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Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring

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Abstract Stable isotope signatures of lactating females and their nursing offspring were measured on 11 species, including herbivores, carnivores, hibernators, and non-hibernators. We hypothesized that: (1) nursing offspring would have stable isotope signatures that were a trophic level higher than their mothers, and (2) this pattern would be species-independent. The plasma of adult females had a $\delta^{15}N$ enrichment over their diets of 4.1±0.7‰, but offspring plasma had a mean $\delta^{15}N$ enrichment over maternal plasma of 0.9±0.8‰ and no C enrichment (0.0±0.6‰). The trophic level enrichment did not occur between mother and offspring because milk was depleted in both $\delta^{15}N$ (1.0±0.5‰) and $\delta^{13}C$ (2.1±0.9‰) relative to maternal plasma. Milk to offspring plasma enrichment was relatively small ($\delta^{15}N$ enrichment of 1.9±0.7‰ and $\delta^{13}C$ enrichment of 1.9±0.8‰) compared to the trophic level enrichment between the adults and their diets. While some species did have significant differences between the isotope signatures of mother and offspring, the differences were not related to whether they were hibernators or non-hibernators, carnivores or herbivores. Investigators wanting to use stable isotopes to quantify weaning or other lactation processes or diets of predators when both adults and nursing offspring are consumed must first establish the parameters that apply to a particular species/environment/diet combination.

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Introduction

Stable isotope methodology has been extraordinarily helpful in understanding animal migration, diet, food webs, and nutrient flow (Hobson and Montevecchi 1991; Ben-David et al. 1997; Wassenaar and Hobson 1998; Hilderbrand et al. 1999a, 1999b). Dietary estimates can be made from the analyses of an animal's blood, hair or other tissue because of the isotopic fractionation in which the heavier isotope increases with each additional trophic level. However, one error in most dietary studies of omnivores and carnivores has been the potential isotope fractionation between mother and offspring of nursing mammalian prey. Theoretically, the nursing offspring is consuming its mother and, therefore, would be a trophic level higher than its mother until weaning begins. During the weaning process, the offspring's isotope signature should approach that of its mother in proportion to the percent of its total nourishment coming from the adult-type diet.

Dietary estimates of predators that are derived solely from the stable isotope signature of the adult prey will overestimate that item's content if either offspring or a mixture of offspring and adult prey are consumed and if the offspring prey have a significantly higher isotope signature than the adult prey. This is potentially important when predators [e.g., grizzly bears (*Ursus arctos horribilis*) or wolves (*Canis lupus*)] feed seasonally on highly abundant offspring of grazing and browsing herbivores. However, if neonates and adults have different isotope signatures, that difference might also be used to quantify: (1) the proportion of newborn and adult prey in a predator's diet, and (2) the relative nutritional importance of milk when sequential blood samples can be taken.

In the only controlled study done of isotope fractionation between mothers and offspring, fingernails of human neonates did not differ in either $\delta^{13}C$ or $\delta^{15}N$ from those of their mothers for the portions grown

during gestation (Fogel et al. 1989). During lactation, the infants' fingernails developed a +2.4‰ $\delta^{15}N$ enrichment relative to their mother but retained C isotope signatures identical to their mothers. The $\delta^{15}N$ isotopic difference slowly declined with weaning.

Fossil bones (30,000–50,000 years old) of European cave bear (*Ursus spelaeus*) neonates/fetuses and cubs had isotope signatures that were 2.4–1.5 more depleted in $\delta^{13}C$ and 5.1–3.1 more enriched in $\delta^{15}N$ than adults (Nelson et al. 1998; Liden and Angerbjorn 1999). The differential between adult cave bears and their offspring largely disappeared by the second year, i.e., as yearlings. Nelson et al. (1998) hypothesized that the different patterns of isotopic enrichment between the cave bear and human were the result of the many metabolic adaptations associated with ursid hibernation. They predicted that hibernating, modern bears would also have the same isotopic disparity between mothers and offspring and that comparable measures on living bears would confirm their cave bear interpretations.

The following interspecific study was conducted to determine if isotopic fractionation occurs between mothers, neonates, and nursing offspring. We sampled a wide range of mammals, including herbivores, carnivores, hibernators, and non-hibernators.

