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

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Abstract: Stable nitrogen and carbon isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$, respectively) of plasma, serum, clotted red blood cells, and unclotted red blood cells from six captive northern fur seals (*Callorhinus ursinus*) and two fish species in their diet, Atlantic herring (*Clupea harengus*) and Icelandic capelin (*Mallotus villosus*), were measured. The $\delta^{15}N$ values from the fur seal blood components ranged from 15.5 ± 0.1‰ (mean ± SE; all RBCs) to 16.7 ± 0.1‰ (plasma), and $\delta^{13}C$ values ranged from –18.3 ± 0.1‰ (serum) to –17.5 ± 0.1‰ (clotted RBCs). Fur seal blood components had higher $\delta^{15}N$ and $\delta^{13}C$ values than their diet. Mean enrichments of ^{15}N between fur seal RBCs and plasma/serum and their prey were +4.1 and +5.2‰, respectively, while mean ^{13}C enrichments were +0.6‰ (serum), +1.0‰ (plasma), and +1.3 to +1.4‰ (all RBCs). Fur seal blood components did not differ in $\delta^{15}N$ or $\delta^{13}C$ between the sexes. One female (Baabs) was pregnant at the time of sampling and was resampled 4 months later, when she was approximately 3 months post parturient. The $\delta^{15}N$ values of the blood components taken from Baabs during pregnancy were all higher by 0.6‰ (unclotted RBCs) to 1.3‰ (plasma) than those of samples taken during lactation, while her $\delta^{13}C$ values were nearly the same (RBCs) for the two time periods or lower during lactation by 0.5‰ (serum) and 0.7‰ (plasma). This study was the first to analyze isotope ratios of blood components from captive fur seals and their prey. The subsequent establishment of fractionation values between captive fur seals and their diet can be used to interpret trophic level and habitat usage of otariids feeding in the wild.

Résumé : Les rapports des isotopes stables d'azote ($^{15}N/^{14}N$) et de carbone ($^{13}C/^{12}C$) ont été déterminés dans le plasma, le sérum, les érythrocytes coagulés et les érythrocytes non coagulés de six otaries à fourrure du Nord (*Callorhinus ursinus*) en captivité, ainsi que chez leurs proies, le hareng atlantique (*Clupea harengus*) et le capelan d'Islande (*Mallotus villosus*). Les valeurs de $\delta^{15}N$ dans les composantes du sang de l'otarie à fourrure varient de 15,5 ± 0,1 ‰ (moyennes ± erreur type; tous les érythrocytes) à 16,7 ± 0,1 ‰ (plasma) et celles de $\delta^{13}C$, de –18,3 ± 0,1 ‰ (sérum) à –17,5 ± 0,1 ‰ (sang coagulé). Les composantes sanguines de l'otarie à fourrure présentent des valeurs de $\delta^{15}N$ et de $\delta^{13}C$ supérieures à celles de leurs proies. Les enrichissements moyens en ^{15}N dans les érythrocytes et le plasma/sérum des otaries par comparaison à leurs proies sont respectivement de +4,1 ‰ et de +5,2 ‰, alors que les enrichissements en ^{13}C sont de +0,6 ‰ dans le sérum, de +1,0 ‰ dans le plasma, et de +1,3 à +1,4 ‰ dans les érythrocytes. Il n'y a pas de différences sexuelles dans les valeurs de $\delta^{15}N$ et de $\delta^{13}C$. Une femelle (Baabs) était enceinte au moment des prélèvements et elle a pu être examinée de nouveau 4 mois plus tard, soit environ 3 mois après la mise bas. Les valeurs de $\delta^{15}N$ des composantes sanguines de Baabs durant la grossesse étaient toutes supérieures de 0,6 ‰ (érythrocytes non coagulés) à 1,3 ‰ (plasma) à celles mesurées durant l'allaitement, alors que les valeurs de $\delta^{13}C$ étaient ou bien à peu près les mêmes (érythrocytes) durant les deux périodes, ou alors inférieures de 0,5 ‰ (sérum) et de 0,7 ‰ (plasma) durant l'allaitement. Notre étude est la première à analyser les rapports isotopiques des composantes sanguines chez des otaries à fourrure en captivité et chez leurs proies. L'établissement éventuel des valeurs de fractionnement entre les otaries à fourrure en captivité et leurs proies pourra être utilisé pour interpréter les données sur le niveau trophique et l'utilisation de l'habitat chez des otariidés qui se nourrissent en nature.

