Marine Mammal Science

MARINE MAMMAL SCIENCE, **(*): ***–*** (*** 2010)

DOI: 10.1111/j.1748-7692.2010.00372.x

Assessment of gestation, lactation and fasting on stable isotope ratios in northern elephant seals (*Mirounga angustirostris*)

SARAH HABRAN
MARE Centre,
Laboratory for Oceanology B6c,
University of Liège, B-4000 Liège, Belgium
E-mail: s.habran@ulg.ac.be

CATHY DEBIER
Unité de Biochimie de la Nutrition,
Institut des Sciences de la vie,
Université catholique de Louvain,
Place Croix du Sud 2/8, B-1348 Louvain-la-Neuve, Belgium

DANIEL E. CROCKER
Department of Biology,
Sonoma State University,
Rohnert Park, California 94928, U.S.A.

DORIAN S. HOUSER
BIOMIMETICA,
7951 Shantung Drive,
Santee, California 92071, U.S.A.

GILLES LEPOINT
JEAN-MARIE BOUQUEGNEAU
KRISHNA DAS
MARE Centre,
Laboratory for Oceanology B6c,
University of Liège, B-4000 Liège, Belgium

ABSTRACT

Effects of physiological processes such as gestation, lactation and nutritional stress on stable isotope ratios remain poorly understood. To determine their impact, we investigated these processes in simultaneously fasting and lactating northern elephant seals (*Mirounga angustirostris*). Stable carbon and nitrogen isotope values were measured in blood and milk of 10 mother-pup pairs on days 5 and 22 of lactation. As long- and short-term integrators of diet, blood cells and serum may reflect foraging data or energy reserves from late gestation and lactation, respectively. Limited changes in isotopic signatures of maternal blood over the lactating period were highlighted. Nitrogen isotope fractionation associated with mother-to-offspring

transfer of nutrients was generated between mother and offspring during gestation and lactation. This fractionation was tissue and time-specific, it varied between early and late lactation from +0.6‰ to +1.3‰ in blood cells and from +1.1‰ to nonsignificant value in serum. Therefore, if pups appear to be good proxies to investigate the female trophic ecology especially for C sources, much more caution is required in using $\delta^{15}N$ values. Further studies are also needed to better define the relative impact of fasting and lactation on the enrichment or depletion of isotopes in different tissues.

Key words: northern elephant seal, *Mirounga angustirostris*, stable isotopes, lactation, fasting, gestation.

Stable carbon and nitrogen isotope ratios ($^{13}C/^{12}C$ reported as $\delta^{13}C$ and $^{15}N/^{14}N$ reported as $\delta^{15}N$, respectively) in animal tissues have been widely used to study trophic relationships and foraging locations in terrestrial and marine food webs (Hobson and Stirling 1997, Burns *et al.* 1998, Burton and Koch 1999, Kelly 2000, Kurle and Worthy 2001, 2002, Das *et al.* 2003, Dehn *et al.* 2007). Isotopic ratios in the various foods consumed are reflected in the animal's tissues, proportionate to the amount assimilated for each food source, after accounting for fractionation against heavier isotopes in the digestion and assimilation process (DeNiro and Epstein 1978, 1981). Stable nitrogen isotopes undergo fractionation between predator and prey, leading to an enrichment in ^{15}N with increasing trophic level (DeNiro and Epstein 1981, Minagawa and Wada 1984), thereby denoting an animal's trophic position. The $\delta^{13}C$ value is close to that of the diet and is used to indicate relative contributions to the diet of two different potential primary sources in a trophic network, indicating for example the aquatic *vs*. terrestrial, inshore *vs*. offshore, or pelagic *vs*. benthic contribution to food intake (Hobson *et al.* 1995, Dauby *et al.* 1998). Geographic differences in $\delta^{13}C$ values can be used to indicate foraging locations of animals in marine environments (Kelly 2000, Kurle and Worthy 2002). Stable isotope methodology, however, is dependent on physiological and biochemical assumptions that are not sufficiently taken into consideration (Cherel *et al.* 2005). Indeed, nutritional stress and physiological processes have been shown to affect the isotopic composition of tissues and can therefore complicate isotopic data interpretation (Hobson and Clark 1992*b*, Hobson *et al.* 1993, Kelly 2000, Kurle and Worthy 2001, Fuller *et al.* 2004, 2005, Cherel *et al.* 2005).

Many wild animals fast during their breeding, weaning, molting, or migration periods (Mrosovsky and Sherry 1980). Using their endogenous nutrient stores, fasting animals literally "feed on themselves" (Cherel *et al.* 2005). These animals undergoing nitrogen recycling preferentially return ^{15}N to their nitrogen pool, thereby enriching their tissues in ^{15}N by utilizing a more ^{15}N-rich nitrogen source for amino acid synthesis (Minagawa and Wada 1984, Kelly 2000, Fuller *et al.* 2005). Thus, conditions such as nutritional stress (Hobson and Clark 1992*b*, Hobson *et al.* 1993, Voigt and Matt 2004, Fuller *et al.* 2005) or disease (Katzenberg and Lovell 1999) have been shown to increase $\delta^{15}N$ values when an organism presents a net catabolic state associated with lean muscle degradation (or negative nitrogen balance). On the contrary, a net anabolic state associated with protein synthesis (or positive nitrogen balance) during gestation (Fuller *et al.* 2004) or lactation (Kurle 2002) causes a decrease in the nitrogen isotope ratio of the maternal body protein pool. Since the metabolic state induces variation in the nitrogen isotope ratio of the body protein pool (Fuller *et al.*

2004, 2005), it is expected that $\delta^{15}N$ values of animals will be dependent not only on their food sources and trophic levels, but also on their nutritional and physiological status (Cherel *et al.* 2005). Gestation, lactation, or fasting can thus lead to erroneous interpretations of $\delta^{15}N$ values within the context of feeding ecology if the effect of these processes is not taken into account.

