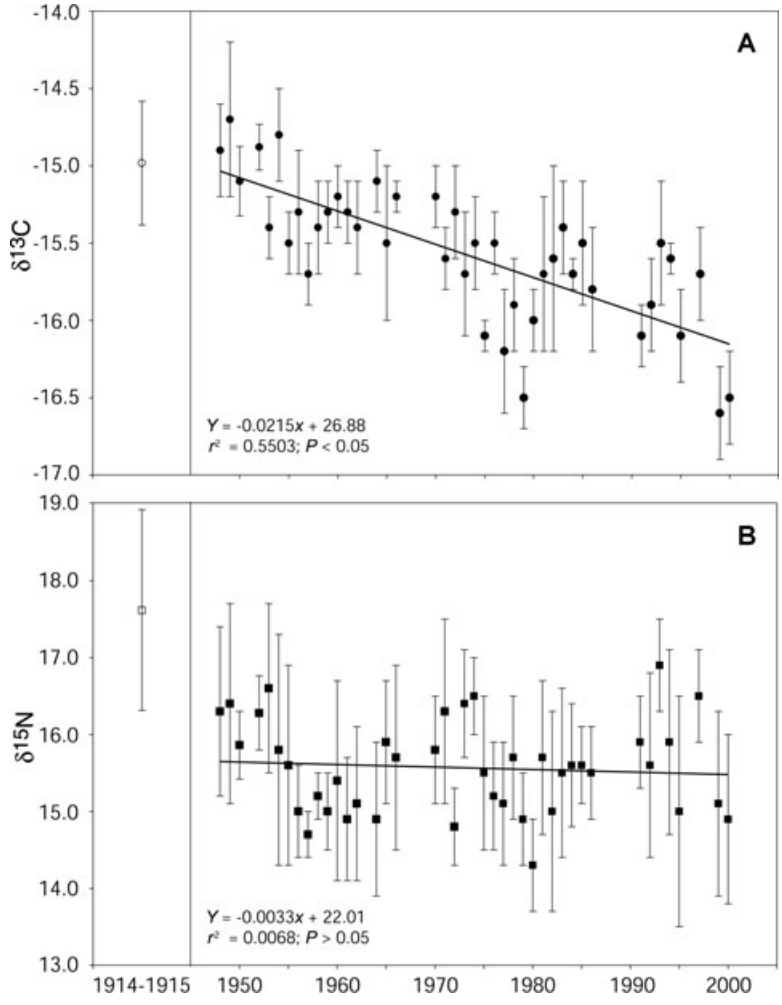


Figure 5. Historic time series of mean (±SD) tooth dentin $\delta^{13}C$ (A) and $\delta^{15}N$ (B) values of 3-yr-old northern fur seals (*C. ursinus*) sourced from Reef Rookery on Saint Paul Island (Pribilof Islands, Alaska). The third growth layer (*i.e.*, annulus) of five randomly chosen individuals were analyzed per year. The solid lines and equations are the linear models for each time series.

profound impacts on marine mammal populations. For example, at the last glacial maximum, just 20,000 yr ago, the Pribilof islands (where most northern fur seals breed today) were not islands at all, but rather were uplands at the edge of a vast low lying plain extending from Siberia to Alaska that was inhabited by a host of large carnivores (lions, sabertooths, gray wolves, brown bears, short-faced bears) (Manley 2002, Guthrie 2004). For the last 10,000 yr (the Holocene), climatic variations have been more subdued, but not absent. For example, based on the distributions of pinnipeds and cetaceans at archaeological sites in the Aleutians, Crockford and Frederick (2007) argue that the Pribilofs would have been surrounded by sea ice in

the early summer from 4,700 to 2,500 yr ago, precluding their use as a rookery site for northern fur seals.

Isotopic records from fossils and sediments shed light on the response of marine mammals to past worlds, and illuminate their behavior within them. At the most basic level, they can offer a crude proxy for the importance of animals at rookery sites when fossils are not preserved. For example, Erskine *et al*. (1998) studied the sources of nitrogen to plants on subantarctic Macquarie Island, currently home to a large rookery of southern elephant seals (*Mirounga leonina*), as well as sea bird rookeries. They discovered strong ^{15}N-gradients in plants, with very high values near marine mammal and sea bird rookeries, reflecting direct deposition of marine nitrogen from feces and guano, and much lower values in upland sites, perhaps due to deposition of ^{15}N-depleted ammonia volatizing from penguin rookeries. Bergstrom *et al*. (2002) then studied peat cores from beneath inland herb fields uplifted 20–90 m above sea level by active tectonics. At depth in these cores, in sections representing time periods in the middle Holocene, they found palynofloral evidence for nitrophiles and other plants that thrive under the disturbed conditions at rookeries, as well as strong ^{15}N-enrichment in fossil peat samples. They concluded that in the middle Holocene the sites were occupied by southern elephant seal or sea bird rookeries, a conclusion supported by the presence of seal fur in some cores. Liu *et al*. (2004) conducted a similar study on King George Island in the South Shetland Islands. They demonstrated a clear inverse relationship between sediment δ^{15}N values and the concentration of seal hairs in sediment cores, and detected two large shifts in both measures of seal abundance over the past 1,300 yr.

Isotopic data have been used to understand shifts in the ecology of northern fur seals in the eastern north Pacific (Burton *et al*. 2001, 2002; Moss *et al*. 2006; Newsome *et al*. 2007*a*). This species was common at archaeological sites from southern California to the Aleutian Islands, yet today it breeds almost exclusively on offshore islands at high latitudes and it forages offshore in pelagic waters that would have been inaccessible to native human hunters. In all sites where they co-occur, prehistoric adult female northern fur seals have lower δ^{13}C values than nearshore-foraging harbor seals, suggesting that female northern fur seals were foraging in deep, offshore waters over their entire range. Thus, the apparent availability of fur seals to prehistoric human hunters was not because they foraged close to shore. Furthermore, prehistoric adult female northern fur seals cluster isotoptically into three groups: a southern group (California) with high δ^{13}C and δ^{15}N values, a northern group (eastern Aleutian/Gulf of Alaska/Pacific Northwest) with intermediate values, and a western Aleutian group with very low isotope values. These isotopic distinctions among seals from different regions suggest that ancient northern fur seal females were less migratory than animals from the modern Pribilof Islands rookery and confirm that prehistoric fur seals from California were not immigrants from northern waters but instead were year-round residents. This conclusion is supported by archaeometric data showing that archaeological sites contain many unweaned pups, confirming the presence of temperate-latitude breeding colonies in California, the Pacific Northwest, and the eastern Aleutian Islands. SIA of ontogenetic series from ancient temperate-latitude rookeries indicates that young were weaned at 12 mo or more, as in most other eared seals, and not in 4 mo as in surviving populations of northern fur seals. Thus the collapse of ancient temperate latitude rookeries coincided with a major change in the life history and reproductive biology of the species. The relative roles of human

hunting *vs.* climatic factors in explaining these ecological and behavioral shifts are unclear and the focus of ongoing research.