[Traduit par la Rédaction]

Introduction

Stable carbon and nitrogen isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$, respectively) in animal tissues can be used to make inferences about trophic relationships and foraging locations in terrestrial and marine food webs (Chamberlain et al. 1997; Burns et al. 1998; Burton and Koch 1999; Fry et al. 1999; Hobson et al. 2000; Kelly 2000; Kurle and Worthy 2001,

Received 27 August 2001. Accepted 8 April 2002. Published on the NRC Research Press Web site at http://cjz.nrc.ca on 31 May 2002.

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 DOI: 10.1139/Z02-069

2002). Stable nitrogen isotopes undergo fractionation between predator and prey, leading to a predictable enrichment of ^{15}N with increasing trophic level (Ehleringer and Rundel 1989; Wada et al. 1991; Vander Zanden et al. 1997; Yoshii et al. 1999), thereby denoting an animal's trophic position. The use of carbon-isotope ratios as trophic indicators is limited in marine environments because isotopic fractionation of carbon between predator and prey is weak for higher level consumers and is more influenced by factors other than trophic position (see Kelly 2000). However, carbon-isotope ratios in a marine system are directly influenced by factors that act at the level of primary production (i.e., phytoplankton size, geometry, and growth rate (Laws et al. 1995; Pancost et al. 1997; Popp et al. 1998), the occurrence of phytoplankton blooms (Nakatsuka et al. 1992), and the amount of primary productivity (Descolas-Gros and Fontugne 1990; Laws et al. 1995; Bidigare et al. 1997; Schell 2000)), leading to geographic differences in $\delta^{13}C$ values (for delta notation see Methods) that can be used to indicate foraging locations of animals in marine environments (Wada et al. 1987; Fry 1988; Gearing 1991; Hobson and Welch 1992; Fogel and Cifuentes 1993; Yoshii et al. 1999; Kelly 2000; Kurle and Worthy 2002).

Traditional methods of studying feeding ecology include fecal or stomach-content analyses, which are limited in their use, partly because they only represent the food that an animal ate most recently. Isotope ratios in a predator's tissues reflect an integration of the isotope content of all prey over a period of time, the duration of that period being dependent on the biochemical turnover rate of the tissue being analyzed (Schell et al. 1989; Hildebrand et al. 1996; Hobson et al. 1996; Burton and Koch 1999; Kurle and Worthy 2002). Tissues with high rates of biochemical turnover provide dietary information assimilated from recent feeding bouts, while tissues with lower turnover rates integrate feeding data from more remote periods of time (Tieszen et al. 1983; Hobson and Clark 1992*a*). Isotope information from dietary sources takes up to two to three isotope half-lives to become completely incorporated into a particular predator tissue (Hobson 1993; Hildebrand et al. 1996). The half-lives for stable carbon and nitrogen isotopes refer to the time taken for the respective isotope ratios measured in a specific tissue to shift from those derived from one particular diet to those derived from another (see Tieszen et al. 1983). For example, Hildebrand et al. (1996) found that carbon and nitrogen isotopes in plasma from American black bears (*Ursus americanus*) had a half-life of 4 days, and it took 10 days for a change to an isotopically distinct diet to become fully integrated into their plasma. Black bear red blood cells (RBCs) had a longer half-life (up to 28 days; Hildebrand et al. 1996) and required more time to incorporate dietary changes. Therefore, I expected that the $\delta^{15}N$ and $\delta^{13}C$ values of plasma and serum from the captive fur seals in this study would reflect nutrients incorporated beginning approximately 1–2 weeks prior to collection, while those of the RBCs would reflect the diet beginning as far back as 2–3 months.

A further benefit of using stable-isotope analysis is that, depending on tissue type, sampling can be done in a nondestructive manner, which is especially useful when examining threatened or endangered species. Blood is particularly valuable because its collection is minimally invasive, it can be separated into components that illustrate different isotope-turnover rates and thus represent varying time scales, and it is frequently incorporated as a routine part of wild seal and sea lion assessment work (National Marine Fisheries Service 1992, 1993; Castellini et al. 1993; Aguirre et al. 1999; Johanos and Ragen 1999).