Materials and methods

Paired blood and/or milk samples were collected from mothers and offspring of 11 species [moose (*Alces alces*), caribou (*Rangifer tarandus*), black-tailed deer (*Odocoileus hemionus*), coyotes (*Canis latrans*), grizzly bears (*Ursus arctos horribilis*), domestic rabbits (*Oryctolagus cuniculus*), rats (*Rattus norvegicus*), cows (*Bos taurus*), sheep (*Ovis aries*), pigs (*Sus scrofa*), and cats (*Felis catus*)]. The domestic animals and grizzly bears were sampled at Washington State University and the University of Idaho, moose and caribou at the Moose Research Center, Kenai, Alaska, and coyotes and black-tailed deer at the USDA/APHIS Field Stations in Logan, Utah and Olympia, Washington. All animals were held on a constant diet throughout the study that was characteristic of the particular species (e.g., domestic rats and rabbits on laboratory chow, ungulates on pelleted plant diets or long hay, bears on omnivore chow, and coyotes and cats on commercial dog and cat foods).

Samples were collected from three to five mother/neonate pairs to provide isotopic signatures representative of late gestation and lactation. Blood was collected in heparinized vacutainers or capillary tubes depending on animal size, centrifuged, and separated into plasma and red blood cells (RBCs). For eight of the 11 species, samples were collected only once at 12–14 days postpartum when all neonates were consuming only milk. Because neonates from the smaller species frequently start consuming solid food after ~2 weeks, an early sampling was the only time that all species could be compared at the same chronological age. The remaining three species (grizzly bears, moose, and caribou) were sampled more intensively to determine if later sampling in the larger species would have changed conclusions based on the 12- to 14-day sampling and because of our interest in using isotopes to understand the nutritional ecology of bears (Hilderbrand et al. 1996, 1998, 1999a, 1999b, 1999c; Jacoby et al. 1999). However, because neonates of the large ungulates begin consuming adult-type foods at ~30 days (Reese and Robbins 1994), early sampling during the lactation cycle is required if one wishes to measure the isotope responses associated only with milk consumption.

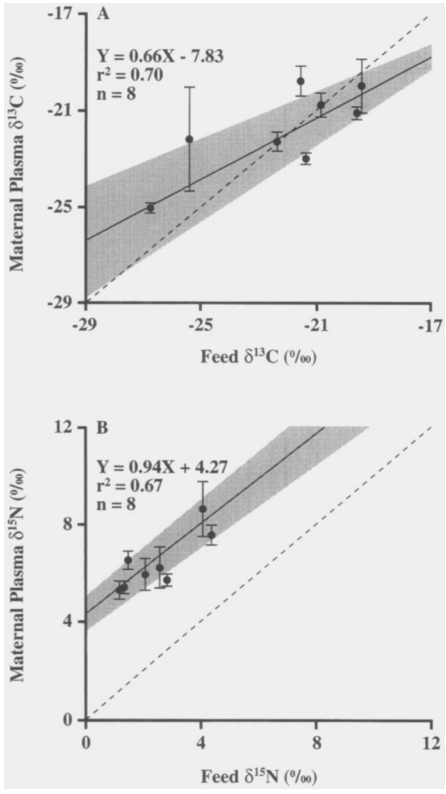

Fig. 1 The relationship between the C isotope signature (**a**) and N isotope signature (**b**) of adult female moose, caribou, grizzly bears, black-tailed deer, domestic rabbits, sheep, pigs, and rats and their diet. *Dashed line* is the 1:1 relationship. *Shaded area* is the 95% confidence interval

For moose and caribou, samples were collected from mothers and calves on days 14, 28, 42, 70, and 98. Grizzly bears began hibernation on 10 November when all feeding ceased and gave birth between mid January and early February. Mothers and cubs (twins in all cases) were sampled 14, 38 and 62 days postpartum. Hibernation ended mid to late March when food was given. One additional sample was taken approximately 92 days postpartum (30 days after daily feeding began) and just before the cubs began to eat an omnivore chow.

Because whole blood has constituents that have different turnover rates (Hobson and Clark 1993; Hilderbrand et al. 1996), the isotope signature of RBCs collected 12–14 days postpartum will reflect metabolic events that occurred in late gestation and early lactation. Plasma from the same blood sample will have an isotope signature indicative of the preceding 7–10 days and, thus, will represent only lactation.

