

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 ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) 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 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, but these two tissues had significantly higher $\delta^{15}\text{N}$ values and lower $\delta^{13}\text{C}$ values in comparison to red blood cells or fur. In general, $\delta^{15}\text{N}$ values decreased with increasing age; $\delta^{15}\text{N}$ values for pups were between 1.2‰ (fur) to 2.0‰ (serum) greater in comparison to adult females. In general, $\delta^{13}\text{C}$ values increased with age (except red blood cells). Pups had mean $\delta^{13}\text{C}$ 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 ($^{15}\text{N}/^{14}\text{N}$) and carbon ($^{13}\text{C}/^{12}\text{C}$) 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, $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values corresponding to higher trophic levels. The ratio of stable carbon isotopes varies little with trophic position. Only $\sim 0.5\text{--}1.1\text{‰}$ enrichment per trophic level has been observed for $\delta^{13}\text{C}$ values in marine food webs (e.g., Fry and Sherr 1984; Wada et al. 1991; Kurle 2002). However, $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values that can be used to indicate consumer foraging locations ($\delta^{13}\text{C}$ 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 $\sim 2\text{ cm} \times 2\text{ cm}$ patch near the dorsal rump region of each animal using electric clippers or

Table 1. Mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ 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	
	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
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 anticoagulant 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 ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) 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 XL¹ mass spectrometer in the light stable isotope facility at Carnegie Institution of Washington (Washington, DC). Isotopic results are expressed in δ notation: $\delta^{15}\text{N}$ or $\delta^{13}\text{C} = 1000 * [(R_{\text{sample}}/R_{\text{standard}}) - 1]$, where R_{sample} and R_{standard} are the $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$ 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 acetanilide standard was 0.2‰ for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values differed among sample types (blocked ANOVA: $F = 172.96$, $df = 3$, $P < 0.001$ for $\delta^{15}\text{N}$; and $F = 340.61$, $df = 3$; $P < 0.001$ for $\delta^{13}\text{C}$). Plasma and serum had higher (enriched) $\delta^{15}\text{N}$ and lower (depleted) $\delta^{13}\text{C}$ values compared to RBCs and fur (Fig. 1). Subsequent tests indicated that there were no significant differences between $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ values among age classes. In general, $\delta^{15}\text{N}$ 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

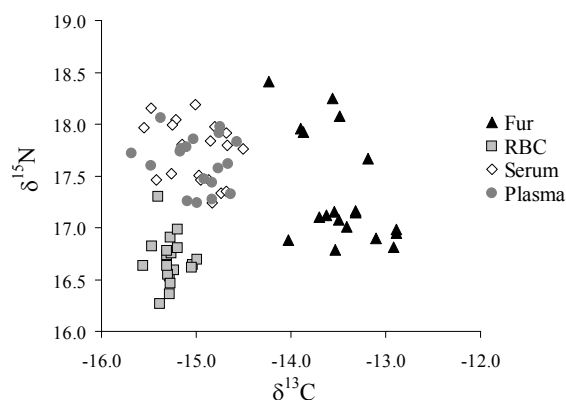


Figure 1. The relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of different tissues sampled from California sea lions at San Miguel Island during 2006 and 2007. Pup values were excluded for clarity.

(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 $\delta^{15}\text{N}$ values compared to the other age classes (Tukey's HSD: for all comparisons $P < 0.001$; Table 1). The magnitude of difference of $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values and significantly lower mean serum $\delta^{13}\text{C}$ values compared to all the other age classes (ANOVAs; for all comparisons $P < 0.02$). There were no significant differences in mean $\delta^{13}\text{C}$ values among older age classes (ANOVAs; for all comparisons $P > 0.52$; Table 1). The magnitude of difference of $\delta^{13}\text{C}$ values between pups and adult females was similar among all tissues (~ 0.5 ‰).

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 $\delta^{15}\text{N}$ 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). $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values among blood constituents in captive northern fur seals (*Callorhinus ursinus*) that were fed an isotopically homogeneous diet throughout the study.

Overall, $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values among different tissues.

Among Age Classes

Significant differences in mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values for fur and similar $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values. However, their $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values for all tissues, indicating that they were feeding at approximately the same trophic level. There were greater magnitudes of difference in $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values, pups had significantly lower $\delta^{13}\text{C}$ values than those of older age classes (except for RBCs); $\delta^{13}\text{C}$ 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; Kurlle 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 $\delta^{13}\text{C}$ -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 (Aurióles-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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