The last example involves an extinct species, Steller's sea cow (*Hydrodamalis gigas*). This was the largest sirenian species (up to 5 m long) and the only one inhabiting temperate and subarctic waters. Steller's sea cow was discovered by western explorers on the Commander Islands in 1741 and was driven to extinction by overhunting by 1768 (Anderson and Domning 2002, Turvey and Risley 2006). The species had a wider distribution in the Pleistocene, from Japan to the Aleutians to southern California. In a study of archaeological and paleontological materials, Savinetsky *et al*. (2004) discovered that sea cows were more abundant in warm intervals and argued that cooling may have limited them to the Commander Islands prior to contact with Western hunters. Other authors have attributed range retraction to hunting by native peoples (Anderson and Domning 2002). In any case, the relict population observed in the 18th century fed in kelp forests; it is unclear if such behavior characterized the species across its entire geographic range.

Corbett *et al*. (2008) measured the isotopic composition of historical and fossil specimens attributed to Steller sea cows to understand the generality of kelp feeding and as a tool to understand whether bone fragments attributed to the species were correctly identified. Specimens that were unambiguously identified as sea cows (historical specimens from the Commander Islands and Pleistocene-aged fossils from the Aleutians and California) have collagen and bioapatite $\delta^{13}C$ values and collagen $\delta^{15}N$ values consistent with a diet rich in kelp. Thus the sea cows in the relict population on the Commander Islands had diets similar to those of animals in warmer regions where they may have been more abundant. In contrast, among the archaeological materials, only the samples from Kiska Island resembled extant or paleontological sea cows. Based on isotopic data, Corbett *et al.* (2008) argued that a number of other putative sea cows were in fact baleen or toothed whales.

Deep Time

We will examine three examples of deep-time isotopic paleoecology. The first is an exploration of the habitat and feeding preferences of desmostyilans. The Desmostylia are an extinct order of mammals related to sirenians and proboscideans (Domning 2002*a*). They are recovered from nearshore and, sometimes, offshore deposits along the Pacific coast of Asia and North America that range in age from 30 mya to 10 mya. The posture of these hippopotamus-sized animals, which have four weight-bearing limbs, is controversial, leading to debate about how much time they spent out of the water. Their dentition is also unusual, with thick enamel and pillar-like cusps on high-crowned teeth, and procumbent tusk-like incisors and canines. Most researchers think they were herbivores, though some suggest a diet rich in mollusks or other hard-shelled invertebrates.

Clementz *et al*. (2003) analyzed the isotopic composition of tooth enamel from the genus *Desmostylus* and co-occurring terrestrial and marine species to address the debate surrounding its ecology. *Desmostylus* had much higher $\delta^{13}C$ values than coeval terrestrial or marine mammals, suggesting a diet that consisted of submerged aquatic vegetation (sea grass or kelp). Fossil marine mammals and *Desmostylus* had low $\delta^{18}O$ variability, indicating that *Desmostylus* spent as much time in water as a seal. Finally, the strontium isotope composition of marine organisms reflects that of the ocean and is relatively invariant when compared with values from land animals.

The mean and variation in strontium isotope values for *Desmostylus* were similar to those for terrestrial, not marine, mammals. Clementz *et al*. (2003) concluded that *Desmostylus* spent time in estuarine or freshwater environments. Overall, isotopic data suggest that *Desmostylus* was an aquatic herbivore that spent a considerable portion of its life foraging in estuarine or freshwater ecosystems. The paleoecology of other desmostylians, including those found more commonly in offshore deposits, has not been examined isotopically and may differ from that of *Desmostylus*.

Isotopic methods have also been used to illuminate sirenian origins and evolution. At present, there are no isotopic data for the least derived sirenians, the Prorastomidae, which include taxa with four weight-bearing limbs such as *Pezosiren* (Domning 2001, 2002*b*). However, relatively high $\delta^{13}C$ and $\delta^{18}O$ values from another extinct clade, the Eocene-aged Protosirenidae, indicate that these fully aquatic mammals inhabited marine ecosystems, where they foraged in sea grass beds (MacFadden *et al*. 2004, Clementz *et al*. 2006) (Fig. 6A). Isotopic data reveal that Eocene-aged members of the Dugongidae (*e.g.*, *Eosiren*, *Eotheroides*, *Halitherium*), which include extant dugongs and Steller's sea cow, were also marine animals foraging on sea grass. The Miocene-aged dugongid, *Metaxytherium*, from Florida also fed largely on marine sea grass, though some individuals consumed emergent C3 plants from freshwater systems, and the large tusked dugongid, *Corystosiren*, consumed carbohydrate-rich rhizomes of sea grasses and other submerged aquatic plants. *Metaxytherium* is the sister-group to the lineage containing *Hydrodamalis*, so kelp foraging appears to have arisen during the middle to late Miocene. In the Trichechidae, the most primitive genus, *Potamosiren*, has low $\delta^{13}C$ and $\delta^{18}O$ values, consistent with foraging in freshwater ecosystems. Members of the genus *Trichechus*, including extant manatees, have very catholic dietary and habitat preferences, ranging from fully freshwater to fully marine (MacFadden *et al*. 2004) (Fig. 6B). By the close of the Pliocene, these species were the only sirenians to persist in the Caribbean and West-Atlantic region. In the face of increasing environmental change, the generalized diet and habitat preferences of *Trichechus* may have favored its survival over that of the more specialized dugongids. In contrast, specimens of *Metaxytherium* sampled from the Mediterranean across the Messinian Salinity Crisis show a significant decrease in body size that is correlated with higher enamel $\delta^{13}C$ and $\delta^{18}O$ values; these findings demonstrate that some dugongids were able to weather significant salinity changes while maintaining a constant diet through ecophenotypic dwarfing (Clementz *et al*. 2009). However, as in the Caribbean and West-Atlantic region, subsequent and significantly greater climate and environmental change at the end of the Pliocene may have been an important factor accounting for the eventual extinction of dugongids in the Mediterranean. Overall, isotopic data support the following scenario for sirenian evolution. The modest radiation of sirenians began in marine ecosystems focused on sea grass, and then expanded late in its history to include marine kelps and freshwater habitats and vegetation.