Controlled isotopic studies of animals on known diets indicate that there is predictable heavy-isotope enrichment between prey and predator, especially for ^{15}N, but the enrichment can vary depending upon the tissue and the species analyzed (Steele and Daniel 1978; Tieszen et al. 1983; Hobson and Clark 1992*b*; Hildebrand et al. 1996; Hobson et al. 1996). Currently, there are few data on stable isotope fractionation patterns in large mammals (Hildebrand et al. 1996; Hobson et al. 1996), and none for seals in the family Otariidae. The potential for conflict between fisheries and pinnipeds in Alaskan waters (especially Steller sea lions (*Eumetopias jubatus*) and northern fur seals (*Callorhinus ursinus*)) has heightened the need for trophic information about the animals within those ecosystems. Stable-isotope analysis will be of greater use as a tool for examining feeding ecology with further validation from studies on captive animals. Here I obtained blood from captive northern fur seals held on a known diet for at least 6 months, and I determined carbon- and nitrogen-fractionation values between diet and fur seal plasma, serum, unclotted packed RBCs, and clotted packed RBCs. Isotope ratios for multiple tissues in wild northern fur seals were determined by Burton and Koch (1999) and Kurle and Worthy (2001, 2002), and, in an attempt to draw meaningful conclusions regarding prey selection, they speculated that $\delta^{15}N$ and $\delta^{13}C$ fractionation values between prey and predator tissues were 2–4‰ and 0–1‰, respectively. More exact values between otariids and their prey were unavailable. A goal of this study was to measure carbon- and nitrogen-fractionation values between diet and blood components from captive fur seals in order to estimate the feeding ecology of wild otariids.

Methods

Blood samples were collected in June 2000 from six northern fur seals, one of whom was pregnant ("pregnant Baabs"), held in captivity at the Seattle Aquarium (see Table 1). Additional samples were collected from Baabs in mid-October 2000 ("lactating Baabs") while she was lactating following the birth of a male pup on 7 July 2000. All individuals were held on a constant diet of two-thirds thawed Atlantic herring (*Clupea harengus*) and one-third thawed Icelandic capelin (*Mallotus villosus*) for at least 6 months prior to sampling. The four females received 3.5 kg of fish per day, while the two males, Buster and Al, received 4–15 and 14–15 kg/day, respectively. Blood samples were obtained during routine collections from accessible veins in the rear flippers using butterfly blood-collection kits and vacuumed serum- and plasma-collection tubes. Samples were centrifuged for 5 min and cellular components were harvested for analysis. Plasma, unclotted packed RBCs ("unclotted RBCs"), serum, and clotted packed RBCs ("clotted RBCs") were the four components of interest. Plasma and unclotted RBCs were obtained from blood collected in vacuumed collection tubes that contained the anticlotting factor sodium heparin, which does not

cause isotope alteration (Hobson et al. 1997*a*), while the serum tubes contained no additives.

Approximately 200 μL of plasma, unclotted RBCs, and serum was pipetted from each sample and decanted onto separate precombusted glass-fiber filter papers. Clotted RBCs were scooped from the tube with a spatula and placed on filter papers. Papers were labeled, placed in closed petri dishes, and oven-dried at ~50°C for 24 h. They were subsequently stored in airtight scintillation vials at room temperature until analysis (following Hobson et al. 1997*a*). Approximately 1.0–1.4 mg of plasma or serum and 0.7–0.9 mg of clotted and unclotted RBCs was then scraped from the filter papers with forceps and sealed in 5 × 9 mm tin cups. Although some filter paper may have adhered to each sample, isotope analysis performed on the precombusted filter paper alone showed no measurable nitrogen or carbon isotopes, therefore the filter paper was not considered to be a contaminant.

Whole prey items were ground in blenders and the resulting homogenate was subsampled. Prey subsamples were freeze-dried for 24 h and all lipids were removed using petroleum ether (as recommended by Dobush et al. 1985) in a Soxhlet extractor for 2 h. Samples were dried under a fume hood for 1 h to evaporate any remaining solvent and then ground to a powder by hand. Approximately 1.0–1.9 mg of each sample was weighed and sealed into a 5 × 9 mm tin capsule. Blood components and fish samples were analyzed using a Carlo Erba NA 1500 CHN Combustion Analyzer interfaced to a Finnigan Delta C mass spectrometer at the Stable Isotope/Soil Biology Laboratory, University of Georgia Institute of Ecology. The average precision for the seal and fish data was 0.07 and 0.10‰ for nitrogen and 0.13 and 0.16‰ for carbon, respectively.