Plasma, RBCs, milk, and feed samples were freeze-dried, ground into a fine powder, and loaded into tin boats (2.0 mg for plasma and RBCs and 4 mg for milk). Samples were analyzed for $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) on a Micromass Optima isotope ratio mass spectrometer (analytical precision: ±0.1‰ for C and ±0.2‰ for N) at the United States Geological Survey (USGS) Laboratory in Denver, Colorado. Results are reported relative to PeeDee limestone ($\delta^{13}C$) or atmospheric N ($\delta^{15}N$) as follows:

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

where δX is $\delta^{13}C$ or $\delta^{15}N$, and R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio (Peterson and Fry 1987).

To determine if enrichment or depletion of $\delta^{13}C$ or $\delta^{15}N$ were occurring between diet, mother, milk, and offspring across species, linear regressions were developed between each of two variables. Slopes were tested to determine if they differed from 1 and intercepts

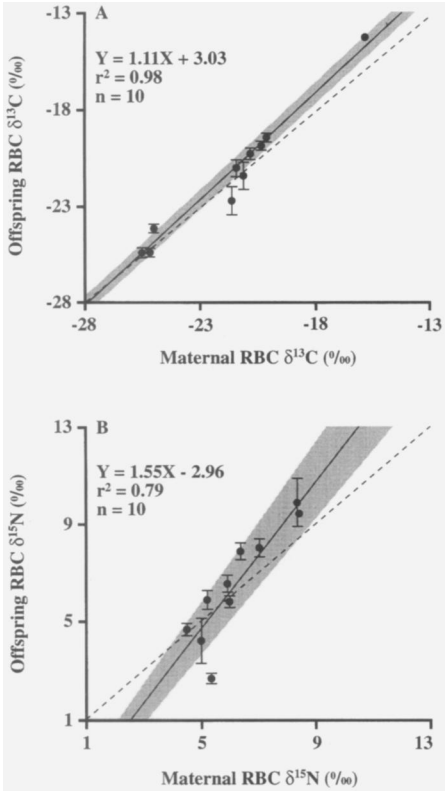

Fig. 2 The C isotope signature (**a**) and N isotope signature (**b**) of red blood cells (*RBCs*) from adult female moose, caribou, grizzly bears, black-tailed deer, domestic rabbits, sheep, cows, pigs, rats, and cats and their offspring at 12–14 days after birth

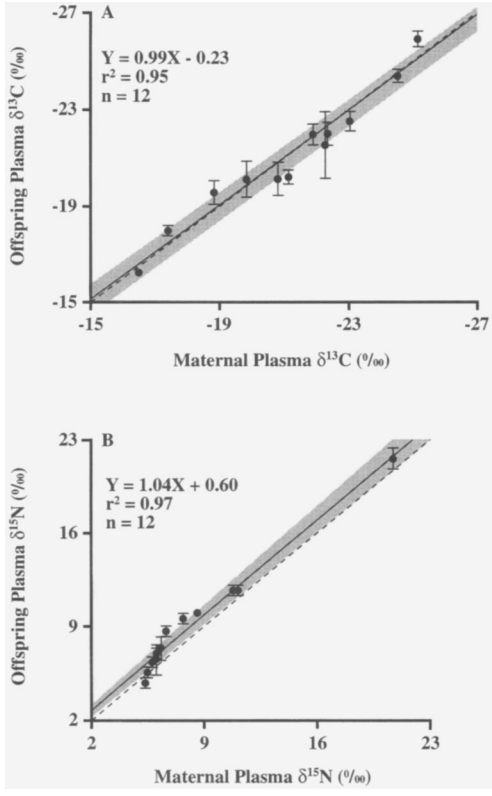

Fig. 3 The C isotope signature (**a**) and N isotope signature (**b**) of plasma from adult female moose, caribou, grizzly bears, black-tailed deer, coyotes, domestic rabbits, sheep, cows, pigs, rats, and cats and their offspring at 12–14 days after birth (current study). Isotope signatures of plasma from free-ranging, adult, female polar bears and their cubs (Polischuk et al., 2001) were also included. All offspring had consumed only milk

were compared to 0 (ANCOVA; SAS PROC GLM). Confidence intervals (95%) were calculated for each regression. Values for each species were then compared to the regression confidence interval using a two-tailed *t*-test to identify those species that deviated significantly from the interspecific regression. Because the same grizzly bears, moose and caribou were repeatedly sampled in the time-sequence study, the potential for serial correlation was tested using SAS PROC AUTOREG. Serial correlation did not occur. Therefore, the standard ordinary least squares analyses of covariance was used to test for differences (SAS PROC GLM). Significance was set at $P \leq 0.01$ because of the need for distinct signatures between mother and offspring to be useful in most ecological applications. Means are reported ±1 SD.