Nitrogen and carbon stable isotope analysis has also been used to explore lactation patterns in mammalian females (Nelson *et al.* 1998, Polischuk *et al.* 2001, Sare *et al.* 2005, Newsome *et al.* 2006), to explore tactics of energy acquisition for lactation (Dalerum *et al.* 2007), or to explore the ecology of adult mammal populations by sampling their offspring (Aurioles *et al.* 2006, Ducatez *et al.* 2008). In some cases, reliable conclusions from ^{15}N data may be limited by the scant understanding of the effects of various physiological variables on isotope assimilation (Dalerum *et al.* 2007). The isotopic fractionations associated with mother-to-offspring transfer of nutrients during gestation, lactation, and weaning are still poorly understood (Newsome *et al.* 2006). Theoretically, offspring "feeding" on maternal tissue would be a trophic level higher than its mother until weaning. This ^{15}N-enrichment of the offspring over its mother during gestation or nursing has been shown in different species (Hobson and Sease 1998, Nelson *et al.* 1998, Polischuk *et al.* 2001, Dalerum *et al.* 2007). However, according to Jenkins *et al.* (2001), this fractionation between offspring and mother is species-specific and the model of increased trophic level of nursing offspring in relation to mother may be too simplistic. The magnitude of animal-to-diet ^{15}N-enrichment may be a function of the protein quality (Robbins *et al.* 2005) or the C:N ratio (*i.e.*, nitrogen concentration) of the diet (Pearson *et al.* 2003). For carbon isotopes, the trophic level prediction may be complicated by the fact that milk in some marine mammals has a high lipid content (Newsome *et al.* 2006), and lipids are ^{13}C-depleted relative to proteins (DeNiro and Epstein 1978). Lipids probably have little impact on $\delta^{15}N$ values given their low nitrogen content, so the consumption of milk rich in lipids would not affect the ^{15}N-enrichment.

Marine mammals such as pinnipeds come ashore to give birth and to suckle their young, and most of them mate and molt on land. These haul-out periods involve some intense physiological processes for several true seal species like elephant seals. Northern elephant seal (*Mirounga angustirostris*) females give birth shortly after arrival on rookeries in California and Mexico and nurse a single pup for an average of 24–28 d (Le Boeuf *et al.* 1972, Le Boeuf and Laws 1994). During this period, they fast completely from food and water while secreting a fat-rich milk synthesized from their body reserves (Le Boeuf and Ortiz 1977). Lactating females may lose more than a third of their initial body mass, while pups gain around 90 kg during lactation (Crocker *et al.* 2001). Pups are abruptly weaned when females return to sea to forage. Mating occurs at the end of nursing period. Elephant seals utilize embryonic diapause, with implantation of the blastocyst thought to occur during the molt (Crocker *et al.* 2001). Active embryonic growth occupies about 7.5 mo (Laws 1956), when elephant seal females spend their time foraging in the ocean.

In order to assess the effect of gestation on the isotopic composition, we used a tissue with a medium isotopic turnover rate, such as blood cells that illustrate a foraging period of 2–3 mo in large mammals (Hilderbrand *et al.* 1996). Blood cells collected from the beginning of lactation in mother-pup pairs should reflect the diet and potential effect of physiological processes occurring during the last months of gestation. In contrast, blood serum typically represents information over a period of a few days to a week (Hobson and Clark 1992*a*, 1993, Hilderbrand *et al.* 1996). Therefore, blood serum and milk collection during lactation allowed

the assessment of how lactation and fasting affect stable carbon and nitrogen isotope ratios in northern elephant seal mothers and pups.

Materials and Methods

Field Techniques

This study was conducted at Año Nuevo State Reserve, California (37°06′30″N, 122°20′10″W), during the 2005 breeding season (January–February). On arrival at the rookery, females were marked with hair dye (Clairol, Stamford, CT). Dates of birth were recorded by observing the marked females each day. Ten mother-pup pairs were captured on day 5 of lactation (early lactation) and then, were recaptured on day 22 of lactation (late lactation). Mothers were immobilized with an intramuscular injection of Telazol (1 mL per 100 kg of estimated body mass) and immobilization was maintained with intravenous injections of Ketamine (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) and Diazepam (Elkins-Sinn, Cherry Hill, NJ). Blood samples were collected from the extradural vein. Milk was collected from the teat using a clean cut-off syringe after a subcutaneous injection of 40 IU of oxytocin (10 U mL^{-1}, American Pharmaceuticals Partners, Los Angeles, CA) near the mammary gland. At the first capture (on day 5), pups were hand captured, while at the second capture, pups were immobilized with Telazol at the same mass-specific dosage used for the adult females. Blood samples were collected from the extradural vein of the pups on days 5 and 22 of lactation. At each capture, both mother and pup were measured using a measuring tape, and weighed using a scale (capacity 1,000 ± 1 kg and 500 ± 0.2 kg) suspended from a tripod. The pup's sex was determined.

After each procedure, the mother and pup were released and monitored until the female was completely mobile. All samples were kept on ice in the field (at 4°C). At the end of each day, whole blood samples were centrifuged for 20 min and the cellular component was harvested for analysis. Serum was aliquoted into 1.8 mL Nunc tubes (Nunc Cryotubes, Nalge Nunc Ink, Roskilde, Denmark) and samples were stored at −20°C for later analysis.

Stable Isotope Ratio Measurements

Blood cells, serum, and milk samples were freeze-dried, ground with a mortar and pestle into powder, and loaded into tin boats (1.5–2.0 mg for blood cells and serum and 4–5 mg for milk). Lipids were not removed from tissue samples. Stable isotope measurements were performed with an isotope ratio mass spectrometer (V. G. Optima, Micromass) coupled to an N-C-S elemental analyzer (Carlo Erba) for automated analyses. Stable isotope abundances are expressed in delta (δ) notation as the deviation from standards in parts per thousand (‰) according to the following equation:

$$\delta X = \left[\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right] \times 1{,}000$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Standard values were based on the Vienna PeeDee Belemnite (v-PDB) for $\delta^{13}C$ measurements and atmospheric nitrogen for $\delta^{15}N$ measurements. Reference materials were

IAEA-N1 ($\delta^{15}N = 0.4 \pm 0.2‰$) and IAEA CH-6 (sucrose) ($\delta^{13}C = -10.4‰ \pm 0.2‰$). Internal standards (glycine) were inserted into all runs at regular intervals to calibrate the system and to assess drift over time. Standard deviations of internal standard replicates were 0.1‰ and 0.3‰ for carbon and nitrogen, respectively.