The natural abundance of ^{13}C or ^{15}N in a sample is expressed in delta notation:

$$[1] \qquad \delta X\ (‰) = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000$$

where δX is the parts-per-thousand ("per mil") difference in isotopic composition between the sample and the standard, and R_{sample} and R_{standard} are the heavy-to-light isotope ratios (i.e., $^{15}N/^{14}N$ and $^{13}C/^{12}C$) of the sample and standard, respectively (DeNiro and Epstein 1981; Owens 1987; Ehleringer and Rundel 1989; Boutton 1991).

To account for the differential contributions of the two prey types, the average isotope-fractionation factors between prey and predator for $\delta^{15}N$ and $\delta^{13}C$ were calculated using the following equation:

$$[2] \qquad \delta X\ (‰) = (B_{\text{predator}}) - [(1/3\,C_{\text{prey}}) + (2/3\,H_{\text{prey}})]$$

where δX is the corrected fractionation factor between prey and predator, B_{predator} is the $\delta^{15}N$ or $\delta^{13}C$ value of the blood component of interest, and C_{prey} and H_{prey} are the $\delta^{15}N$ or $\delta^{13}C$ values of the capelin and herring, respectively. It was assumed that there was no differential digestion of the two prey types.

Statistical treatment of data

Analysis of variance (ANOVA) was used to determine the equality of the isotope ratios among the four blood components. Tukey–Kramer multiple-comparison tests were used to sort and group the isotope ratios of the blood components from each animal, and Mann–Whitney tests were performed to ascertain possible sex differences in the $\delta^{15}N$ and $\delta^{13}C$ ratios in the four blood components. Paired *t* tests were used to measure the equivalence of isotope ratios between pairs of blood components from all of the animals, and *t* tests were applied to determine differences between the isotope ratios from the two prey types (StatView for Windows, Abacus Concepts, Berkeley, Calif., 1992–1996). Significance was tested at the $\alpha = 0.05$ level.

Results

The $\delta^{15}N$ and $\delta^{13}C$ values for all of the animals sampled are shown in Table 1. The isotope ratios from lactating Baabs were excluded from further analyses because of the difference in sampling dates, and the remaining data were analyzed. The $\delta^{15}N$ values from the clotted and unclotted RBCs did not differ between the sexes ($P = 0.355$ and $P > 0.999$, respectively; Mann–Whitney tests), therefore the values from both sexes were pooled in further analyses. In addition, Mann–Whitney tests indicated that there were no statistically significant differences in $\delta^{15}N$ values from plasma and serum components between the sexes ($P = 0.064$ for both). An ANOVA ($P < 0.001$) verified that the isotope ratios from the four blood components were different, and that values for plasma and serum differed from those for the two RBC components (all $P < 0.05$; Tukey–Kramer test). Further testing confirmed that there were no significant differences between $\delta^{15}N$ values from plasma and serum ($P = 0.773$; paired *t* test) or between those from clotted and unclotted RBCs ($P = 0.923$; paired *t* test) in any of the animals. Paired *t* tests (all $P \leq 0.002$) demonstrated that $\delta^{15}N$ values from serum and plasma were higher than those from the two RBC components by 0.6‰ (Woodstock) to 1.8‰ (Buster).

There were no differences in $\delta^{13}C$ values of any blood component between the sexes ($P = 0.355$–0.643; Mann–Whitney tests). An ANOVA ($P < 0.001$) verified that the isotope ratios from the four blood components were different, and paired *t* tests confirmed that $\delta^{13}C$ values were the same between the unclotted RBCs and plasma ($P = 0.209$) and between the two RBC components ($P = 0.427$). The $\delta^{13}C$ values of clotted RBCs were higher than those of plasma ($P = 0.028$) by an average of 0.4‰, and $\delta^{13}C$ values of serum were lower than those of plasma ($P = 0.010$) by an average of 0.4‰, unclotted RBCs ($P = 0.003$) by an average of 0.7‰, and clotted RBCs ($P < 0.001$) by an average of 0.8‰ (paired *t* tests) (Table 1).