Our final deep-time case study involves the evolution of aquatic habitat preferences and diets in cetaceans. A series of papers (Thewissen *et al*. 1996, Roe *et al*. 1998, Clementz *et al*. 2006) has explored the ecology of Eocene-aged Archaeocete whales in five families: Pakicetidae, Ambulocetidae, Remingtonocetidae, Protocetidae, and Basilosauridae (see Thewissen and Williams 2002 for descriptions of each family). *Pakicetus*, a wolf-sized piscivore from Pakistan with cursorial fore and hind limbs, has low $\delta^{13}C$ values, low mean $\delta^{18}O$ values, and low $\delta^{18}O$ variability, all consistent with an aquatic wading animal that fed on freshwater aquatic prey (Fig. 7). Ambulocetids were amphibious, sea-lion sized cetaceans, with large weight-bearing

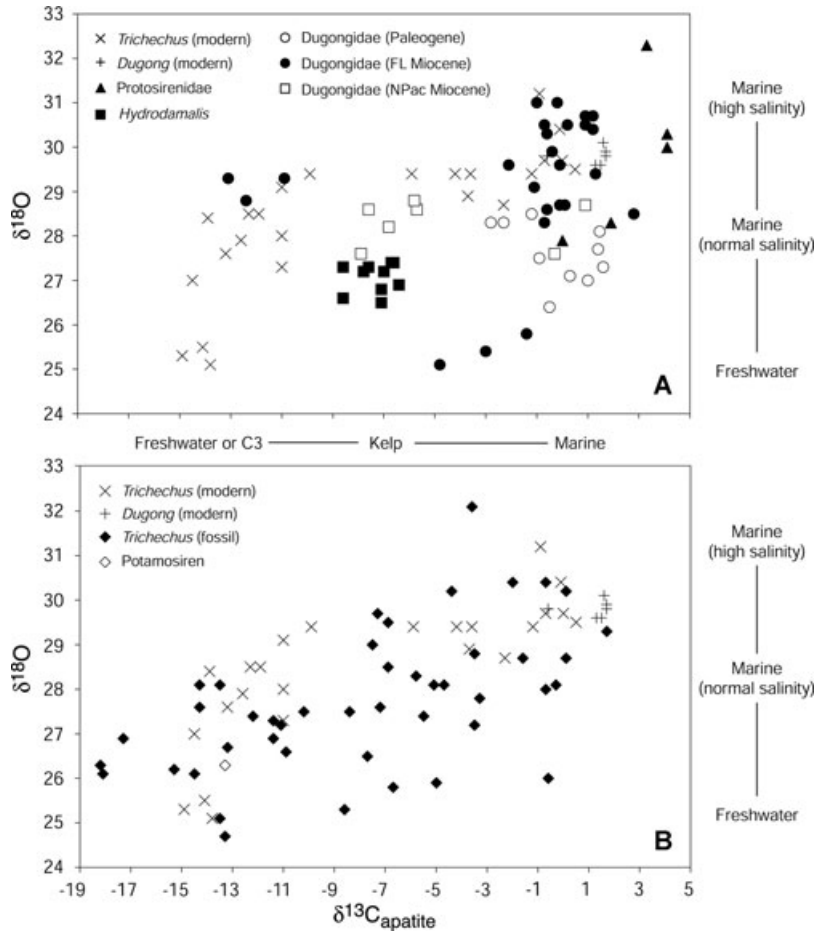


Figure 6. Carbon and oxygen isotope data for bioapatite carbonate from modern and fossil sirenians. A. Data for modern sirenians fall along an array from fully marine, sea grass feeders (*Dugong* from Australia) to fully freshwater animals feeding on emergent C3 plants and aquatic vegetation (the most ^{13}C and ^{18}O depleted manatees {*Trichechus*} from Florida). Paleogene-aged protosirenids plot near modern dugongs, as do most Paleogene-aged dugongids (*Eosiren*, *Eotheroides*, and *Halitherium* from the Tethys region). Subtle differences in $\delta^{18}O$ values among these animals likely relate to differences in the salinity driven by strong evaporation in shallow tropical seas, as well as shifts in the $\delta^{18}O$ value of seawater between the Paleogene and Neogene due to glaciation. Miocene dugongids from Florida (*Metaxytherium*) are variable; most individuals overlap modern dugongids, but several individuals fed on sea grass or macroalgae in freshwater, and others fed on C3 vegetation but lived in water with marine $\delta^{18}O$ values. Miocene dugongids from the California and Oregon coast have normal marine $\delta^{18}O$ values but $\delta^{13}C$ values range from high values consistent with sea grass consumption (*Dioplotherium*) to intermediate $\delta^{13}C$ values consistent with kelp consumption (*Dusisiren*). Holocene *Hydrodamalis* fed on kelp. B. The first trichechid, the Miocene-aged *Potamosiren* from Colombia, was a freshwater animal feeding on C3 or aquatic plants. Pleistocene-aged *Trichechus* from Florida show a wide range of values covering the full range from modern dugongs to the manatees with the lowest isotope values. Data from MacFadden *et al*. (2004), Clementz *et al*. (2006), Corbett *et al*. (2008), and new data (Miocene dugongids from Californa and Oregon).

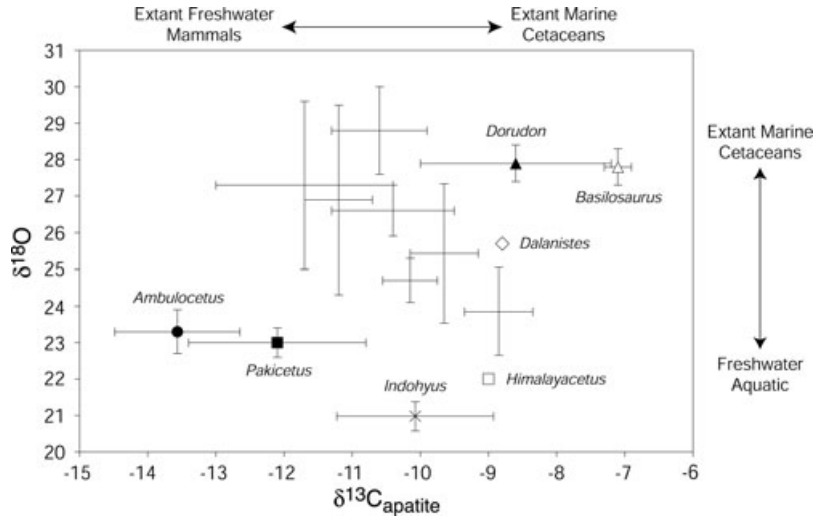


Figure 7. Mean carbon and oxygen isotope values (±SD) for fossil cetaceans (*Pakicetus*, *Ambulocetus*, *Dalanistes*, *Himalayacetus*, *Dorudon*, *Basilosaurus*), the fossil artiodactyl, *Indohyus*, and a variety of co-occurring terrestrial mammals (unlabeled crosses) including *Sorocyon*, *Cambaytherium*, *Pilgrimella*, and *Anthracobune* from the early Eocene to Middle Eocene of India or Pakistan and *Saghatherium*, *Arsinoitherium*, and *Bothriogenys* from the early Oligocene of Egypt. Single points indicate that a single specimen was analyzed. Note that all terrestrial mammals have high $\delta^{18}O$ standard deviations, whereas all cetaceans and *Indohyus* have low values, indicating aquatic lifestyles. The low $\delta^{13}C$ and $\delta^{18}O$ values for *Indohyus* and all cetaceans except *Dorudon* and *Basilosaurus* are consistent with freshwater diets and habitats. Expected ranges from modern animals from Clementz and Koch (2001). Fossil data from Clementz *et al*. (2006) and Thewissen *et al*. (2007).