Statistical Analyses

A Kolmogorov–Smirnov test was used to determine whether data departed from normality. To evaluate the variability during lactation for each variable and each sampled tissue, paired *t*-tests were used to compare means in early (day 5) and late (day 22) lactation. Analysis of variance (ANOVA) with repeated measures was used to determine equality of the isotope ratios among the different sampled tissues. A test was carried out for each sampling (on days 5 and 22) and means were compared pairwise, using Scheffe's *post hoc* test. The Bravais–Pearson correlation coefficient was used to test correlations between two variables. Statistical analysis of the data was performed using Statistica software (Statsoft Inc., version 7.1) and $P < 0.05$ was considered as significant. Results are presented as means ± standard deviation (SD).

RESULTS

Biometry of the northern elephant seal mothers and pups is shown in Table 1. The mean $\delta^{15}N$ and $\delta^{13}C$ values in blood cells, serum and milk for early (day 5) and late (day 22) lactation are presented in Figure 1. The $\delta^{15}N$ values in pup blood components and milk differed significantly between day 5 and day 22 of lactation. Pup blood cells had higher $\delta^{15}N$ values on day 22 ($+0.7 \pm 0.3‰$, paired *t*-test; $P < 0.001$, $n = 9$), while pup serum had lower $\delta^{15}N$ values on day 22 ($-0.5‰ \pm 0.3‰$, paired *t*-test; $P < 0.001$, $n = 8$). Milk also had lower $\delta^{15}N$ values in late lactation ($-0.4‰ \pm 0.3‰$, paired *t*-test; $P = 0.002$, $n = 10$). No significant difference was observed for $\delta^{15}N$ values in maternal blood cells and serum between both samplings (paired *t*-tests; $P = 0.695$, $n = 10$, and $P = 0.526$, $n = 9$, respectively). Only the $\delta^{13}C$ values in maternal serum differed significantly between early and late lactation ($-0.2‰ \pm 0.1‰$, paired *t*-test; $P = 0.003$, $n = 9$). The $\delta^{13}C$ values in maternal and pup blood cells, pup serum, and milk remained similar between both samplings (paired *t*-tests; $P = 0.145$, $n = 10$; $P = 0.485$, $n = 9$; $P = 0.068$, $n = 8$; and $P = 0.056$, $n = 10$, respectively).

Table 1. Biometry of northern elephant seal mothers and pups on days 5 and 22 of lactation; mean ± SD (range).

	Lactation duration (day)	25 ± 2 (22–29)	
		Day 5	Day 22
Mothers	Body weight (kg)	453 ± 55 (401–588)	330 ± 49 (283–448)
	Standard length (cm)	256 ± 14 (240–284)	257 ± 12 (232–278)
Pups (6♂, 4♀)	Body weight (kg)	43 ± 3 (37–48)	114 ± 8 (97–122)
	Standard length (cm)	127 ± 5 (117–133)	146 ± 8 (129–152)
	Axial girth (cm)	87 ± 7 (75–99)	128 ± 5 (119–136)

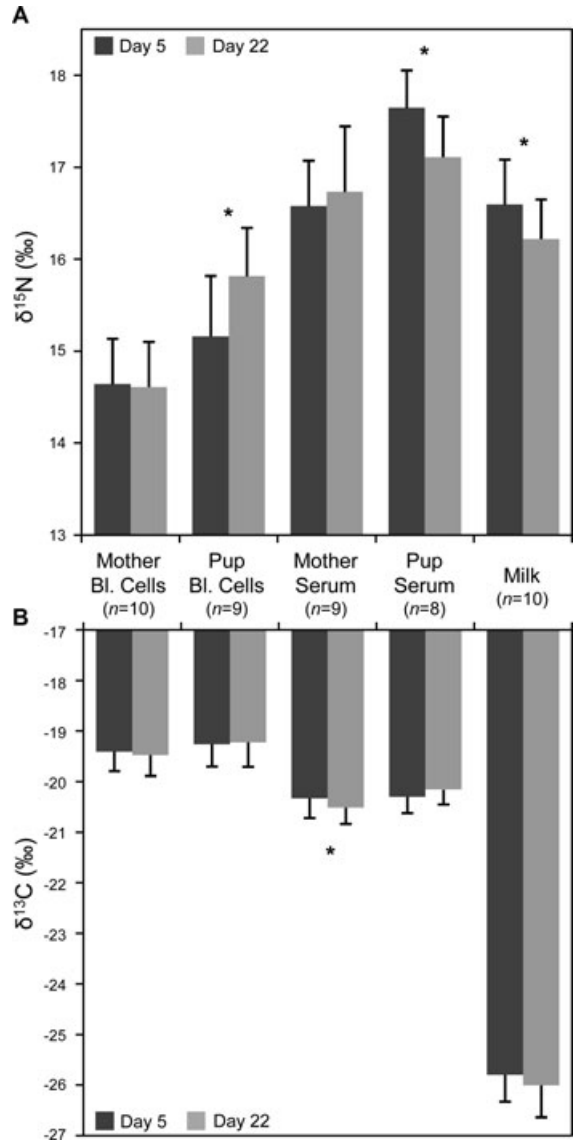

Figure 1. Mean (± SD) $\delta^{15}N$ (A) and $\delta^{13}C$ (B) (‰) in blood cells, serum and milk of northern elephant seal mothers and pups on days 5 (dark bars) and 22 (light bars) of lactation. *Significant difference between values on day 5 and values on day 22 (paired *t*-test). (Table with values in Appendix).