Results

The plasma of adult females had a $\delta^{15}N$ enrichment over their diets of 4.1±0.7‰ (Fig. 1; slope relative to 1, $P=0.84$; intercept relative to 0, $P<0.01$). Female plasma was not enriched in $\delta^{13}C$ relative to the diet (slope and intercept, $P=0.08$).

Maternal and neonatal RBCs collected 12–14 days after birth had C and N signatures that were not different from the 1:1 relationship (Fig. 2, $\delta^{15}N$, slope $P=0.09$, intercept $P=0.13$; $\delta^{13}C$, slope $P=0.06$, intercept $P=0.03$). C and N signatures of maternal and neonatal plasma at 12–14 days after birth were also indistinguishable from the 1:1 relationship (Fig. 3, $\delta^{15}N$, slope $P=0.50$, intercept $P=0.26$; $\delta^{13}C$, slope $P=0.88$, intercept $P=0.88$) because of the balancing between the depletion of milk relative to maternal plasma in both $\delta^{13}C$ or $\delta^{15}N$ (Fig. 4) and the trophic enrichment between milk and offspring plasma (Fig. 5). Milk had a mean $\delta^{15}N$ depletion relative to the lactating female's plasma of 1.0±0.5‰ and a mean $\delta^{13}C$ depletion of 2.1±0.9‰. Offspring plasma had a mean $\delta^{15}N$ enrichment over the milk of 1.9±0.7‰ and a mean $\delta^{13}C$ enrichment of 1.9±0.8‰. Finally, maternal plasma to offspring plasma had a mean $\delta^{15}N$ enrichment of 0.9±0.8‰ and no C enrichment (0.0±0.6‰). In eight of the ten interspecific comparisons, hibernating grizzly bears did not differ from the interspecific regressions (Figs. 1, 2, 3, 4, 5, $P=0.17$–0.84).

The $\delta^{13}C$ signatures of paired mother and offspring samples from caribou, moose, and grizzly bears did not differ during the first 3 months of lactation (Fig. 6, $P=0.12$–0.89). $\delta^{15}N$ signatures in mother and offspring caribou differed by 1.9±0.1‰ during the first 70 days ($P<0.01$), but the difference decreased to 0.6±0.1‰ at 98 days after birth. The N isotope signatures of moose calves and their mothers never differed ($P>0.01$). N isotope signatures of grizzly bear cubs were enriched relative to their mothers by 1.6±0.3‰ for plasma and

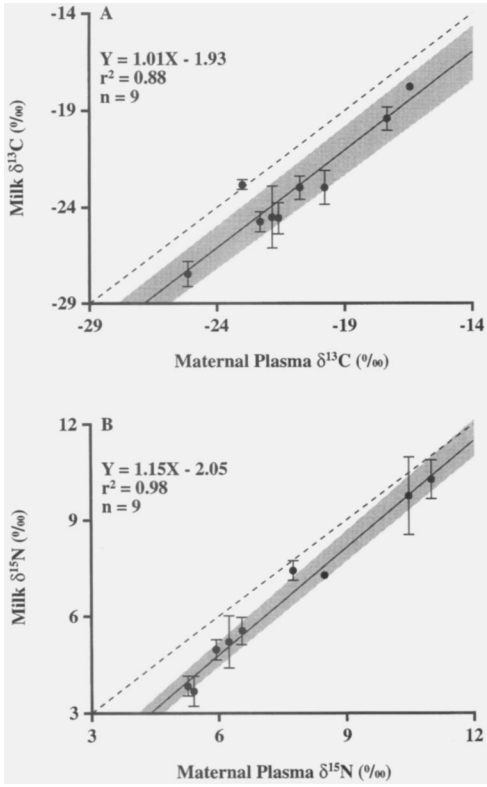

Fig. 4 The C isotope signature (**a**) and N isotope signature (**b**) of plasma and milk produced by lactating moose, caribou, grizzly bears, black-tailed deer, coyotes, domestic rabbits, sheep, pigs, and cats