fore and hind limbs and large hands and feet modified for swimming. Despite being recovered from marginal marine deposits, these animals have mean $\delta^{18}O$ values suggesting they ingested fresh water and low $\delta^{13}C$ values consistent with freshwater aquatic prey. Remingtonocetids also had large hind limbs, but unlike ambulocetids, they had small eyes and long snouts. They are also found in marginal marine settings, and have mean $\delta^{18}O$ and $\delta^{13}C$ values indicating a greater reliance on estuarine or marine resources. Protocetids are a morphologically diverse group showing a range of aquatic adaptations. Some had well developed hind limbs, but others may not have been able to support their weight on land. They are known from more fully marine deposits and are the first cetaceans known from outside the Indo-Pakistani region. Isotopic data from the few protocetid specimens that have been analyzed support a more fully marine lifestyle. Finally, dorudontines and basilosaurines (subfamilies within the Basilosauridae) were large, fully aquatic cetaceans with reduced hind limbs. Mean $\delta^{18}O$ and $\delta^{13}C$ values support their reconstruction as fully marine mammals that did not frequent freshwater ecosystems and were primarily foraging nearshore.

Thus in contrast to sirenians, which first exploited marine ecosystems and only invaded freshwater late in their radiation, cetaceans first evolved in freshwater habitats with a variety of amphibious forms, but then rapidly evolved into fully aquatic animals inhabiting chiefly marine habitats. Recently, Thewissen *et al.* (2007)

explored the first few steps in this transition in a study of the ecology of *Indohyus*, an Eocene-aged raccoon-sized artiodactyl from India in the family Raoellidae. Phylogenetic analysis revealed that raoellids are the sister-group of Cetacea. Raoellids had extremely thick cortical bone in their limbs (osteosclerosis), an adaptation observed in secondarily aquatic species that is thought to provide ballast for buoyancy control. Both mean values and variance in $\delta^{18}O$ values are low in raoellids relative to associated terrestrial taxa, confirming that they were largely aquatic. Yet the dentition of raoellids is not highly modified for consumption of aquatic prey. They were most likely herbivores or, perhaps, omnivores consuming a mix of plants and invertebrates. Their $\delta^{13}C$ values resemble those of associated terrestrial herbivores, unlike those of pakicetids, which clearly obtained nutrients from freshwater aquatic food webs. Thewissen *et al*. (2007) hypothesize that raoellids may have taken to fresh water to escape predators, like the modern African mouse deer. In any case, aquatic lifestyles precede the origin of Cetacea. Cetacean origins, as represented by the pakicetids, occurred when a raoellid-like ancestor switched from herbivory-omnivory to a diet of aquatic prey.

Analytical Considerations In Studies of Modern Taxa

Lipid Extraction

With the growing demand for SIA in ecological research, there has been a significant increase in the number of laboratories and research groups. As such, there is a need for a standardization of tissue collection and preparation protocols to improve the quality and reliability of interlaboratory comparisons. Foremost among these considerations is the issue of lipid extraction, but other points worth considering include methods of preservation in the field and in the lab (*e.g.*, desiccation, roasting, freeze-drying, and use of preservatives) and homogenization of samples. While definitely not a complete list, these points are commonly presented in the literature and therefore warrant discussion.

There is no disciplinary standard when it comes to the decision on whether or not tissues should be lipid extracted prior to SIA. Most studies on marine mammals cite the importance of lipid extraction when trying to interpret differences in $\delta^{13}C$ values among tissues. The concentration of lipids, which have $\delta^{13}C$ values that are up to 5‰ lower than associated proteins, varies among tissues. Thus lipid-rich tissues, such as liver, muscle and various blood components (*e.g.*, serum and plasma), likely have lower $\delta^{13}C$ values than lipid-poor tissues (*e.g.*, hair, dentin, and whiskers). Interpreting differences in $\delta^{13}C$ values among tissues can be difficult, since they can either be due to systematic tissue-specific differences in lipid concentration or temporal changes in ecology, or a combination of these possibilities. The $\delta^{15}N$ values of lipids are not significantly different than associated proteins because lipids are primarily composed of carbon, oxygen, and hydrogen and only contain small amounts of nitrogen in cell walls and lipoprotein membranes (Lehninger 1982).

Lipid extraction is especially important in the interpretation of experiments designed to determine trophic or tissue-specific discrimination factors (Hobson *et al.* 1996, Kurle 2002, Kurle and Worthy 2002, Lesage *et al.* 2002, Zhao *et al.* 2006, Stegall *et al.* 2008). Kurle (2002) found significant differences in $\delta^{13}C$ values of serum and plasma in comparison to red blood cells (RBCs) in captive northern fur seals and attributed this to differences in the amount of lipid present in each blood

component. In comparison to RBCs, total lipids are higher in plasma and serum because these components contain serum albumin, which is a major carrier of fatty acids in the blood (Lehninger 1982). Furthermore, serum does not contain fibrinogen and many other clotting proteins (Schier *et al.* 1996), and thus has a higher lipid to protein ratio than plasma or RBCs, which explains why serum typically has lower $\delta^{13}C$ values than plasma (Kurle 2002; Orr *et al.* 2009). Despite these mechanistic hypotheses, Stegall *et al.* (2008) found no significant difference in $\delta^{13}C$ values between lipid extracted (LE) and nonlipid extracted (NLE) serum from wild Steller sea lion pups and juveniles. Interestingly, this study also found no differences in $\delta^{13}C$ values between LE milk, the assumed dietary source for pups, and NLE or LE serum. The tissue-to-diet discrimination patterns for three species of phocid seals reported in Lesage *et al.* (2002) are confounded by the fact that none of the pinniped tissues analyzed in the study were lipid extracted. As a result, these authors conclude that lipid extraction should be routine when measuring lipid-rich tissues or with tissues in which lipid content may vary with changes in diet or nutritional status. Finally, unpublished data from a California sea otter population that consume moderately lipid-rich diets suggest that variation in diet-vibrissae $\delta^{13}C$ trophic discrimination factors likely relate to dietary lipid content and that consumers that eat more lipid-rich sea urchins may have lower trophic discrimination factors (Newsome *et al*. in review). While indispensable amino acids must be derived from diet and are thus directly routed, it is known that dispensable amino acids may be synthesized *de novo* from other carbon containing compounds (Howland *et al*. 2003, Jim *et al*. 2006). These results suggest that it may not be appropriate to lipid-extract prey samples when using isotopes to examine diet in consumers that consume lipid-rich foods, such as many marine mammals and seabirds.