Tissue Comparison

The $\delta^{15}N$ and $\delta^{13}C$ values differed significantly between blood cells and serum independent of the lactation period. Serum had $\delta^{15}N$ values ~2‰ higher and $\delta^{13}C$ values ~0.9‰ lower than blood cells for mothers and pups (Table 2). The $\delta^{15}N$

Table 2. Results from analysis of variance with repeated measures (ANOVA) and Scheffe's *post-hoc* tests of mean $\delta^{15}N$ and $\delta^{13}C$ values on days 5 and 22 of lactation in tissues of northern elephant seal mothers and pups. The difference between tissue 1 and tissue 2 is noted for each Scheffe's *post-hoc* test.

ANOVA (all $P < 0.001$, $n = 8$)		$\delta^{15}N$		$\delta^{13}C$	
		$F_{4,28} = 131.6$	$F_{4,28} = 64.3$	$F_{4,28} = 1{,}851.9$	$F_{4,28} = 2{,}826.7$
Tissue 1	Tissue 2	Day 5	Day 22	Day 5	Day 22
Maternal serum	Maternal blood cells	$P < 0.001$ 2.0 ± 0.4	$P < 0.001$ 2.1 ± 0.7	$P < 0.001$ -0.8 ± 0.3	$P < 0.001$ -1.0 ± 0.3
Pup serum	Pup blood cells	$P < 0.001$ 2.5 ± 0.5	$P < 0.001$ 1.3 ± 0.2	$P < 0.001$ -0.9 ± 0.2	$P < 0.001$ -0.8 ± 0.2
Milk	Maternal serum	$P = 1.000$ 0.0 ± 0.3	$P = 0.127$ -0.5 ± 0.5	$P < 0.001$ -5.6 ± 0.3	$P < 0.001$ -5.6 ± 0.2
Milk	Pup serum	$P < 0.001$ -1.1 ± 0.5	$P < 0.001$ -1.0 ± 0.3	$P < 0.001$ -5.6 ± 0.3	$P < 0.001$ -6.0 ± 0.2
Pup blood cells	Maternal blood cells	$P = 0.021$ 0.6 ± 0.4	$P < 0.001$ 1.3 ± 0.3	$P = 0.716$ 0.1 ± 0.1	$P = 0.110$ 0.2 ± 0.1
Pup serum	Maternal serum	$P < 0.001$ 1.1 ± 0.3	$P = 0.122$ 0.5 ± 0.7	$P = 1.000$ 0.0 ± 0.1	$P = 0.002$ 0.4 ± 0.2

values in milk were similar to those in maternal serum, but were significantly lower by ~1‰ than those measured in pup serum (Table 2). In contrast, milk had $\delta^{13}C$ values ~5.6‰ lower than both maternal and pup serum (Table 2).

Isotopic Fractionation between Mother and Offspring

Blood components in pups had significantly higher $\delta^{15}N$ values than in mothers (from +0.6‰ to +1.3‰), except for serum on day 22 of lactation (Table 2). The enrichment between mother and offspring increased with time in blood cells (between days 5 and 22: +0.7‰ ± 0.4‰), while it decreased with time in serum. In contrast, the $\delta^{13}C$ values in pup blood components remained similar to those of the mother, except for pup serum on day 22 (Table 2).

Relationships between Mother and Offspring

Carbon and nitrogen signatures of pups and their mothers were positively and linearly correlated in blood cells ($\delta^{13}C$: $r = 0.95$, $P < 0.001$ on day 5; $r = 0.97$, $P < 0.001$ on day 22; $\delta^{15}N$: $r = 0.85$, $P = 0.004$ on day 5; $r = 0.85$, $P = 0.003$ on day 22; for all $n = 9$). Similar relationships were observed in serum, except for $\delta^{15}N$ values on day 22 ($\delta^{13}C$: $r = 0.95$, $P < 0.001$ on day 5; $r = 0.76$, $P = 0.028$ on day 22; $\delta^{15}N$: $r = 0.75$, $P = 0.030$ on day 5; $r = 0.17$, $P = 0.683$ on day 22; for all $n = 8$; Fig. 2A). A significant positive relationship was observed between $\delta^{15}N$ values in milk and pup serum only on day 22 ($r = 0.81$, $P = 0.015$, $n = 8$; Fig. 2B).

Variation of C:N Ratio

Carbon-nitrogen ratio (C:N) in maternal and pup blood cells was 3.3 ± 0.0 and was constant during lactation (paired *t*-test; $P = 0.6$, $n = 10$ for mothers and $n = 9$

