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VARIATION IN STABLE CARBON AND NITROGEN ISOTOPE VALUES FROM MULTIPLE TISSUES OF CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*)

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Abstract—Stable nitrogen and carbon isotope analyses have increasingly been used to investigate the foraging ecology of marine mammals, particularly in response to the limitations of using conventional dietary analysis techniques. In this study, we compared nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) values of four different tissues (i.e., fur, red blood cells, serum, and plasma) from California sea lions (*Zalophus californianus*; $n = 58$) of various age classes (i.e., pup, yearling, juvenile, and adult). Metabolically active tissues with relatively high turnover rates (plasma and serum) had similar $\delta^{15}N$ and $\delta^{13}C$ values, but these two tissues had significantly higher $\delta^{15}N$ values and lower $\delta^{13}C$ values in comparison to red blood cells or fur. In general, $\delta^{15}N$ values decreased with increasing age; $\delta^{15}N$ values for pups were between 1.2‰ (fur) to 2.0‰ (serum) greater in comparison to adult females. In general, $\delta^{13}C$ values increased with age (except red blood cells). Pups had mean $\delta^{13}C$ values that were 0.4‰ (plasma) to 0.5‰ (fur and serum) lower than mean values of adult females. Stable isotope analysis of various tissues can provide dietary information at the individual level on different temporal and spatial scales, as well as reveal patterns of the foraging ecology among groups of animals.

INTRODUCTION

The limitations and biases of using traditional methods to examine the diet of pinnipeds (e.g., analysis of stomach contents of harvested or recently dead individuals, stomach lavage, regurgitations, fecal analysis, and direct observation) have been well documented (Jobling and Breiby 1986; Jobling 1987; Dellinger and Trillmich 1988; Harvey 1989; Gales and Cheal 1992; Harvey and Antonelis 1994; Cottrell et al. 1996; Tollit et al. 1997; Bowen 2000; Orr and Harvey 2001; Arim and Naya 2003; Gudmundson et al. 2006). A noteworthy limitation of these methods is that each analysis represents only a "snapshot" of prey consumed prior to collection (Hobson et al. 1997). Although fecal samples, which are now routinely collected to assess the diet of pinnipeds, provide information about dietary composition, they do not provide information about the age or sex of the consumer; therefore, dietary changes within individual or intraspecific comparisons are not possible. Biochemical methods (e.g., serology, fatty acid signature analysis, and stable isotope analysis) have been developed to overcome some of the problems associated with using conventional techniques (e.g., Hobson 1990; Wada et al. 1991; Iverson et al. 1995; Kirsch et al. 2000). Stable isotope analysis (SIA) is based on the idea that the stable isotope composition of a consumer's diet is reflected in its tissues. Because different tissues have dissimilar isotopic turnover rates, an isotopic approach can provide time-integrated data on average dietary intake, rather than a recent "snapshot" of information derived from traditional proxies (Kirsch et al. 2000).

The analysis of naturally occurring stable isotope ratios of nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) is now frequently used to investigate changes in feeding patterns and habitat utilization of animals in space and time (Hobson et al. 1996; Das et al. 2003; Dalerum and Angerbjörn 2005; Sinisalo et al. 2008). Due to the preferential excretion of ^{14}N in urine, $\delta^{15}N$ values increase by ~3–5% per trophic level in marine food webs (DeNiro and Epstein 1978; Minagawa and Wada 1984; Owens 1987;

Wada et al. 1991; Hobson and Welch 1992; Vander Zanden and Rasmussen 2001; Kurle 2002). Therefore, the relative amount of ^{15}N in tissues reflects the trophic level at which the consumer is feeding, with higher $\delta^{15}N$ values corresponding to higher trophic levels. The ratio of stable carbon isotopes varies little with trophic position. Only ~0.5–1.1‰ enrichment per trophic level has been observed for $\delta^{13}C$ values in marine food webs (e.g., Fry and Sherr 1984; Wada et al. 1991; Kurle 2002). However, $\delta^{13}C$ values are affected by factors that act at the base of the food web, such as phytoplankton size and growth rate (Schoeninger and DeNiro 1984; Laws et al. 1995; Bidigare et al. 1997), resulting in geographic differences in $\delta^{13}C$ values that can be used to indicate consumer foraging locations ($\delta^{13}C$ enrichment: fresh water > marine, nearshore > offshore, benthic > pelagic; Rau et al. 1982; Rau et al. 1983; Fry and Sherr 1984; Wada et al. 1991; France 1995; Hobson et al. 1997; Burton and Koch 1999). For example, primary producers in nearshore/benthic areas are relatively more ^{13}C enriched compared to those in offshore/pelagic regions where nutrients are relatively limited, phytoplankton growth rates are lower, and the base of the food web is more enriched in ^{12}C (Fry and Wainright 1991; France 1995; Pancost et al. 1997).