When samples have not been lipid extracted but C/N ratios are available, $\delta^{13}C$ values can be corrected for lipid content using different algorithms (McConnaughey and McRoy 1979). This method allows one to choose an absolute difference between pure protein and lipid and makes the assumption that pure protein has a theoretically derived atomic C/N ratio. While results of these studies are mixed with respect to the effects of lipid extraction on tissue $\delta^{13}C$ values, we suggest that future studies minimize these confounding factors by using an accepted protocol to remove lipids from all samples.

We offer a few simple rules as a guide when deciding how marine mammal tissues and associated prey should be prepared for SIA. Overall, our suggestions are based on the type of consumer tissue(s) analyzed, which for marine mammals often depends on logistical considerations related to sample availability. For consumers, samples should be prepared such that pure protein or pure lipid is analyzed. For example, protein-rich tissues known to contain a considerable amount of lipids (*e.g.*, skin, muscle, internal organs, plasma, serum, and bone collagen) should be lipid-extracted prior to SIA. In contrast, whole blood (RBCs) and metabolically inert tissues constructed of keratin (*e.g.*, fur and vibrissae) or tooth collagen (*e.g.*, dentin) do not require lipid extraction because they do not contain considerable lipids. Lipid extraction is not necessary for studies focused on deeper time scales where tooth hydroxyapatite (*e.g.*, enamel) is the only trustworthy substrate.

In regards to prey, it would be ideal to perform isotopic analyses of lipid extracted (LE) and nonlipid extracted (NLE) subsamples from individual prey samples when possible. At the very least, isotopic differences between LE and NLE subsamples should be characterized for any lipid-rich prey type ($>15\%$ lipids on a dry basis) in situations where consumers are eating a significant portion ($>50\%$ edible biomass) of

such prey. This is especially important when analyzing consumer tissues that reflect bulk diet, such as bioapatite or lipid. In cases where lipid-rich prey are not substantial components of diet, we suggest that all potential prey items be lipid-extracted when examining consumer protein that has also been lipid-extracted. For sirenians, which forage primarily on aquatic plants and algae, the low lipid (and protein) content of the food items means that lipid extraction of food is not necessary. Depending upon the goals of the study, vegetation may either be homogenized or subsampled based on the different structures within the plants and algae (*e.g.*, leaves, blades, rhizomes, *etc.*). Additional care must be taken when sampling marine plants and algae that may accumulate marine carbonates. These samples should be repeatedly rinsed in DI water to remove most soluble carbonates. Heavily calcified species may require initial rinses in weak HCl (1 M or less) to enhance subsequent carbonate removal by rinsing in DI water (Kennedy *et al*. 2005). Finally, the animal epiphytes on plants consumed by such herbivores must be removed and analyzed separately.

A number of different lipid-extraction protocols are used in isotopic ecology. All of them involve treatment of samples in organic solvents such as chloroform, methanol, or petroleum ether. Some studies have found that petroleum ether is a superior solvent because it removes a smaller fraction of nonlipid material during the extraction process (Dobush *et al.* 1985), but the majority of published studies use a combination of chloroform and methanol using some modified version of the method of Bligh and Dyer (1959). Samples can be treated with repeated rinses of organic solvents and sonicated in a fume hood at ambient temperature, rinsed for 12–24 h at higher temperatures using a Soxhlet apparatus, or treated using one of a variety of automated extraction devices that use microwave oven or ultrasound assisted extraction, supercritical fluid extraction, or pressurized supercritical fluid extraction. There is no systematic period of time samples should be subject to solvents, as it depends on the lipid content of the tissue being analyzed. The most reliable proxy for determining whether or not samples have been adequately lipid extracted is through comparison of sample C/N ratios with those expected from "pure" tissues. For example, the theoretical weight percent C/N ratios of collagen and keratin are approximately 2.8 and 3.0, respectively. If sample C/N ratios are significantly higher than that expected from pure tissues, they likely contain lipids. As an independent proxy for data quality and for comparison of results among studies, it is *essential* for authors to present the mean C/N ratios and associated error of all tissue types subject to SIA.

Sample Preservation

Considerable time can pass between sample collection and analysis, and sample preservation is needed to retain the original stable isotope composition. Methods of preservation are strongly dependent upon the tissue type. For instance, keratinous tissues, such as hair, vibrissae, nails, or feathers, are highly resistant to decay and can often be easily stored under dry conditions (Hobson *et al.* 1996, Hirons *et al.* 2001*b*, Smith *et al.* 2003, Cerling *et al.* 2004). In contrast, blood and tissue samples, which have greater water content and are highly susceptible to degradation and isotope alteration, must be preserved soon after collection. Multiple studies have assessed which methods provide the best preservation of soft tissue stable isotope values (Hobson *et al.* 1997*a*, Gloutney and Hobson 1998, Kaehler and Pakhomov 2001, Edwards *et al.* 2002, Sarakinos *et al.* 2002, Feuchtmayr and Grey 2003, Kelly

et al. 2006, Barrow *et al.* 2008). Blood, epidermis and muscle were the common materials subjected to these tests, which compared preservation by freezing, freeze-drying, oven-drying, and preservation in dimethyl sulfoxide (DMSO) buffer, ethanol, formalin, and NaCl aqueous solutions. Overwhelmingly, the best methods of preservation were freezing, freeze-drying, and oven-drying. Barrow *et al*. (2008) provide a summary of results for carbon and nitrogen isotope preservation for twenty different methods and show that freezing and drying (air-, oven- or freeze-drying) lead to no significant alteration. All other methods alter the $\delta^{13}C$ and $\delta^{15}N$ values of the analyzed tissues. The extent of this alteration varied widely among methods, but for some, such as ethanol or formalin, the effects appear to be consistent and correctable (Edwards *et al*. 2002) and previous studies (Todd *et al*. 1997) have shown that careful preparation using either sonication or Soxhelet extraction can remove DMSO from tissue samples. These finding bode well for the increasing interest in SIA of historical specimens in museums and research collections. With appropriate corrections and sample preparation methods, it is possible to use these specimens to study the ecology of historical populations of marine mammals.