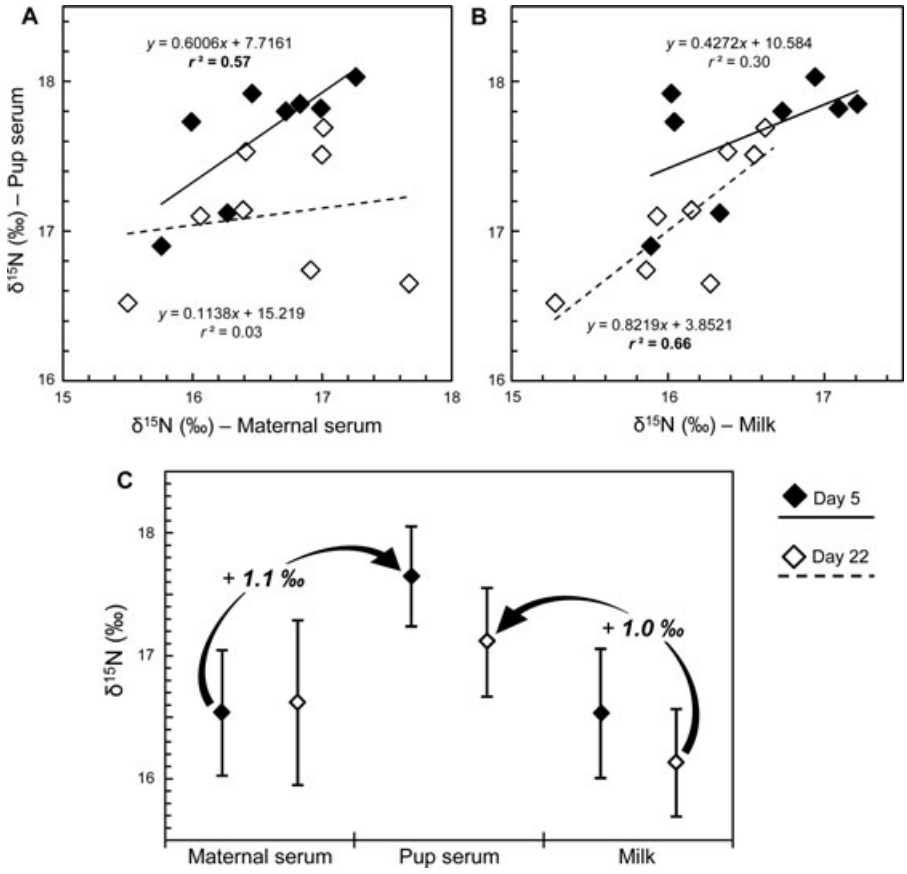

Figure 2. (A) Relationships between $\delta^{15}N$ values (‰) in maternal serum and pup serum and (B) between $\delta^{15}N$ values (‰) in milk and pup serum (Bravais–Pearson correlation). (C) Mean (± SD) $\delta^{15}N$ (‰) in maternal and pup serum and milk during lactation (on days 5 and 22, $n = 9$). The arrows show enrichments in ^{15}N in pup serum in relation to maternal serum and milk.

for pups). Maternal serum displayed a C:N ratio of 4.0 ± 0.1 on day 5 and 4.2 ± 0.1 on day 22. These values differed significantly (paired *t*-test; $P < 0.001$, $n = 9$). The C:N ratio in pup serum was constant during lactation, with 3.8 ± 0.1 and 3.9 ± 0.1 on days 5 and 22, respectively (paired *t*-test; $P = 0.166$, $n = 8$). In milk, this ratio differed between both samplings, with 18.5 ± 2.1 on day 5 and 39.2 ± 5.1 on day 22 (paired *t*-test; $P < 0.001$, $n = 10$). No relationship between $\delta^{13}C$ values and C:N ratio was observed in blood components or milk (for all $P > 0.05$).

Discussion

Due to their varying protein and therefore isotopic turnover rates, blood components illustrate foraging data from different time periods (Kelly 2000). Stable

isotopes in blood cells are believed to reflect combined effects of both retrospective diet and physiological processes during the last 2–3 mo of gestation in northern elephant seals. Serum with its high turnover rate reflected nutrients incorporated approximately one week prior and up to sampling. Interestingly, the $\delta^{15}N$ and $\delta^{13}C$ values varied systematically between blood cells and serum for mothers as well as pups. These $\delta^{15}N$ and $\delta^{13}C$ variations could reflect a change of the maternal diet between their foraging area and their breeding area. Before the breeding season, northern elephant seal females feed in deep oceanic waters of the North Pacific Ocean as far as several thousand kilometers from the Californian coast (Aurioles *et al.* 2006). On their way back to breeding sites, females shift from a pelagic diet to a more inshore or benthic diet. The inshore or benthic C sources are known to be enriched in ^{13}C relative to offshore or pelagic C sources (Rau *et al.* 1992, Gannes *et al.* 1998, Kelly 2000). Consecutively, higher $\delta^{13}C$ values were expected in serum compared to blood cells. Furthermore, if we hypothesized that females fast during their return migration, enhanced $\delta^{13}C$ values in serum should be expected (see Introduction). However, higher $\delta^{13}C$ values were measured in blood cells than in serum. The specific biochemical composition of blood cells and serum might shed some light on such a discrepancy. Several authors have indeed observed such differences between both these components even on a constant diet (Kurle 2002, Lesage *et al.* 2002, Zhao *et al.* 2006). A likely cause would be differences in the amount of lipid present in each blood component (Kurle 2002). Circulating lipids in the blood are transported in serum by different macromolecules such as serum albumin and lipoproteins, and lipid content is higher in serum than it is in blood cells of pinnipeds (14.6% *vs.* 3.8%, respectively; Lesage *et al.* 2002). As a potential indicator of lipid content, the C:N ratios calculated from carbon and nitrogen percentages in this study corroborate these statements (4 in serum *vs.* 3.3 in cells). So a higher lipid content can generate the lower $\delta^{13}C$ values since lipids are known to be ^{13}C-depleted during biochemical fractionation (DeNiro and Epstein 1978, Tieszen *et al.* 1983). Nevertheless, no relationship between $\delta^{13}C$ values and C:N ratio was observed in blood components. On the other hand, differences in proteins and amino acid patterns between blood cells and serum might lead to variations in $\delta^{15}N$ values. Each amino acid has a wide range of isotopic values for nitrogen (Fantle *et al.* 1999, Zhao 2002). The most abundant proteins in serum and blood cells are serum albumin and hemoglobin, respectively (Wheater *et al.* 2001).

Effect of Gestation on Stable Isotope Ratios

The $\delta^{15}N$ values in blood cells showed that gestation in northern elephant seals generates a nitrogen isotope fractionation between mother and offspring. The ^{15}N-enrichment in relation to mothers was $+0.6‰ \pm 0.4‰$ at first sampling (day 5). This enrichment increased between both samplings to reach $+1.3‰ \pm 0.3‰$ later (Table 2). The nitrogen isotope fractionation between mother and offspring linked to gestation is reported in several species of terrestrial and marine mammals, but it is not systematic and may vary by species (+5‰ in bones of European cave bears, *Ursus spelaeus* [Nelson *et al.* 1998]; +1.5‰ and +3.5‰ in liver and muscle, respectively, of harbor porpoises, *Phocoena phocoena* [Fontaine 2002]). The phenomenon could be explained by the progressive ^{15}N-enrichment of the fetus from maternal tissue while the maternal $\delta^{15}N$ values are decreasing such that the offset between the mother-fetus pair is essentially balanced (Fuller *et al.* 2004). Previous study has demonstrated

that gestation generates a decrease in $\delta^{15}N$ values in maternal human hair (Fuller *et al.* 2004) due probably to maternal nitrogen conservation during pregnancy. In this study, we did not observe any variation in $\delta^{15}N$ and $\delta^{13}C$ values in maternal blood cells measured on days 5 and 22 of lactation. However, the sampling interval may seem rather short and unsuitable to conclude that the gestation did not modify isotopic signatures in maternal blood cells. Unfortunately no point of comparison with nonparturient females is available. It would be interesting to investigate molting elephant seal females since the isotopic signature of their blood cells will not yet have integrated the potential effect of active embryonic growth. Some previous marine mammal studies also reported the absence of difference in $\delta^{15}N$ and $\delta^{13}C$ values with those obtained in other, nonparturient females in northern fur seals, *Callorhinus ursinus* (Kurle 2002) and harbor porpoises (Fontaine 2002).