Although the isotopic composition of an animal is determined by its diet, there is substantial isotopic variation among tissues. Isotopic signatures assessed from consumer tissues with high turnover rates (e.g., blood plasma, liver) reflect recent dietary intake, whereas tissues with slower turnover rates (e.g., muscle, bone collagen) reflect feeding over a longer period of time (Tieszen et al. 1983). This is a result of nutrients of tissues being assimilated at time scales dependent upon the metabolism and protein turnover rate of a particular tissue (Kurle and Worthy 2002; Dalerum and Angerbjörn 2005). Because different tissues equilibrate to dietary isotopic changes at different rates, it should be possible to examine the diet of a consumer over multiple temporal scales by examining the isotopic composition of multiple tissues (Phillips and Eldridge 2006).

Stable isotopes have been used to describe the foraging ecology of several pinniped species (e.g., Hobson et al. 1996, 1997; Hobson and Sease 1998; Burton and Koch 1999; Kurle 2002; Newsome et al. 2006; Zhao et al. 2006; Sinisalo et al. 2008); however, few studies have utilized multiple tissues with dissimilar turnover rates collected from wild populations. Here, we did not compare sea lion isotope values to those of their prey, which is necessary to provide more direct information about trophic level or foraging location. Instead, we examined the pattern of variation in nitrogen and carbon isotope values within individuals and among different age classes of California sea lions (*Zalophus californianus*) to establish the use of SIA as a useful tool for the study of temporal diet shifts within and among individual sea lions.

MATERIALS AND METHODS

Field Collection

California sea lion tissues were collected from individuals at San Miguel Island, California (SMI; 34.03°N, 120.44°W), during 2006 and 2007. The animals were captured as part of ongoing studies conducted by the National Marine Fisheries Service's National Marine Mammal Laboratory at SMI, which supports one of the two largest *Zalophus californianus* rookeries in the world. Tissues collected were fur, red blood cells (RBCs), plasma, and serum. The growth rate of fur is slower than turnover rates for blood components (i.e., metabolically active tissues), and RBCs have slower turnover rates than serum or plasma (Tieszen et al. 1983; Hobson and Clark 1993; Hilderbrand et al. 1996; Klaassen et al. 2004). Therefore, the isotopic measurement of fur should provide long-term dietary information reflecting integration over several months. For animals that undergo an annual molting cycle like all otariids (assuming the entire shaft was collected and completely homogenized), the isotopic signature of fur will reflect diet since the previous molt. For pinnipeds, plasma and serum have high turnover rates and are likely to provide dietary information from 1 to 2 weeks prior to collection (Kurle 2002), whereas RBCs are likely to represent the average isotopic composition of diet integrated over two or more months (Kurle 2002; Zhao et al. 2006).

Samples of all four tissues were obtained from 58 individuals (Table 1). Fur was obtained by cutting an ~2 cm x 2 cm patch near the dorsal rump region of each animal using electric clippers or

Table 1. Mean $\delta^{15}N$ and $\delta^{13}C$ values of tissues collected from California sea lions of various age classes that were captured and sampled at San Miguel Island, California, during September 2006 through January 2007. Values in parentheses indicate standard error.

Age Class	Fur		RBC		Serum		Plasma	
	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Pup (< 1 yr; n = 39)	18.4 (0.1)	-13.9 (0.1)	18.4 (0.1)	-14.9 (0.0)	19.3 (0.1)	-15.4 (0.0)	19.4 (0.1)	-15.3 (0.0)
Yearling (1 - 2 yr; n = 3)	18.1 (0.1)	-13.6 (0.1)	17.0 (0.1)	-15.3 (0.1)	17.9 (0.1)	-14.9 (0.3)	17.7 (0.1)	-15.0 (0.4)
Juvenile (2 - 4 yr; n = 9)	17.2 (0.1)	-13.5 (0.1)	16.7 (0.0)	-15.3 (0.0)	17.9 (0.1)	-15.1 (0.1)	17.8 (0.1)	-15.1 (0.1)
Adult (≥4 yr; n = 7)	17.2 (0.2)	-13.4 (0.2)	16.5 (0.1)	-15.2 (0.1)	17.4 (0.0)	-14.9 (0.1)	17.6 (0.3)	-14.9 (0.1)