Homogenization

Another aspect of tissue preparation and handling for SIA that must be considered is the need for homogenization of samples. For most tissues, particularly skin biopsies, homogenization is a critical step in preparation and is needed to ensure comparability of isotope values among individuals within a population and within communities. Variation in the amino acid or lipid composition of different layers or portions of a tissue sample can lead to large differences in the stable isotope values of replicates analyzed from these specimens. To overcome this problem, homogenization of dried samples through powdering is recommended using a mortar and pestle, a ball-mill, or some other method of grinding. Homogenization may not be warranted for all studies; variation in the stable isotope composition of metabolically inert materials (*e.g.*, vibrissae, baleen plates, *etc*.) can provide a record of variation over seasons to years. A mean value can be easily calculated from such time series if tissue growth dynamics are understood.

Future Directions

While isotopic turnover and tissue-dependent fractionation studies of birds and terrestrial mammals provide useful guides for interpreting data from marine mammals, more can be learned through study of (1) marine mammals born and/or raised in captivity, or (2) wild populations that are accessible during the breeding season.

Potential Impacts of Dynamics on the Isotopic Ecology of Marine Mammals

Many marine mammals have extremely dynamic life cycles that may leave distinct signals in isotopic records. Many are capital breeders, in which foraging and reproduction do not overlap spatially or temporally; they undertake extraordinary annual (or biannual) migrations between productive foraging grounds and suitable, safe places to give birth and raise offspring. An example is the annual life cycle of the northern elephant seal in the northeast Pacific Ocean (life history summary based on Le Boeuf *et al.* 2000), which make biannual 6,000–10,000 km foraging trips to

the North Pacific Convergence (females) or southern Alaska and eastern Aleutian Island (males) shelves, returning to the California coast twice each year to reproduce (December–February) and molt (May–July). Adult female elephant seals arrive on the breeding colony in prime body condition, give birth within a few days, and suckle their offspring for approximately 1 mo. During the nursing period, adult females can lose up to 50% of their body weight, as stored energy in the form of blubber (*i.e.*, lipid) and muscle (*i.e.*, protein) is converted into lipid-rich milk for their pups. Pups remain at the breeding colony for 2–3 mo after the females have left, burning through their own fat stores acquired during the nursing period, until hunger takes its toll and they venture into the North Pacific to find solid food. Adult males, especially those that defend territories and mate, also undergo a prolonged fast and can also lose exceptional amounts of blubber and muscle (up to 50% of their body weight) over the course of the 3-mo breeding season.

These profound physiological shifts may be traced using SIA because they likely result in unique, nonconventional isotopic fractionations within individuals or between mothers and their offspring that could change over the course of the breeding season. As discussed above, the tissues of an animal that catabolizes ^{13}C-depleted lipid stores, such as a fasting pup or adult male, should have lower δ^{13}C values than those of an animal that consumes solid prey, whereas fasting animals that catabolize ^{15}N-enriched body proteins may have higher δ^{15}N values than those that metabolize exogenous protein. The rate at which such fasting signals are incorporated into metabolically active tissues will depend on (1) the turnover time of the tissue, which might be slower for an animal that experiences an extended catabolic state, and (2) the relative rate of nitrogen loss, which may vary between males (*i.e.*, urine) and females (*i.e.*, urine and milk). Accurate interpretation of isotopic data from tissues collected at the breeding colony, when elephant seals are easily accessible, depends on an understanding of such isotopic patterns. More generally, we might expect differences in isotopic discrimination between capital breeders (most phocids and large cetaceans) versus income breeders (otariids, small cetaceans, and sea otters), as they may experience dissimilar physiological demands throughout the course of their annual life cycle.

Seasonal and Interannual Isotopic Time Series

A unique perspective into the lives of marine mammals may be obtained through the analysis of continuously growing but metabolically inert tissue such as vibrissae, baleen, or tooth dentin. Proper sampling of these tissues generates a time series of isotopic information that provides insight on seasonal or interannual changes in diet and/or habitat use that is otherwise difficult to collect using traditional techniques, such as direct observation or gut/scat content analysis. For example, serial analysis of a relatively fast growing and easily sampled tissue such as vibrissae (see Fig. 8) can provide insights on seasonal variation in individual diets, movement patterns, or physiological state. Comparison of temporal intraindividual to interindividual isotopic variation can also be used to assess the prevalence of dietary specialization within or among populations (Lewis *et al.* 2006, Newsome *et al.* 2009*b*).

Baleen and vibrissae function as foraging and sensory structures, respectively, and are maintained from year to year with nearly continuous growth. As noted above, Schell *et al.* (1989) generated high-resolution, multiyear isotopic records for bowhead whales by subsampling consecutive segments of baleen. These records were used to

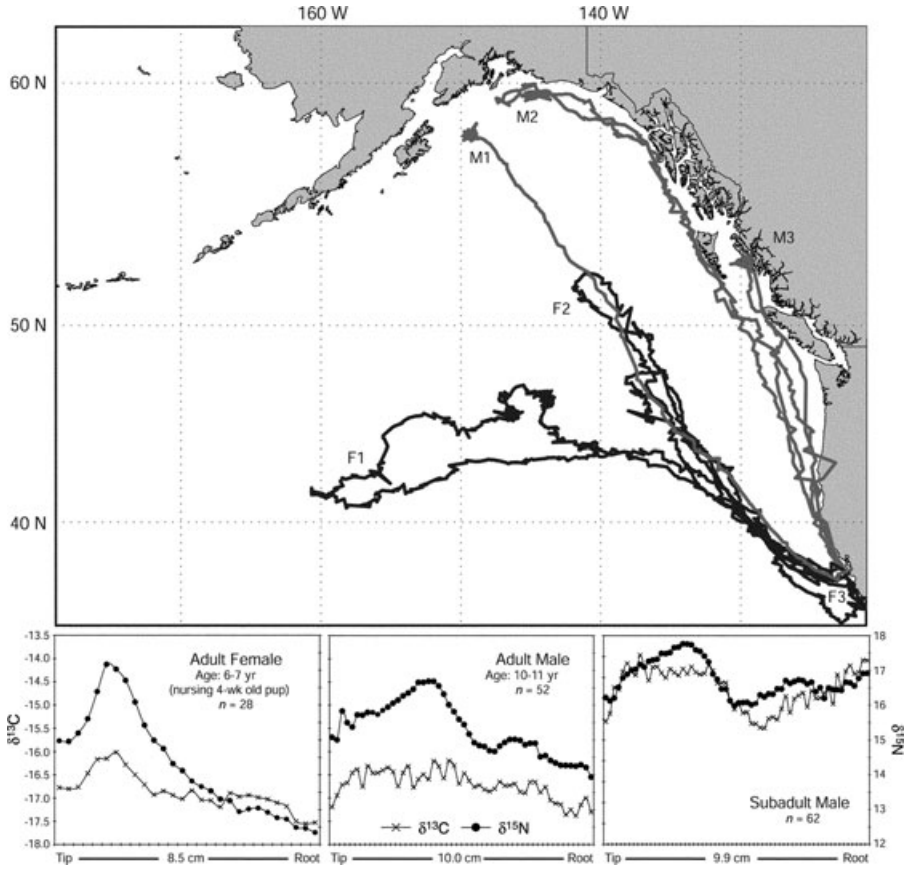


Figure 8. Compilation of satellite tracking and isotope data for northern elephant seals in the northeast Pacific Ocean. Top panel presents satellite tracks for six individuals during the post-breeding foraging trip (M1–M3 are males; F1–F3 are females) showing that males and females generally utilize different habitats; males typically forage on the continental shelf while most females forage in open-ocean pelagic settings along the North Pacific Convergence (~40°–50°N). Bottom panels present vibrissae $\delta^{13}C$ (X, left axis) and $\delta^{15}N$ (•, right axis) profiles for three elephant seal individuals, two males and one female. The total length (cm) and total number of segments analyzed from each vibrissa is noted. Note that isotope and satellite data are for different individuals. Satellite tracking data were generously provided by Dan Costa, Jason Hassrick, and the Tagging of Pacific Pelagics (TOPP) program.