Effect of Lactation Period on Stable Isotope Ratios

Northern elephant seals, like several other true seal species, fast during lactation. Lactating and fasting females must thus meet both their own metabolic requirements and, through milk production, the nutrient requirements of their pups for neonatal and post-weaning development (Kovacs and Lavigne 1986). From a physiological point of view, whereas fasting in phocids is characterized by protein sparing and reductions in metabolic rates, lactation is characterized by dramatic increases in metabolism and a significant transfer of nutrients and water to mammary gland for the synthesis of milk (Crocker *et al.* 1998). Lactating and fasting females are therefore faced with a dual challenge and show a progressive increase in the rate of protein catabolism across lactation (Crocker *et al.* 1998), although their total free amino acid concentration in plasma has been shown to decline by 11% as fasting progresses (Houser and Crocker 2004). According to this information, we expected to find higher $\delta^{15}N$ values in maternal serum in late lactation since a net catabolic state associated with lean muscle degradation results in an increase in the nitrogen isotope ratio (see Introduction). Surprisingly, we found no significant change in the nitrogen isotope ratio of maternal serum between early and late lactation. This discrepancy is likely to be a consequence of a counteracting effect of lactogenesis. Indeed, lactogenesis may cause ^{15}N depletion in maternal tissues. Kurle (2002) observed this process in a captive northern fur seal (*Callorhinus ursinus*)—that had never been nutritionally stressed—where $\delta^{15}N$ values in blood components were depleted during lactation in relation to those during gestation. Whereas $\delta^{15}N$ values may decrease in maternal tissues during lactation, milk produced in marine mammals might be enriched in ^{15}N over maternal tissues (Kurle 2002). For instance, milk protein from female polar bears (*Ursus maritimus*) is significantly more enriched in ^{15}N than their plasma (Polischuk *et al.* 2001). However, contrary to what was expected, we did not observe any variation in $\delta^{15}N$ values between maternal serum and milk. In contrast, milk was uniformly ^{15}N-depleted during lactation independently of maternal serum. Although milk protein concentration remains fairly constant during lactation (8%–15% of milk by weight, Riedman and Ortiz 1979, Davis *et al.* 1995, Crocker *et al.* 2001), the stage of lactation has a slight effect on milk amino acid pattern, with changes in the amino acids histidine, isoleucine, methionine, and valine (Davis *et al.* 1995). These slight variations in milk amino acid pattern may explain modifications in $\delta^{15}N$ values in the milk of northern elephant seals during lactation.

Lactogenesis makes a large demand on lipid reserves, as produced milk is copious and energy-rich. The milk fat content rises during lactation from approximately 15% to 55%, while the water content falls from 75% to a level of 35% (Riedman and Ortiz 1979). Milk energy and nutrient contents are derived entirely from maternal body stores, and are shipped to the pup *via* the different compartments of transfer (maternal serum → milk → pup serum). Circulating fatty acid and triglyceride concentrations in maternal serum show high levels and increase across lactation (McDonald and Crocker 2006). This is in agreement with the higher C:N ratio that we measured in late lactation and the slight decrease observed in $\delta^{13}C$ values of maternal serum during lactation ($-0.2‰ \pm 0.1‰$), since lipids are known to be ^{13}C-depleted during biochemical fractionation (DeNiro and Epstein 1978, Tieszen *et al.* 1983). Milk showed low $\delta^{13}C$ values, easily explained by its high fat content. However, no variation in $\delta^{13}C$ values was observed between early and late lactation despite the high increase in the C:N ratio in milk supporting the lipid content increase throughout lactation.

Although lipids probably have little impact on $\delta^{15}N$ values given their low nitrogen content, they may greatly influence $\delta^{13}C$ values when present in appreciable amounts (Lesage *et al.* 2002). As Kurle (2002) and Lesage *et al.* (2002) made clear, lipids should be extracted from lipid-rich tissues, or with tissues having a lipid content that may vary unpredictably between individuals, such as blood serum or plasma. This would allow the determination of the extent to which lipid content in the blood and milk influences stable isotope ratios (Kurle 2002).

A nitrogen isotope fractionation between mother and offspring was measured in serum on day 5. Surprisingly, we observed a decrease in $\delta^{15}N$ values of pup serum throughout lactation ($-0.5‰$) and therefore in the ^{15}N fractionation between mother and offspring. With its high turnover rate, serum likely reflects the nutrients incorporated during the week previous to collection. Serum collected on day 5 of lactation would reflect information since a few days before birth, whereas serum collected on day 22 would reflect information since the middle of lactation. Although milk intake already started on day 5, gestation was still influencing the isotopic signature in serum during which her offspring was directly nourished from nutrients within the maternal blood. This may explain why we observed a relationship between $\delta^{15}N$ values in maternal serum and pup serum only on day 5 (Fig. 2A), whereas there was no relationship between milk and pup serum (Fig. 2B). A ^{15}N fractionation between mother and offspring occurred on day 5 ($+1.1‰$ in pups, Fig. 2C). In contrast, on day 22 the relationship between maternal and pup serum was no longer observed (Fig. 2A). The isotopic signature of pup serum became dependent on the isotopic signature of the milk (Fig. 2B) since the pup is nourished *via* maternal milk. Similarly, a ^{15}N fractionation occurred between milk and pup serum ($+1.0‰$ in pups on day 22, Fig. 2C). The ^{15}N fractionation between maternal and pup tissues is thus maintained during lactation with a similar magnitude. The ^{15}N-depletion in pup serum during lactation might reflect the diet shift (from maternal blood to milk) and the effect of a net anabolic state associated with growth and protein synthesis (see Introduction). Same observations of decreasing pup $\delta^{15}N$ values during lactation have been reported in hair of northern elephant seals (Aurioles *et al.* 2006) and bones of European cave bears (Nelson *et al.* 1998).