scissors applied to the base of the fur without removing the follicle. Samples were stored dry in envelopes until further processing in the laboratory. Blood samples were obtained during routine collections from the caudal-gluteal vein. Plasma and RBCs were collected in tubes containing the anticlotting agent sodium heparin, which has been determined not to alter isotopic values (Hobson et al. 1997; Kurle 2002). Serum was collected from tubes containing a clot activator for serum separation, also known not to cause isotopic alterations (Kurle 2002). Blood components were obtained after 10 min of centrifugation. Approximately 1 mL of each component was decanted into a 2 mL cryovial and frozen at -40°C until further processing.

Stable Isotope and Statistical Analyses

Once in the laboratory, fur samples were placed in scintillation vials and cleaned by washing them with a mild detergent solution, followed by a rinse with de-ionized (DI) water. Surface contaminants were removed using a solvent wash (2:1 chloroform:methanol) and another DI water rinse. Samples were then placed in a lyophilizer and dried for 24 to 48 h. Frozen blood samples also were placed into the lyophilizer. Once dried, all samples were powdered and homogenized using a mortar and pestle (fur) or glass rod (blood components). Samples were weighed into tin capsules (8 x 5 mm) to a target weight of 1.0 ± 0.2 mg.

Nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope values of all tissues analyzed in this study were determined using a Carlo-Erba elemental analyzer (NC 2500)[1] interfaced with a Finnegan Delta Plus XL[1] mass spectrometer in the light stable isotope facility at Carnegie Institution of Washington (Washington, DC). Isotopic results are expressed in δ notation: $\delta^{15}N$ or $\delta^{13}C = 1000* [(R_{sample}/R_{standard})-1]$, where R_{sample} and $R_{standard}$ are the $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratios of the sample and standard, respectively. The standards used were atmospheric N_2 for nitrogen or Vienna-Pee Dee Belemnite limestone (V-PDB) for carbon. The units are expressed as parts per thousand or per mil (‰) and are calibrated to international standards through repeated measurements of an organic standard of known isotopic composition. The within-run standard deviation of an acetalinide standard was 0.2‰ for both $\delta^{15}N$ and $\delta^{13}C$ values.

Tissues within an individual were compared amongst each other using a blocked one-way analysis of variance (blocked ANOVA). Additionally, we used one-way ANOVAs to compare $\delta^{15}N$ or $\delta^{13}C$ values among age classes (i.e., pup (< 1 yr), yearling (1–2 yr), juvenile (2–4

1. References to trade names do not imply endorsement by the National Marine Fisheries Service, NOAA.

yr), and adult (≥ 4 yr)). When significant differences were detected, pairwise multiple comparisons were conducted using Tukey's Honest Significant Difference (HSD) method (Zar 1999). Significance was tested at the $\alpha = 0.05$ level. Statistical analyses were performed using R Program Language (ver. 2.4.1; R Development Core Team 2006).

RESULTS

Within Individual

The $\delta^{15}N$ and $\delta^{13}C$ values differed among sample types (blocked ANOVA: $F = 172.96$, $df = 3$, $P < 0.001$ for $\delta^{15}N$; and $F = 340.61$, $df = 3$; $P < 0.001$ for $\delta^{13}C$). Plasma and serum had higher (enriched) $\delta^{15}N$ and lower (depleted) $\delta^{13}C$ values compared to RBCs and fur (Fig. 1). Subsequent tests indicated that there were no significant differences between $\delta^{15}N$ or $\delta^{13}C$ values for plasma and serum (Tukey's HSD: $P = 1.00$ and 0.32, respectively), however significant differences remained between these two tissues and the others (Tukey's HSD: for all comparisons $P < 0.002$).

Among Age Classes

There were significant differences in mean $\delta^{15}N$ values among age classes. In general, $\delta^{15}N$ values decreased with increase in age (Table 1). Subsequent tests indicated that there were differences among all age combinations except between adult-juvenile and yearling-pup for fur (Tukey's HSD: 1.00 and 0.67, respectively; all other comparisons $P < 0.007$; Table 1). For the remaining tissues, only pups had significantly different mean $\delta^{15}N$ values compared to the other age classes (Tukey's HSD: for all comparisons $P < 0.001$; Table 1). The magnitude of difference of $\delta^{15}N$ values of pups and adult females was least for fur (1.2‰) compared to blood components (≥ 1.8‰).