The small sample size prevented statistical comparison of the isotope ratios from Baabs while she was pregnant and while she was lactating, but some observations were made. The $\delta^{15}N$ values for all blood components from pregnant Baabs were higher than those from lactating Baabs by 0.6‰ (unclotted RBCs) to 1.3‰ (plasma) (Table 1). The $\delta^{13}C$ values for the blood components from lactating Baabs were nearly the same (all RBC values from both sampling periods ranged from –17.7 to –17.6‰) or lower (plasma and serum $\delta^{13}C$ values were 0.7 and 0.5‰ lower, respectively) than those for pregnant Baabs (Table 1).

The $\delta^{15}N$ values of the Atlantic herring and Icelandic capelin (11.8 ± 0.2 and 11.1 ± 0.2‰, respectively (mean ± SE))

Table 1. Stable nitrogen and carbon isotope ratios ($\delta^{15}N$ and $\delta^{13}C$; ‰) of blood components from captive northern fur seals (*Callorhinus ursinus*).

	Sex	Year of birth	Clotted RBCs		Unclotted RBCs		Plasma		Serum	
			$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
Baabs[a]										
Pregnant	F	1988	15.7	–17.7	15.5	–17.6	16.5	–17.7	16.6	–18.2
Lactating	F	1988	15.0	–17.7	14.9	–17.7	15.2	–18.4	15.4	–18.7
Diana	F	1980	15.7	–17.7	15.7	–17.5	16.5	–18.4	16.5	–18.7
Female 2	F	1976	15.4	–17.1	15.6	–17.7	16.7	–17.5	16.4	–18.0
Woodstock	F	1989	15.1	–17.5	15.4	–17.7	16.4	–17.9	16.0	–18.3
Al	M	1993	15.8	–17.5	15.7	–17.5	17.0	–18.0	17.2	–18.3
Buster	M	1983	15.5	–17.4	15.2	–17.5	16.8	–17.4	17.0	–18.1
Mean ± SE[b]			15.5 ± 0.1	–17.5 ± 0.1	15.5 ± 0.1	–17.6 ± 0.0	16.7 ± 0.1	–17.9 ± 0.2	16.6 ± 0.2	–18.3 ± 0.1
Avg. fractionation factor between predator and prey[b]			+4.1	+1.3	+4.1	+1.4	+5.2	+1.0	+5.2	+0.6
Fractionation factor between lactating Baabs and prey			+3.5	+1.2	+3.4	+1.2	+3.7	+0.5	+3.9	+0.2

[a]Baabs was sampled twice: in June 2000 while she was pregnant ("pregnant Baabs" in the text) and in October 2000 while she was lactating ("lactating Baabs").
[b]Calculated without values from lactating Baabs.

were different ($P = 0.012$; *t* test), but were close enough to indicate that the two species occupied similar trophic levels. The $\delta^{13}C$ value of the herring (–18.5 ± 0.3‰), however, was significantly higher than that of the capelin (–19.8 ± 0.1‰) ($P = 0.001$; *t* test) (Table 2). All mean isotope ratios for the fur seal blood components were higher than the mean prey isotope ratios (see Table 1). Fur seal RBC and plasma/serum components had mean $\delta^{15}N$ values that were higher than those of their prey by 4.1 and 5.2‰, respectively. The mean $\delta^{13}C$ values of fur seal RBCs, plasma, and serum were higher than those from the fish by ~1.4, 1.0, and 0.6‰, respectively.

Discussion

This study demonstrated consistent ^{15}N enrichment between prey and fur seal RBCs (mean = +4.1‰) and plasma and serum (mean = +5.2‰). The low variability between individuals suggests that these values can be used to assess trophic levels of wild northern fur seals when analyzing specific blood components. Since $\delta^{15}N$ values allow the assessment of an animal's trophic level, the application of known ^{15}N enrichment factors between blood components from fur seals and their diet will enable researchers to better estimate the likelihood that a particular prey species will form part of a wild fur seal's diet. Up to now, isotope studies on wild fur seals (see Kurle and Worthy 2001, 2002) have relied on predator/prey isotope-fractionation factors derived from captive phocid seals (Hobson et al. 1996) and on hypothesized values from trophic-structure studies (Rau et al. 1983; Minagawa and Wada 1984; Fry 1988; Wada et al. 1991; Hobson and Welch 1992).