In the congeneric southern elephant seal, Ducatez *et al.* (2008) investigated the winter trophic ecology and maternal investment with the help of stable isotope analysis in maternal and pup blood. Although they analyzed whole blood instead of separating blood cells and serum, similar results were obtained in the northern

and southern elephant seal (NES and SES, respectively). First, limited changes in isotopic signatures of maternal blood over the lactating period were highlighted in both species. Overall, pup blood isotopic composition in NES also reflects that of the mother in a predictable manner. Indeed, significant positive and linear relationships were found for $\delta^{15}N$ and $\delta^{13}C$ values in blood cells and serum between pups and mothers, except for $\delta^{15}N$ values in serum in late lactation (day 22). An identical increase in $\delta^{15}N$ values between pups and mothers was observed: $+1.3‰ \pm 0.3‰$ in whole blood for SES (Ducatez *et al.* 2008) and $+1.3‰ \pm 0.3‰$ in blood cells for NES (in this study). However, if NES pups appear to be good proxies to investigate the female trophic ecology especially for C sources, much more caution is required in using $\delta^{15}N$ values and no generalization can be made. The nitrogen isotope fractionation between mother and offspring is tissue and time-specific. It varied between early and late lactation from $+0.6‰$ to $+1.3‰$ in blood cells and from $+1.1‰$ to nonsignificant value in serum of NES. Some processes during lactation remain misunderstood in tissues with a high rate of protein turnover, such as serum. Further studies are needed to better define the relative impact of fasting and lactation on the enrichment or depletion of isotopes in different tissues.

Acknowledgments

This study was performed at the Año Nuevo State Reserve, a part of the University of California Natural Reserve System. The authors are grateful to R. Biondo for his technical assistance. The authors would also like to thank C. Champagne and M. Fowler for their assistance with sample collection and the park rangers of Año Nuevo for their cooperation in this study. We also thank S. Caut for his valuable comments on the manuscript. We would additionally like to acknowledge the Clairol Corporation for providing hair dye that was use as a marking solution. K.D. is a F.R.S.-FNRS Research Associate. S.H. is a F.R.S.-FNRS Research fellow. This study was supported by NSF grant #0213095 and by FRFC grant #2.4502.07 (F.R.S.-FNRS). Research was conducted under National Marine Fisheries Service marine mammal research permit #786-1463 and all animal handling procedures were approved by the Institutional Animal Care and Utilization Committee of the University of California, Santa Cruz. This paper is MARE publication 184.

Literature Cited

Aurioles, 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.

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.

Cherel, Y., K. A. Hobson, F. Bailleul and R. Groscolas. 2005. Nutrition, physiology, and stable isotopes: New information from fasting and molting penguins. Ecology 86:2881–2888.

Crocker, D. E., P. M. Webb, D. P. Costa and B. J. Le Boeuf. 1998. Protein catabolism and renal function in lactating northern elephant seals. Physiological Zoology 71:485–491.

Crocker, D. E., J. D. Williams, D. P. Costa and B. J. Le Boeuf. 2001. Maternal traits and reproductive effort in northern elephant seals. Ecology 82:3541–3555.

Dalerum, F., N. C. Bennett and T. H. Clutton-Brock. 2007. Longitudinal differences in ^{15}N between mothers and offspring during and after weaning in a small cooperative mammal, the meerkat (*Suricata suricatta*). Rapid Communications in Mass Spectrometry 21:1889–1892.

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

Dauby, P., A. Khomsi and J. M. Bouquegneau. 1998. Trophic relationships within intertidal communities of the Brittany Coasts: A stable carbon isotope analysis. Journal of Coastal Research 14:1202–1212.

Davis, T. A., H. V. Nguyen, D. P. Costa and P. J. Reeds. 1995. Amino acid composition of pinniped milk. Comparative Biochemistry and Physiology. Part B 110:633–639.

Dehn, L. A., G. G. Sheffield, E. H. Follmann, L. K. Duffy, D. L. Thomas and T. M. O'hara. 2007. Feeding ecology of phocid seals and some walrus in the Alaskan and Canadian Arctic as determined by stomach contents and stable isotope analysis. Polar Biology 30:167–181.

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.

Deniro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45:341–351.

Ducatez, S., S. Dalloyau, P. Richard, C. Guinet and Y. Cherel. 2008. Stable isotopes document winter trophic ecology and maternal investment of adult female southern elephant seals (*Mirounga leonina*) breeding at the Kerguelen Islands. Marine Biology 155:413–420.

Fantle, M. S., A. I. Dittel, S. M. Schwalm, C. E. Epifanio and M. L. Fogel. 1999. A food web analysis of the juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. Oecologia 120:416–426.

Fontaine, M. 2002. Sources de variation des concentrations en métaux traces du marsouin commun (*Phocoena phocoena*) (L.) capturés dans des filets le long des côtes norvégiennes. M.S. dissertation, Université de Liège, Liège, Belgium. 64 pp.

Fuller, B. T., J. L. Fuller, N. E. Sage, D. A. Harris, T. C. O'Connell and R. E. M. Hedges. 2004. Nitrogen balance and $\delta^{15}N$: Why you're not what you eat during pregnancy. Rapid Communications in Mass Spectrometry 18:2889–2896.

Fuller, B. T., J. L. Fuller, N. E. Sage, D. A. Harris, T. C. O'Connell and R. E. M. Hedges. 2005. Nitrogen balance and $\delta^{15}N$: Why you're not what you eat during nutritional stress. Rapid Communications in Mass Spectrometry 19:2481–2728.

Gannes, L. Z., C. Martinez Del 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.