There were no significant differences among age classes in mean $\delta^{13}C$ values for fur or plasma except between adult-pup and juvenile-pup (fur), and adult-pup (plasma), respectively (ANOVAs; for all comparisons $P < 0.008$). Pups had significantly higher mean RBC $\delta^{13}C$ values and significantly lower mean serum $\delta^{13}C$ values compared to all the other age classes (ANOVAs; for all comparisons $P < 0.02$). There were no significant differences in mean $\delta^{13}C$ values among older age classes (ANOVAs; for all comparisons $P > 0.52$; Table 1). The magnitude of difference of $\delta^{13}C$ values between pups and adult females was similar among all tissues (~ 0.5‰).

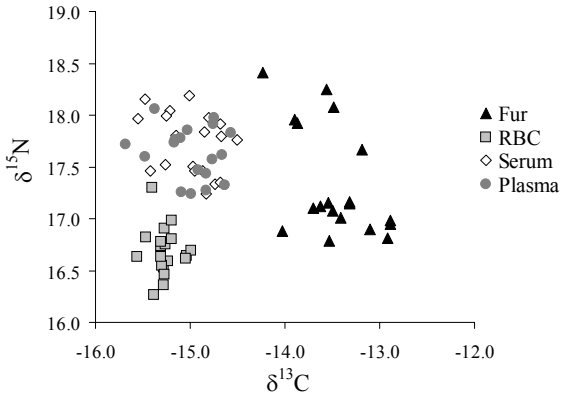

Figure 1. The relationship between $\delta^{13}C$ and $\delta^{15}N$ of different tissues sampled from California sea lions at San Miguel Island during 2006 and 2007. Pup values were excluded for clarity.

DISCUSSION

Stable isotope ratios in consumer tissues are useful for discerning dietary components assimilated over time; however, it is important to be mindful of the physiological, biochemical, and ecological factors that greatly influence the patterns of isotope values among tissue types and individuals (Gannes et al. 1997). Also noteworthy is that isotopic signatures determined at a given point in time do not reflect the current or the previous diet but are time-integrated products of both (Sweeting et al. 2005). Without pertinent information on turnover rates of tissues, isotopic signatures will only be loosely descriptive of diet (Gannes et al. 1997). In this study, we did not measure diet-tissue isotopic fractionations; however, we provided unique information about general isotopic patterns from multiple tissues from wild California sea lions of various age classes.

Within Individual

There were significant differences in isotope values among tissues within individuals. Besides being indicative of changes in dietary intake and trophic level at different time scales, discrepancies

in $\delta^{15}N$ values among tissues of an individual could have resulted from differences in the macromolecular composition (i.e., amino acids, lipids) of tissues (Kurle 2002; Zhao et al. 2006). Results from several studies on captive birds and mammals have indicated that isotope values of different tissues from the same individual do vary in a systematic way, even when the animal is fed an isotopically monotonous diet (Tieszen et al. 1983; Sutoh et al. 1987; Hobson and Clark 1993; Hobson et al. 1996; Kurle 2002; Vanderklift and Ponsard 2003; De Smet et al. 2004; Sponheimer et al. 2006; Zhao et al. 2006). $\delta^{15}N$ values of plasma and serum were similar to each other and more enriched compared to RBCs and fur. These two tissues are similar except plasma contains fibrinogen and other clotting-factor proteins (Schier et al. 1996). Kurle (2002) stated that differences in amino acid composition likely explained differences in $\delta^{15}N$ values among blood constituents in captive northern fur seals (*Callorhinus ursinus*) that were fed an isotopically homogeneous diet throughout the study.

Overall, $\delta^{13}C$ values of fur were more enriched compared to the blood components; RBCs in turn were more enriched compared to serum and plasma. These findings were similar to previous studies of other pinniped species (Hobson et al. 1996, 1997; Kurle 2002; Zhao et al. 2006). As with stable nitrogen isotope values, discrepancies of $\delta^{13}C$ values among tissues of an individual could reflect temporal shifts in diet and/or habitat use or may relate to the differences in the amino acid composition or lipid content in the tissues (Kurle 2002; Zhao et al. 2006). For example, serum albumin is the major carrier of fatty acids in blood (Nelson 1970), and lipids are ^{13}C-depleted in comparison to associated proteins (Kelly 2000), which results in lower $\delta^{13}C$ values (Kurle 2002). Zhao (2002) noted that carbon and nitrogen isotope ratios differ greatly among individual amino acids. Therefore, the lipid and/or amino acid content present in blood could have been a contributing factor in the lower mean $\delta^{13}C$ values of the blood components in comparison to those of fur. Unlike captive animals, wild sea lions usually do not have an isotopically homogeneous diet, therefore changes in their diet (prey species, acquisition location) and tissue composition must be factored in explaining differences in $\delta^{15}N$ and $\delta^{13}C$ values among different tissues.