The carbon isotope fractionation values between the fur seals' blood constituents and their prey were higher than those found between prey and whole blood from captive phocid seals (+1.7‰; Hobson et al. 1996), and were at the middle to high end of those found in marine vertebrates in general (+~0 to 1‰ per trophic level; Minagawa and Wada 1984; Fry 1988; Hobson and Welch 1992). Carbon is thought to be a stronger indicator of habitat usage than of trophic level in marine vertebrate consumers (see Introduction; Rau et al. 1983; Wada et al. 1987; Fry 1988; Hobson and Welch 1992; Hobson 1993; Hobson et al. 1994; Kelly 2000). By applying a combination of the ^{13}C and ^{15}N enrichment factors found in this study, one can better estimate both the trophic level at which wild fur seals are feeding and the type of habitat (i.e., nearshore versus offshore, benthic versus higher in the water column, on-shelf versus off-shelf) in which they are foraging. For example, it may be possible to estimate foraging locations of different populations of otariid species feeding in separate habitats within the Alaska ecosystem. Eulachon (*Thaleichthys pacificus*; $n = 10$) caught in Shelikof Strait, Alaska, in winter 2000 had nearly the same $\delta^{15}N$ values as eulachon ($n = 10$) caught in summer 2000 in the eastern Bering Sea (14.0 ± 0.2 and 13.6 ± 0.1‰, respectively), but their $\delta^{13}C$ values were 1.6‰ higher (–17.8 ± 0.1 and –19.4 ± 0.1‰, respectively) (C.M. Kurle, unpublished data). The $\delta^{15}N$ values from the eulachon indicate that they were feeding at the same trophic level, but their $\delta^{13}C$ values exemplify the different habitats they were utilizing. Otariids feeding on eulachon in one habitat or the other would appear to be feeding at the same trophic level according to their

Table 2. Total lengths (mean ± SE) and $\delta^{15}N$ and $\delta^{13}C$ values (‰; mean ± SE) for whole prey items and weighted mean values for the combined dietary contribution.[a]

	Total length (cm)	Size class	*n*	$\delta^{15}N$	$\delta^{13}C$
Icelandic capelin	14.5 ± 0.3	Medium	5	11.1 ± 0.2	–19.8 ± 0.1
Atlantic herring	26.0 ± 1.0	Medium	5	11.8 ± 0.2	–18.5 ± 0.3
Mean				11.6[a]	–18.9[a]

[a]See eq. 2 in Methods.

$\delta^{15}N$ values, but their $\delta^{13}C$ values would be disparate. Knowing that the ^{13}C enrichment between an otariid's blood components and its prey can help researchers better separate the influence of trophic level from that of foraging on prey which come from different habitats. This should lead to better interpretation of the results of future studies that attempt to trace fur seals' movements using stable carbon isotope signatures.

My findings suggest that tissues from male and female fur seals have the same isotope signatures when the seals are fed a constant diet, which mirrors Hobson et al.'s (1996) results from captive phocid seals. This is also consistent with the results of previous studies on wild fur seals that attributed divergent isotope ratios between the sexes to males and females feeding at different trophic levels and in different locations (Kurle and Worthy 2001, 2002).

Isotope studies on captive birds and mammals illustrate that isotope ratios of different tissues from the same individual are rarely uniform, even when animals are held on consistent diets (Tieszen et al. 1983; Sutoh et al. 1987; Hobson and Clark 1992*a*; Hobson et al. 1996). In captivity, these animals received a consistent diet for up to 6 months (up to 10 months for lactating Baabs) previous to and throughout the study period. Therefore, despite the fact that plasma/serum and RBC components reflect diet incorporated over different time periods, no isotopic change directly related to prey intake should appear. The discrepancy between the $\delta^{15}N$ values from the plasma/serum and RBC samples in this study was likely due to differences in the primary proteins and subsequent amino acids present in the different blood components, because $\delta^{13}C$ and $\delta^{15}N$ values of individual amino acids have a wide range (Macko et al. 1987; Hare et al. 1991; Fantle et al. 1999). For example, the most abundant proteins in plasma and RBCs are serum albumin and hemoglobin, respectively (Seitz 1969; Mathews et al. 2000). A comparison of the serum albumin and hemoglobin sequences from dogs (*Canis familiaris*) showed distinct differences in the numbers of amino acids present in each protein (GenBank database (Benson et al. 2000); hemoglobin alpha and beta chains and serum albumin, GenBank accession Nos. HADG, HBDG, and AJ133489, respectively; Brimhall et al. 1977). Complete sequences of neither protein exist for otariids (see hemoglobin alpha I and II and beta chains from the Galápagos fur seal (*Arctocephalus galapagoensis*), GenBank accession Nos. P4139, P41330, and B61434, respectively; Jahan et al. 1991), so it is impossible to make a direct comparison with their albumin and hemoglobin. It is likely, however, that amino acid discrepancies exist between the proteins in northern fur seals, contributing to the different signatures between blood constituents.