examine seasonal shifts in foraging ecology, habitat use, and eventually used to estimate whale growth rates, offering phenomenal insights into the life of the species (Best and Schell 1996, Hobson and Schell 1998, Hoekstra *et al.* 2002, Lee *et al.* 2005).

At present, the largest caveat to studies of isotopic records from serial-sampled baleen or vibrissae is the lack of accurate species-specific growth rates for such tissues. This makes it impossible to know with certainty the time frame over which serial baleen or vibrissae samples reflect ecological information. In his studies of baleen,

Schell overcame this difficulty because he could detect annual cycles that provided an internal chronometer. Growth rate data for vibrissae are becoming available for some pinnipeds. Zhao and Schell (2004) calculated an average growth rate for vibrissae from captive harbor seals of 0.075 mm/d (~2.7 cm/yr) over a 6-mo period (December–May). Hirons *et al.* (2001*b*) calculated a growth rate of approximately 0.08 mm/d (~3.0 cm/yr) and ~0.12 mm/d (4.4 cm/yr), respectively, for wild harbor seals and Steller sea lions, which are similar to growth rates calculated for leopard seals (0.10 mm/d or ~3.7 cm/yr) by Hall-Aspland *et al*. (2005*a*). In addition to providing an average growth rate, these studies suggest that growth rates are nonlinear. Growth rates for newly replaced vibrissae are likely faster than those for established vibrissae, hence growth rates for distal sections near the tip of the vibrissae are higher than proximate sections near the base/root.

Visual analysis of growth layers in primary tooth dentin to age marine mammals was first developed on northern fur seals (Scheffer 1950) and has been successfully applied to studies of other marine mammals. Fortunately, primary dentinal growth layers are metabolically inert and are not remodeled, thus collagen or apatite derived from consecutive annuli in mammalian teeth can provide annually resolved ontogenetic time series from individual animals. Sophisticated micro-drilling systems are commercially available that can sample growth layers as small as approximately 300-μm thick. Individual growth layers in the teeth of some large odontocetes and pinnipeds can be 1.0–1.2-mm thick, which may allow for subannual resolution. Growth layer thickness does decrease with age such that it may be impossible to sample individual annuli deposited during the adult life stage, and material from several annuli may have to be combined to produce enough material for SIA (Niño-Torres *et al.* 2006, Knoff *et al.* 2008). Furthermore, some marine mammal species are sexually dimorphic, which can result in tooth dentin growth layers in adult male teeth being much thicker than those in a female of comparable age.

This technique has been used to assess ontogenetic dietary shifts of Steller sea lions (Hobson and Sease 1998), northern fur seals (Hobson and Sease 1998, Newsome *et al.* 2006), California sea lions (Newsome *et al.* 2006), sperm whales (*Physeter macrocephalus*) (Mendes *et al.* 2007*a*, *b*), killer whales (Newsome *et al.* 2009*a*), longbeaked common dolphin (*Delphinus capensis*) (Niño-Torres *et al.* 2006), and bottlenose dolphins (*T. truncatus*) (Knoff *et al.* 2008), as well as dietary shifts associated with weaning that were discussed above. Stable Pb isotopes in walrus (*Odobenus rosmarus*) dentin have been used to determine stock distinctions and movement patterns in the Canadian Arctic (Outridge *et al.* 2003, Stewart *et al.* 2003).

Satellite Telemetry and SIA: Validation and Expansion

Another fruitful future research direction will be to integrate a rapidly growing, high-resolution database on movement and diving derived from satellite telemetry and time-depth recorders with SIA to better understand foraging and to ground truth the use of isotopic data as proxies for habitat use and diet. Satellite tracking offers a rich archive of information at the individual level, but its high cost makes it difficult to deploy to assess behavior at the population level or to examine changes in behavior over multiple years. As described in detail above, SIA is a promising tool for assessing differences in habitat use over relatively large spatial scales (*i.e.*, ocean basin), yet finer scale resolution may be possible by comparing individual isotopic

information with high-resolution satellite-derived tracking information. We focus on northern elephant seals to highlight this productive avenue of research. Some of the first published satellite tracks of marine mammals were of northern elephant seals to determine sex-related differences in habitat use and migratory paths, and more recently, to characterize individual foraging behavior over successive years (Stewart and DeLong 1995, Le Boeuf *et al.* 2000). These data suggest that there may be individual-level differences in habitat use and that individuals may return to specific foraging grounds season after season.[1] Figure 8 shows six tracks from adult northern elephant seals (three adult females and three adult males) from the breeding colony at Point Año Nuevo in central California and $\delta^{13}C$-$\delta^{15}N$ time series of serially sampled elephant seal whiskers from various sex/age classes collected from the same rookery. Unfortunately we do not yet have satellite tracks and isotopic data from the same individuals. For elephant seals, there are large (2–3‰) differences in isotope values among sex and age classes that likely relate to individual-level differences in diet, habitat use, and/or physiological demands. The adult male and female have similar mean $\delta^{13}C$ but different $\delta^{15}N$ values, and the female has a larger overall range and variance, especially for $\delta^{15}N$. Slightly lower mean $\delta^{15}N$ values in the adult female may relate to differences in trophic level or physiological state; adult females use open-ocean pelagic habitats (*e.g.,* North Pacific Convergence) and consume fish and squid while adult males consume benthic invertebrates on shelf habitats during the nonbreeding season (Le Boeuf *et al.* 2000, Fig. 8). Potential physiological isotopic effects related to pregnancy have not been investigated in northern elephant seals but as noted above, studies of humans show that pregnancy leads to a decrease in hair $\delta^{15}N$ values (Fuller *et al.* 2004). Future comparison of vibrissae time series from nulliparous and pregnant females that forage in approximately the same location and likely consume the same types of prey could provide insight on any isotopic effects associated with the physiological demands of pregnancy. The subadult male, on the other hand, has significantly higher $\delta^{13}C$ and $\delta^{15}N$ values in comparison to the adult male and female, most likely because this individual foraged at lower latitudes than these adults.