Among Age Classes

Significant differences in mean $\delta^{15}N$ and $\delta^{13}C$ also were observed among age classes. Our limited samples sizes for particular age classes (i.e., yearlings and adults) prohibit definitive conclusions, however interesting patterns persisted nonetheless. Pups had higher $\delta^{15}N$ values for all tissues compared to those of older animals. This was expected because most, if not all, of their diet is obtained from their mothers in the form of milk. Milk is derived from remobilized body tissues of lactating females. Because pups, in essence, were feeding on their mothers' tissues, they were feeding at a higher trophic level than older conspecifics. Yearlings had intermediate $\delta^{15}N$ values for fur and similar $\delta^{15}N$ values for blood components compared to those of older animals. As previously mentioned, fur can reflect integrated dietary information over several months (depending on when collected). Therefore, it is likely that the isotopic-composition of fur from yearlings partially reflected their pre-weaned milk diet, resulting in higher $\delta^{15}N$ values. However, their $\delta^{15}N$ values for plasma, serum, and RBC were not statistically different from those of juveniles and adults. Because the isotopic signatures of blood components reflect recent diet, this pattern indicates that yearlings were weaned at the time of sampling and were feeding at a similar trophic level as older individuals. Juvenile and adult sea lions had similar $\delta^{15}N$ values for all tissues, indicating that they were feeding at approximately the same trophic level. There were greater magnitudes of difference in $\delta^{15}N$ values of blood components compared to those of fur between pups and juveniles/adults. This exemplifies that the choice of tissue(s) is important, particularly when assessing ontogenetic or other temporal shifts in diet.

In contrast to $\delta^{15}N$ values, pups had significantly lower $\delta^{13}C$ values than those of older age classes (except for RBCs); $\delta^{13}C$ values for yearlings, juveniles, and adults were statistically nonsignificant. The most likely reason for this pattern is that pups were still dependent on their mothers and were sustained on a lipid-rich milk diet, which is ^{13}C depleted in comparison to a relatively protein-rich piscivorous diet (Tieszen et al. 1983;

Tieszen and Boutton 1988; Polischuk et al. 2001; Kurle 2002). There was an opposite trend for RBCs, which might be due to their lower proportion of lipids in comparison to plasma and serum. The fact that individuals of older age classes had similar $\delta^{13}C$-values may indicate that they were feeding in similar areas. All of the non-pup animals sampled in this study were females. Findings from telemetry studies of different-aged females instrumented on San Miguel Island indicated that many of these animals feed on epi-pelagic prey in shallow waters (Feldkamp et al. 1989; Melin et al. 2008; Orr unpublished data).

In summary, examining the stable carbon and nitrogen isotopes from various tissues provides a better temporal understanding of the foraging ecology of a species compared to using conventional dietary proxies (e.g., fecal analysis) or isotopic data from a single tissue. The application of stable isotope analysis can be strengthened when used in conjunction with other techniques. For example, fecal analysis can be used to "ground truth" the use of isotopic proxies to assess the foraging ecology of a consumer at a particular location (Burns et al. 1998; Porras-Peters et al. 2008). The choice of tissue(s) to analyze will affect conclusions about a consumer's diet. SIA is very useful when researchers are interested in estimating the diet or the change therein of a particular individual or group of animals within an age or sex class over different time periods. Determining the diet of various age classes might be of special concern for those species whose status is dependent on juvenile survival. Using stable isotopes, researchers can determine when weaning occurs and ascertain differences in the foraging ecology of juveniles and adults. Also, SIA is useful when only a segment of the population is accessible for sampling. For example, it is often difficult to capture and collect tissue samples from large top consumers (e.g., adult female pinnipeds); however, it may be relatively easy to acquire samples from their small, dependent offspring. The diet and movement patterns of adult females can be indirectly determined by analyzing the tissues of their offspring after suitable isotopic fractionations are considered (Aurioles-Gamboa et al. 2006; Porras-Peters et al. 2008). It is vital to have an understanding of diet-consumer tissue isotopic fractionations so that proper interpretations and comparisons can be made within and among individuals, species, or projects.