The $\delta^{13}C$ values of serum and plasma were significantly lower than those of RBCs for all of the fur seals. Variability in amino acid content may be partially responsible, but another, more likely cause may be differences in the amount of lipid present in each blood component. Serum albumin is the major carrier of fatty acids in the blood and is found in plasma and serum (Lehninger 1982). Therefore, total lipids are higher in plasma and serum portions of blood than they are in RBCs (see also Nelson 1970, 1971), and higher concentrations of lipids in tissues lead to lower $\delta^{13}C$ values because lipids have proportionally less ^{13}C than proteins (see Kelly 2000; Kurle and Worthy 2002). Additionally, serum is similar to plasma, but lacks fibrinogen and most of the other clotting-factor proteins (Schier et al. 1996). The removal of these proteins may elevate the ratio of lipid to protein, which would lead to a further depletion of ^{13}C in serum compared with plasma. This may account for the fact that serum $\delta^{13}C$ values were slightly lower than those for plasma. The variability seen in the $\delta^{13}C$ values from the plasma and serum may also be attributed to possible differences between individuals in the amount of lipid present in their blood at the time of sampling. Differential lipid loads may slightly affect the $\delta^{15}N$ values between the blood components, but it is unlikely to play a major role. As mentioned earlier, serum and plasma carry more lipids than RBCs do, and the serum and plasma from fur seals in this study had higher $\delta^{15}N$ values than the RBCs. Kurle and Worthy (2002) found that northern fur seal blubber with intact lipids had $\delta^{15}N$ values that were lower by ~1.0‰ over those of blubber with its lipids removed, which is the opposite of the results found here, further suggesting that the presence of lipids was not influencing the $\delta^{15}N$ values of the blood components. In this study I followed the protocols put forth in several previous studies (Hobson et al. 1996; Hobson and Stirling 1997; Roth and Hobson 2000) in which lipids were not extracted from the blood components of the mammalian predators but were extracted from the prey in question prior to isotopic analysis. This consistent methodology suggested that lipid extraction of blood components was unnecessary. However, in light of the variation in the $\delta^{13}C$ values from the different blood components seen in this research, I recommend that in future studies, extracting lipids from all blood components prior to analysis be considered, to avoid possible contamination issues and determine the extent to which lipid content in the blood influences stable-isotope ratios.

A comparison of the stable-isotope ratios for Baabs between the two times she was sampled provided some interesting observations. The time line indicated by the blood constituents and when they were collected reflects prey eaten by Baabs over several stages of pregnancy, birth, and lacta-

tion. Using the stable-isotope half-lives for blood components discussed in the Introduction, it is estimated that RBCs collected from pregnant Baabs would reflect the last 3–4 months of pregnancy, while her plasma/serum would reflect the month or so prior to parturition. RBCs from lactating Baabs would reflect very early lactation, while her plasma/serum would reflect mid to late lactation. All blood components from pregnant Baabs had higher $\delta^{15}N$ values than those from lactating Baabs. It has been hypothesized that the nutritional stress of the fasting/feeding regime experienced by wild northern fur seals during lactation (see Costa and Gentry 1986) contributes to elevated $\delta^{15}N$ values in fur seal tissues (Kurle and Worthy 2001). As Baabs was never nutritionally stressed throughout her pregnancy and lactation period, her situation is not directly comparable to that of a reproductive female in the wild. However, it appears that lactation alone does not cause ^{15}N enrichment in female fur seal blood, but may actually do the opposite. Marine mammals may preferentially excrete ^{15}N into their milk rather than diverting it back into their tissues. For example, Polischuk et al. (2001) found that milk protein from female polar bears (*Ursus maritimus*) was significantly more enriched in ^{15}N than their plasma. Lactating Baabs may have been undergoing this process, thereby contributing to her lower overall blood $\delta^{15}N$ values.