Over the past two decades, researchers have amassed a vast amount of high-resolution tracking data on a variety of marine mammals, and tracking campaigns are now underway (*i.e.*, http://www.topp.org). These data represent an opportunity for isotope ecologists to further strengthen and expand their toolkit in marine ecology. In comparison to satellite tags or even traditional observational methods, SIA is a relatively cost-effective and time efficient tool for investigating variation in habitat use, dietary preferences, or physiological conditions at the individual, population, or species level. Marine mammal ecologists who use sophisticated satellite tags and time-depth recorders are beginning to collaborate with oceanographers to map the temperature and chlorophyll structure of remote pelagic regions. In a similar fashion, isotopic time series derived from continuously growing but metabolically inert tissues could provide an isotopic map (*i.e.*, isoscape) of the world's oceans at a variety of temporal scales and trophic levels (Graham *et al*. in press). Such maps would not only refine the spatial resolution at which stable isotopes can be used to assess movement patterns, but might also provide information on oceanographic conditions.

[1]Unpublished data provided by Daniel P. Costa, Department of Ecology and Evolutionary Biology, Institute of Marine Sciences, Long Marine Lab, University of California, Santa Cruz, CA.

Disentangling Spatial and Trophic/Physiological Differences with Compound Specific SIA

Isotopic differences among consumers may be produced by three factors: (1) differences in isotopic value at the base of the food web, (2) differences in diet/trophic level, and (3) differences in physiological state. As noted in our discussion of time series from northern elephant seals, it is often difficult to distinguish among these factors as sources of variation in free-ranging animals, especially those that are migratory. Recent work suggests that this causal knot may be partially disentangled through isotopic analysis of individual amino acids. As noted above, trophic level ^{15}N-enrichment is thought to result from excretion N wastes that are ^{15}N-depleted due to fractionations associated with deamination or transamination. Studies of marine zooplankton have shown that this effect on whole bodies and bulk protein is generated through differential ^{15}N-enrichment of different amino acids (McClelland and Montoya 2002). Several dispensable amino acids central to cycling of nitrogen into and out of the amino acid pool (alanine, glutamate, aspartate) are strongly ^{15}N-enriched relative to diet (referred to here as "trophic" amino acids). Several other amino acids, including both indispensable (lysine, phenylalanine, tyrosine) and dispensable amino acids (glycine, serine) are not ^{15}N-enriched, and therefore provide a direct measure of the δ^{15}N value at the base of the food web (referred to here as "source" amino acids).

Popp *et al*. (2007) suggest that in studies of free-ranging, migratory animals, it should be possible to analyze source amino acids to determine if animals are moving among regions with different isotopic values at the base of the food web. The trophic level of an animal can be determined by comparison to this nonfractionating baseline (*i.e.*, by the difference in δ^{15}N value between source and trophic amino acids). They used this approach to study yellowfin tuna (*Thunnus albacares*) from the eastern tropical Pacific, where there is a very strong gradient in food web δ^{15}N values. The δ^{15}N value of bulk muscle from yellowfin tuna captured along this gradient differ strongly. Popp *et al*. (2007) discovered that the δ^{15}N value of source amino acids changed by a similar amount, but that the spacing between source and trophic amino acids did not change. Thus the shift in value observed in bulk tissue is due entirely to differences at the base of the food web, not to a change in diet or trophic level.

To date, there are no controlled feeding studies on marine mammals (or any mammal, for that matter) to explore whether the distinction between source and trophic amino acids holds. This is an essential first step before this promising method can be applied to marine mammal tissues. Furthermore, Popp *et al*. (2007) assume that the trophic fractionation between source and trophic amino acids should be relatively constant and assume a value of $\sim+7‰$ per trophic step. Yet as noted above, there is considerable evidence that changes in the body nitrogen balance affect the trophic discrimination in bulk tissue, with higher fractionations in catabolic states, and lower fractionations in anabolic states. We predict that these differences in bulk δ^{15}N values are in fact tracking changes in the spacing between source and trophic amino acids for animals in these different physiological states. This prediction needs to be tested, either experimentally or with carefully monitored wild animals. Such effects would make it difficult to discriminate dietary shifts from changes in physiology, but it would be possible to discriminate these two factors from shifts at the base of the food web.

Summary

SIA is an established tool in the ecological sciences to quantify the flow of energy within and among ecosystems, to estimate habitat use and movement patterns qualitatively, and to explore physiological processes from the organismal to the molecular level. In this review, we have tried to outline not only what SIA has taught us about the ecology of extant and extinct marine mammals, but also to identify research topics that require further basic research or are potentially productive areas for future discovery. As method development and standardization is an important aspect of any emerging scientific tool, we also offer our insights as to preparation protocols aimed to provide a reliable guide for the community.

1. The application of stable isotope methods to the ecological and physiological research on marine mammals has grown tremendously over the past 30 yr. Though isotopes of carbon, nitrogen, and oxygen are the most often used, interest in other isotope systems (hydrogen and sulfur) is growing. Within studies of modern ecosystems, these tools have been applied to answer questions of foraging ecology, migratory behavior, and heavy metal and toxin contamination in several species of marine mammals.
2. Better constraints on the discrimination factors between different isotopes in tissues and diet and on the turnover of these isotopes in different tissues have improved the utility and sensitivity of these measurements as proxies for dietary and ecological information. However, estimates of discrimination factors and turnover rates are primarily based on studies of captive pinnipeds, so there is a need for similar work to be conducted on other marine mammals groups (*i.e.*, cetaceans, sirenians, sea otters).
3. With the increased use of SIA by a growing number of research groups, we call for a standardization of the methods for collecting and preparing tissues. This would improve interlaboratory comparison of isotopic results and facilitate the accumulation and synthesis of large data sets covering broad spatial, temporal and ecosystem scales.
4. Though the growth in SIA has primarily focused on studies of living marine mammals, there have been significant advances in the application of SIA to the study of ancient marine mammals. The timescale of these studies ranges from those focused on how the ecology of extant populations and species has shifted over historical timescales in response to anthropogenic and climate related factors to studies addressing the timing of the land-to-sea transition and subsequent diversification of different groups of marine mammals millions of years ago.
5. Promising areas of future research include a greater emphasis on serial sampling of metabolically inert materials (*i.e.*, baleen, vibrissae, tooth dentin) as a means of acquiring high resolution information on the seasonal foraging habits and life histories of marine mammals; a synthesis of SIA results with migratory and movement information recovered via satellite telemetry; and compound specific SIA of individual lipids and amino acids to distinguish the impacts of trophic and physiological factors from those of ecological factors.

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