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REFERENCES

Arim, M., and D.E. Naya. 2003. Pinniped diets inferred from scats: analysis of biases in prey occurrence. Canadian Journal of Zoology 81:67–73.

Aurioles-Gamboa, D., P.L. Koch, and B.J. Le Boeuf. 2006. Differences in foraging location of Mexican and California elephant seals: evidence from stable isotopes in pups. Marine Mammal Science 22:326–338.

Bidigare, R.R., A. Fluegge, K.H. Freeman, K.L. Hanson, J.M. Hayes, D. Hollander, J.P. Jasper, L.L. King, E.A. Laws, J. Milder, F.J. Millero, R. Pancost, B.N. Popp, P.A. Steinberg, and S.G. Wakeham. 1997. Consistent fractionation of ^{13}C in nature and in the laboratory: growth-rate effects in some haptophyte algae. Global Biogeochemical Cycles 11:279–292.

Bowen, W.D. 2000. Reconstruction of pinnipeds diets: accounting for complete digestion of otoliths and cephalopod beaks. Canadian Journal of Fisheries and Aquatic Sciences 57:898–905.

Burns, J.M., S.J. Trumble, M.A. Castellini, and J.W. Testa. 1998. The diet of Weddell seals in McMurdo Sound, Antarctica as determined from scat collections and stable isotope analysis. Polar Biology 19:272–282.

Burton, R.K., and P.L. Koch. 1999. Isotopic tracking of foraging and long-distance

migration in northeastern Pacific pinnipeds. Oecologia 119:578–585.

Cottrell, P.E., A.W. Trites, and E.H. Miller. 1996. Assessing the use of hard parts in faeces to identify harbour seal prey: results of captive-feeding trials. Canadian Journal of Zoology 74:875–880.

Dalerum, F., and A. Angerbjörn. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647–658.

Das, K., G. Lepoint, Y. Leroy, and J.M. Bouquegneau. 2003. Marine mammals from the southern North Sea: feeding ecology data from delta C-13 and delta N-15 measurements. Marine Ecology-Progress Series 263:287–298.

Dellinger, T., and F. Trillmich. 1988. Estimating diet composition from scat analysis in otariid seals (Otariidae): Is it reliable? Canadian Journal of Zoology 66:1865–1870.

DeNiro, M.J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506.

De Smet, S., A. Balcaen, E. Claeys, P. Boeckx, and O. Van Cleemput. 2004. Stable carbon isotope analysis of different tissues of beef animals in relation to their diet. Rapid Communications in Mass Spectrometry 18:1227–1232.

Feldkamp, S.D., R.L. DeLong, and G.A. Antonelis. 1989. Diving patterns of California sea lions, *Zalophus californianus*. Canadian Journal of Zoology 67:872–883.

France, R.L. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Marine Ecology Progress Series 124:307–312.

Fry, B., and E.B. Sherr. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contributions to Marine Science 27:13–47.

Fry, B., and S.C. Wainright. 1991. Diatom sources of ^{13}C-rich carbon in marine food webs. Marine Ecology Progress Series 76:149–157.

Gales, N.J., and A.J. Cheal. 1992. Estimating diet composition of the Australian sea-lion (*Neophoca cinerea*) from scat analysis: an unreliable technique. Wildlife Research 19:447–455.

Gannes, L.Z., C. Martínez de Rio, and P. Koch. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. Comparative Biochemistry and Physiology A 119:725–737.

Gannes, L.Z., D.M. O'Brien, and C. Martinez de Rio. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78:1271–1276.

Gudmundson, C.J., T.K. Zeppelin, and R.R. Ream. 2006. Application of two methods for determining diet of northern fur seals (*Callorhinus ursinus*). Fishery Bulletin 104:445–455.

Harvey, J.T. 1989. Assessment of errors associated with harbor seal (*Phoca vitulina)* faecal sampling. Journal of Zoology, London 219:101–111.

Harvey, J.T., and G.A. Antonelis. 1994. Biases associated with non-lethal methods of determining the diet of northern elephant seals. Marine Mammal Science 10:178–187.

Hilderbrand, G.V., S.D. Farley, C.T. Robbins, T.A. Hanley, K. Titus, and C. Servheen. 1996. Use of stable isotopes to determine diets of living and extinct bears. Canadian Journal of Zoology 74:2080–2088.

Hobson, K.A. 1990. Stable isotope analysis of marbled murrelets: evidence for fresh-water feeding and determination of trophic level. Condor 92:897–903.