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., and R. G. Clark. 1992*a*. Assessing avian diets using stable isotopes I: Turnover of carbon-13 in tissues. Condor 94:181–188.

Hobson, K. A., and R. G. Clark. 1992*b*. Assessing avian diets using stable isotopes II: Factors influencing diet-tissue fractionation. Condor 94:189–197.

Hobson, K. A., and R. G. Clark. 1993. Turnover of ^{13}C in cellular and plasma fractions of blood: Implications for nondestructive sampling in avian dietary studies. Auk 110:638–641.

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., and I. Stirling. 1997. Low variation in blood $d^{13}C$ among Hudson Bay polar bears: Implications for metabolism and tracing terrestrial foraging. Marine Mammal Science 13:359–367.

Hobson, K. A., R. T. Alisauska and R. G. Clark. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: Implications for isotopic analyses of diet. Condor 95:388–394.

Hobson, K. A., W. G. J. Ambrose and P. E. Renaud. 1995. Sources of primary production, benthic-pelagic coupling, and trophic relationships within the Northeast Water Polynya: Insights from delta^{13}C and delta^{15}N analysis. Marine Ecology Progress Series 128:1–10.

Houser, D. S., and D. E. Crocker. 2004. Age, Sex, and reproductive state influence free amino acid concentrations in the fasting elephant seal. Physiological and Biochemical Zoology 77:838–846.

Jenkins, S. G., S. T. Partridge, T. R. Stephenson, S. D. Farley and C. T. Robbins. 2001. Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. Oecologia 129:336–341.

Katzenberg, M. A., and N. C. Lovell. 1999. Stable isotope variation in pathological bone. International Journal of Osteoarchaeology 9:316–324.

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

Kovacs, K. M., and D. M. Lavigne. 1986. Maternal investment and neonatal growth in phocid seals. Journal of Animal Ecology 55:1035–1051.

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, R. M. 1956. The elephant seal (*Mirounga leonina* Linn.): III. The physiology of reproduction. F. I. D. SURVEY. Scientific Reports 15:1–66.

Le Boeuf, B. J., and R. M. Laws. 1994. Elephant seals: An introduction to the genus. Pages 1–26 *in* B. J. Le Boeuf and R. M. Laws, eds. Elephant seals: Population ecology, behavior, and physiology. University of California Press, Berkeley, CA.

Le Boeuf, B. J., and C. L. Ortiz. 1977. Composition of elephant seal milk. Journal of Mammalogy 58:684–685.

Le Boeuf, B. J., R. J. Whiting and R. F. Gantt. 1972. Perinatal behavior of northern elephant seal females and their young. Behaviour 43:121–156.

Lesage, V., M. O. Hammill and K. M. Kovacs. 2002. Diet-tissue fractionation of stable carbon and nitrogen isotopes in phocid seals. Marine Mammal Science 18:182–193.

McDonald, B. I., and D. E. Crocker. 2006. Physiology and behavior influence lactation efficiency in northern elephant seals (*Mirounga angustirostris*). Physiological and Biochemical Zoology 79:484–496.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relationship between d^{15}N and animal age. Geochimica et Cosmochimica Acta 50:2143–2146.

Mrosovsky, N., and D. F. Sherry. 1980. Animal anorexias. Science of the Total Environment 207:837–842.

Nelson, D. E., A. Angerbjörn, K. Lidén and I. Turk. 1998. Stable isotopes and the metabolism of the European cave bear. Oecologia 116:177–181.

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

Pearson, S. F., D. J. Levey, C. H. Greenberg and C. M. Del Rio. 2003. Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. Oecologia 135:516–523.

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 79:499–511.

Rau, G. H., D. G. Ainley, J. L. Bengtson, J. J. Torres and T. L. Hopkins. 1992. $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in Weddell sea birds, seals and fish: Implications for diet and trophic structure. Marine Ecology Progress Series 84:1–8.

Riedman, M., and C. L. Ortiz. 1979. Changes in milk composition during lactation in the northern elephant seal. Physiological Zoology 52:240–249.

Robbins, C. T., L. A. Felicetti and M. Sponheimer. 2005. The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. Oecologia 144:534–540.

Sare, D. T. J., J. S. Millar and F. J. Longstaffe. 2005. Nitrogen- and carbon-isotope fractionation between mothers and offspring in red-backed voles (*Clethrionomys gapperi*). Canadian Journal of Zoology 83:712–716.

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 ^{13}C analysis of diet. Oecologia 57:32–37.

Voigt, C. C., and F. Matt. 2004. Nitrogen stress causes unpredictable enrichments of ^{15}N in two nectar-feeding bat species. Journal of Experimental Biology 207:1741–1748.

Wheater, P. R., B. Young and J. W. Heath. 2001. Histologie fonctionnelle. DeBoek Université, Brussels, Belgium.

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. 433 pp.

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.

Received: 11 May 2009
Accepted: 11 December 2009

APPENDIX

Table A1. Mean (± SD) $\delta^{15}N$ and $\delta^{13}C$ (‰) in blood cells, serum, and milk of northern elephant seal (*Mirounga angustirostris*) mothers and pups on days 5 and 22 of lactation.

		Blood cells			Serum			Milk		
		n	$\delta^{15}N$	$\delta^{13}C$	*n*	$\delta^{15}N$	$\delta^{13}C$	*n*	$\delta^{15}N$	$\delta^{13}C$
Mothers	Day 5	10	14.6 ± 0.5	−19.4 ± 0.4	9	16.6 ± 0.5	−20.3 ± 0.4	10	16.6 ± 0.5	−25.8 ± 0.5
	Day 22	10	14.6 ± 0.5	−19.5 ± 0.4	9	16.7 ± 0.7	−20.5 ± 0.3	10	16.2 ± 0.4	−26.0 ± 0.6
Pups	Day 5	9	15.2 ± 0.7	−19.3 ± 0.4	8	17.6 ± 0.4	−20.3 ± 0.3			
	Day 22	10	15.9 ± 0.5	−19.2 ± 0.5	8	17.1 ± 0.4	−20.2 ± 0.3			