Variation in $\delta^{13}C$ values between Baabs when she was pregnant and lactating were also observed. The $\delta^{13}C$ values from her RBC components were the same, but pregnant Baabs had plasma and serum $\delta^{13}C$ values that were higher by 0.7 and 0.5‰, respectively, than lactating Baabs. The lipid content of Baabs's plasma and serum may have changed between the time when she was pregnant and the time when she was late in her lactation period. Wild northern fur seals produce milk that is much higher in fat content than their diet, indicating that females process, concentrate, and (or) employ their own reserves in producing milk (Costa and Williams 1999). Throughout this process, more lipids may be present in the blood of lactating females, thereby causing their plasma and serum to be depleted in ^{13}C, since, as discussed previously, lipids are known to be isotopically lighter than proteins (see Kelly 2000; Kurle and Worthy 2002). Such a process could account for the distinct differences seen in the $\delta^{13}C$ values of plasma and serum from pregnant and lactating Baabs. Finally, the $\delta^{15}N$ and $\delta^{13}C$ values of the blood components collected from pregnant Baabs do not appear to differ from those of the other, non-parturient females sampled at the same time, indicating that pregnancy may not have an effect on $\delta^{13}C$ and $\delta^{15}N$ values.

Diet-composition data from Atlantic herring and Icelandic capelin demonstrate that the two species have comparable diets consisting of zooplankton (Eschmeyer et al. 1983; Hinrichs 1985; Froese and Pauly 2001), and the relatively low $\delta^{15}N$ values corroborate this. The small disparity in $\delta^{15}N$ values was not large enough to indicate that the two species were feeding at different trophic levels, but may reflect the difference in latitude at which they were feeding. The carbon signatures, however, were very different, which was most likely due to both the discrepancy in latitude and the species' differential proximity to land. The $\delta^{15}N$ and $\delta^{13}C$ values of particulate organic matter and other organisms in marine systems exhibit a decreasing gradient with increase in latitude (Saino and Hattori 1987; Dunton et al. 1989; Saupe et al. 1989; Hobson et al. 1997*b*; Burton and Koch 1999), and carbon signatures are typically higher in nearshore food webs than offshore (McConnaughey and McRoy 1979; Hobson 1993; France 1995). The Atlantic herring were caught on the continental shelf between New Jersey and Cape Cod (between ~39 and ~42°N), while the Icelandic capelin were caught farther from shore, off the coast of Iceland (between ~63 and ~67°N) (J. Kosmark, fish supplier for the Seattle Aquarium at Atlantic/Pacific, Inc., personal communication). The combination of these factors likely caused the herring to have a much higher $\delta^{13}C$ value than the capelin.

In summary, there was consistent mean ^{15}N and ^{13}C enrichment between the fur seals' blood components and their diet. The nitrogen- and carbon-fractionation values between fur seals and their prey established in this study can be used to interpret the trophic level and habitat usage of fur seals feeding in the wild. Possible higher lipid levels in fur seal serum and plasma may have caused their $\delta^{13}C$ values to be lower than those from RBCs, and differential amino acid content probably contributed to the disparity between the $\delta^{15}N$ values from the fur seals' plasma/serum and RBCs.

Acknowledgments

All northern fur seal samples were obtained under National Marine Mammal Laboratory permit No. 782-1399. Thanks are extended to C.J. Casson, S. Larson, A. Smith, and the Seattle Aquarium for access to and blood collection from their captive northern fur seals. Thanks are extended to T. Loughlin and E. Sinclair for continued support, C. Jenkins for help with preparation of tissues for analysis, and T. Maddox at the University of Georgia Institute of Ecology for providing stable-isotope analysis. Thanks are extended to K. Call, G. Duker, C. Jenkins, J. Lee, M. Simpkins, E. Sinclair, and an anonymous reviewer for comments on improving the manuscript.

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