Hobson, K.A., and R.W. Clark. 1993. Turnover of ^{13}C in cellular and plasma factors of blood: implications for non-destructive sampling in avian dietary studies. Auk 110:638–641.

Hobson, K.A., D.M. Schell, D. Renouf, and E. Noseworthy. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Canadian Journal of Fisheries and Aquatic Sciences 53:528–533.

Hobson, K.A., and J.L. Sease. 1998. Stable isotope analyses of tooth annuli reveal temporal dietary records: an example using Steller sea lions. Marine Mammal Science 14:116–129.

Hobson, K.A., J.L. Sease, R.L. Merrick, and J.F. Piatt. 1997. Investigating trophic relationships of pinnipeds in Alaska and Washington using

stable isotope ratios of nitrogen and carbon. Marine Mammal Science 13:114–132.

Hobson, K.A., and H.E. Welch. 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Marine Ecology Progress Series 84:9–18.

Iverson, S.J., O.T. Oftedal, W.D. Bowen, D.J. Boness, and J. Sampugna. 1995. Prenatal and postnatal transfer of fatty acids from mother to pup in the hooded seal (*Cystophora cristata*). Journal of Comparative Physiology B 165:1–12.

Jobling, M. 1987. Marine mammal faeces samples as indicators of prey importance—a source of error in bioenergetics studies. Sarsia 72:255–260.

Jobling, M., and A. Breiby. 1986. The use and abuse of fish otoliths in studies of feeding habits of marine piscivores. Sarsia 71:265–274.

Kelly, J.F. 2000. Stable isotopes of nitrogen and carbon in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78:1–27.

Kirsch, P.E., S.J. Iverson, and W.D. Bowen. 2000. Effect of diet on body composition and blubber fatty acids in captive harp seals (*Phoca groenlandica*). Physiological and Biochemical Zoology 73:45–59.

Klaassen, M., M. Thums, and I.D. Hume. 2004. Effects of diet change on carbon and nitrogen stable-isotope ratios in blood cells and plasma of the long-nosed bandicoot (*Perameles nasuta*). Australian Journal of Zoology 52:635–647.

Koch, P.L., M.L. Fogel, and N. Tuross. 1994. Tracing the diets of fossil animals using stable isotopes. Pages 1–63. *In*: Lajtha, K. and R.H. Michener (eds.), Stable Isotopes in Ecology and Environmental Science. Blackwell Scientific Publications, Boston. MA.

Kurle, C.M. 2002. Stable-isotope ratios of blood components from captive northern fur seals (*Callorhinus ursinus*) and their diet: applications for studying the foraging ecology of wild otariids. Canadian Journal of Zoology 80:902–909.

Kurle, C.M., and G.A.J. Worthy. 2001. Stable isotope assessment of temporal and geographic differences in feeding ecology of northern fur seals (*Callorhinus ursinus*) and their prey. Oecologia 126:254–265.

Kurle, C.M., and G.A.J. Worthy. 2002. Stable nitrogen and carbon isotope ratios in multiple tissues of the northern fur seal *Callorhinus ursinus*: implications for dietary and migratory reconstructions. Marine Ecology Progress Series 236:289–300.

Laws, E.A., B.N. Popp, R.R. Bidigare, M.C. Kennicutt, and S.A. Macko. 1995. Dependence of phytoplankton carbon isotopic composition on growth rate and $[CO_2]_{aq}$: theoretical considerations and experimental results. Geochimica et Cosmochimica Acta 59:1131–1138.

Melin, S.R., R.L. DeLong, and D.B. Siniff. 2008. The effects of El Niño on the foraging behavior of lactating California sea lions (*Zalophus californianus californianus*) during the nonbreeding season. Canadian Journal of Zoology 86:192–206.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of $\delta^{15}N$ along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta 48:1135–1140.

Nelson, G. 1970. The lipid composition of the blood of marine mammals. I. Young elephant seals (*Mirounga augustriostris*) and harp seals (*Pagophilus groenlandicus*). Comparative Biochemistry and Physiology 34:109–116.

Newsome, S.D., M.A. Etnier, D. Aurioles-Gamboa, and P.L. Koch. 2006. Using carbon and nitrogen isotopes to investigate reproductive strategies in Northeast Pacific otariids. Marine Mammal Science 22:556–572.

Orr, A.J., and J.T. Harvey. 2001. Quantifying errors associated with using fecal samples to determine the diet of the California sea lion (*Zalophus californianus*). Canadian Journal of Zoology 79:1080–1087.

Owens, N.P. 1987. Natural variations in ^{15}N in the marine environment. Advances in Marine Biology 24:389–451.

Pancost, R.D., K.H. Freeman, S.G. Wakeham, and C.Y. Robertson. 1997. Controls on carbon isotope fractionation by diatoms in the Peru upwelling region. Geochimica et Cosmochimica Acta 61:4983–4991.

Peterson, B.J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293–320.

Phillips, D.L., and P.M. Eldridge. 2006. Estimating the timing of diet shifts using stable isotopes. Oecologia 147:195–203.

Polischuk, S.C., K.A. Hobson, and M.A. Ramsay. 2001. Use of stable-carbon and –nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. Canadian Journal of Zoology 79:499–2001.

Porras-Peters, H., D. Aurioles-Gamboa, V.H. Cruz-Escalona, and P.L. Koch. 2008. Trophic level and overlap of sea lions (*Zalophus californianus*) in the Gulf of California, Mexico. Marine Mammal Science 24:554–576.

Rau, G.H., A.J. Mearns, D.R. Young, R.J. Olson, H.A. Schafer, and I.R. Kaplan. 1983. Animal $^{13}C/^{12}C$ correlates with trophic level in pelagic food webs. Ecology 64:1314–1318.

Rau, G.H., R.E. Sweeney, and I.R. Kaplan. 1982. Plankton $^{13}C:^{12}C$ ratio changes with latitude: differences between northern and southern oceans. Deep Sea Research 29:1035–1039.

Schier, D., J. Butler, and R. Lewis. 1996. Human anatomy and physiology. McGraw-Hill Companies, Inc., Boston, MA.

Schoeninger, M., and M.J. DeNiro. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochimica et Cosmochimica Acta 48:625–639.

Sinisalo, T., R.I. Jones, E. Helle, and E.T. Valtonen. 2008. Changes in diets of individual Baltic ringed seals (*Phoca hispida botnica*) during their breeding season inferred from stable isotope analysis of multiple tissues. Marine Mammal Science 24:159–170.

Sponheimer, M., T.F. Robinson, T.E. Cerling, L. Tegland, B.L. Roeder, L. Ayliffe, M.D. Dearing, and J.R. Ehleringer. 2006. Turnover of stable carbon isotopes in the muscle, liver, and breath CO_2 of alpacas (*Lama pacos*). Rapid Communications in Mass Spectrometry 20:1395–1399.

Sutoh, M., T. Koyama, and T. Yoneyama. 1987. Variations of natural ^{15}N abundances in the tissues and digesta of domestic animals. Radioisotopes 36:74–77.

Sweeting, C.J., S. Jennings, and N.V.C. Polunin. 2005. Variance in isotopic signatures as a descriptor of tissue turnover and degree of omnivory. Functional Ecology 19:777–784.

Tieszen, L.L., and T.W. Boutton. 1988. Stable carbon isotopes in terrestrial ecosystems research. Pages 167–195. *In*: Rundel, P.W., J.R. Ehleringer and K.A. Nagy (eds.), Stable Isotopes in Ecological Research. Springer-Verlag, Berlin.

Tieszen, L.L., T.W. Boutton, K.G. Tesdahl, and N.A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37.

Tollit, D.J., M.J. Steward, P.M. Thompson, G.J. Pierce, M.B. Santos, and S. Hughes. 1997. Species and size differences in the digestion of otoliths and beaks: implications for estimates of pinniped diet composition. Canadian Journal of Fisheries and Aquatic Sciences 54:105–119.

Vanderklift, M.A., and S. Ponsard. 2003. Sources of variation in consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. Oecologia 136:169–182.

Vander Zanden, M.J., and J.B. Rasmussen. 2001. Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. Limnology and Oceanography 46:2061–2066.

Wada, E., H. Mizutani, and M. Minagawa. 1991. The use of stable isotopes for food web analysis. Critical Reviews in Food Science and Nutrition 30:361–371.

Zar, J.H. 1999. Biostatistical Analysis. Prentice-Hall, Inc., Upper Saddle River, NJ, 663 pp.

Zhao, L. 2002. Tracing amino acid metabolism of harbor seals (*Phoca vitulina*) using stable isotope techniques. [Ph.D. thesis]. University of Alaska Fairbanks, Fairbanks, AK.

Zhao, L., D.M. Schell, and M.A. Castellini. 2006. Dietary macronutrients influence ^{13}C and ^{15}N signatures of pinnipeds: captive feeding studies with harbor seals (*Phoca vitulina*). Comparative Biochemistry and Physiology, Part A 143:469–478.