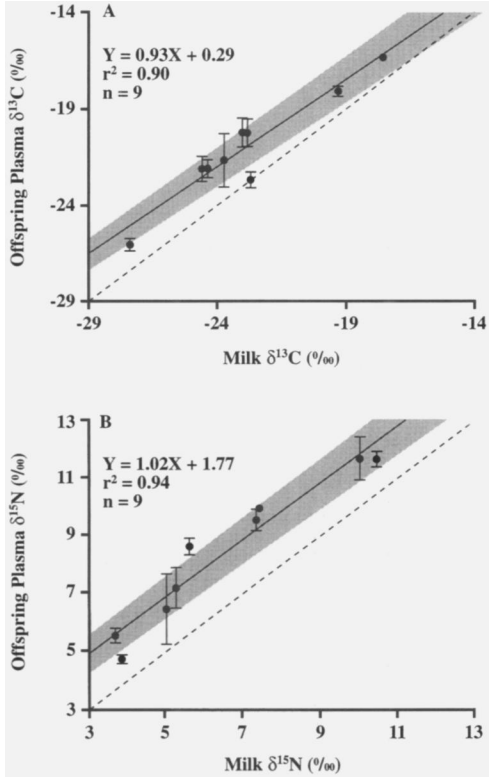

Fig. 5 The C isotope signature (**a**) and N isotope signature (**b**) of milk and the plasma of 12- to 14-day-old nursing moose, caribou, grizzly bears, black-tailed deer, coyotes, domestic rabbits, sheep, pigs, and cats

1.7±0.5 for RBCs on day 14 ($P<0.01$). That difference decreased throughout hibernation for the plasma (0.9±0.2‰ on day 38 and 0.7‰ on day 62, $P<0.01$) before slightly increasing on day 92 (1.2±0.6‰, $P<0.01$). The overall mean $\delta^{15}N$ enrichment between grizzly bear mothers and their offspring during hibernation was 1.2±0.5‰.

Discussion

Hibernating bears and their nursing offspring do not have unique isotope enrichments relative to other mammals. The mean $\delta^{15}N$ difference between the plasma of grizzly bear mothers and their cubs when milk is the only nourishment for the cub (1.2±0.5‰) is similar to that in polar bears (1.0‰) (Polischuk et al., 2001) and the overall interspecific average (0.9±0.8‰), but well below that suggested for cave bears (3.1–5.1‰) (Nelson et al. 1998). Similarly, the above $\delta^{15}N$ enrichments between mother and offspring plasma (~1‰) or milk and offspring plasma (1.9±0.7‰) are small compared to the mean, interspecific $\delta^{15}N$ enrichment between diet and maternal plasma in the current study (4.1±0.7‰). The $\delta^{13}C$ depletion of milk relative to maternal plasma that leads to no difference between maternal and offspring plasma likely occurs because: (1) the main energy source in the milk of many species is fat, and (2) fats are depleted in $\delta^{13}C$ relative to other tissues (Hilderbrand et al. 1996). Thus, we are unable to support the observation regarding cave bears of significant trophic enrichment based on the direct comparison of maternal plasma, milk, and offspring plasma.

One could hypothesize that the $\delta^{15}N$ isotope fractionation between diet and bone (Nelson et al. 1998) and diet and plasma (current study) differ with animal age in such a way that both the cave bear and current results could be true. While most studies found no differential age effect on diet to tissue fractionation for either $\delta^{13}C$ or $\delta^{15}N$ (Minagawa and Wada 1984; Sutoh et al. 1987; Hobson and Clark 1992; Roth and Hobson 2000), Roth and Hobson (2000) found a very small differential effect on $\delta^{15}N$ fractionation (0.1–0.3‰) between diet and different tissues in subadult and adult red foxes (*Vulpes vulpes*). Bone was not sampled in their study. However, even the above, relatively uncommon, age-dependent differences were <10% of the magnitude that would have to occur for both the cave bear and current results to be correct.

Another commonly cited study that is purported to support the trophic level enrichment hypothesis between mother and nursing offspring analyzed tooth annuli of Stellar sea lions (*Eumetopias jubatus*) (Hobson and Sease 1998). Their results are quite equivocal with 11 of

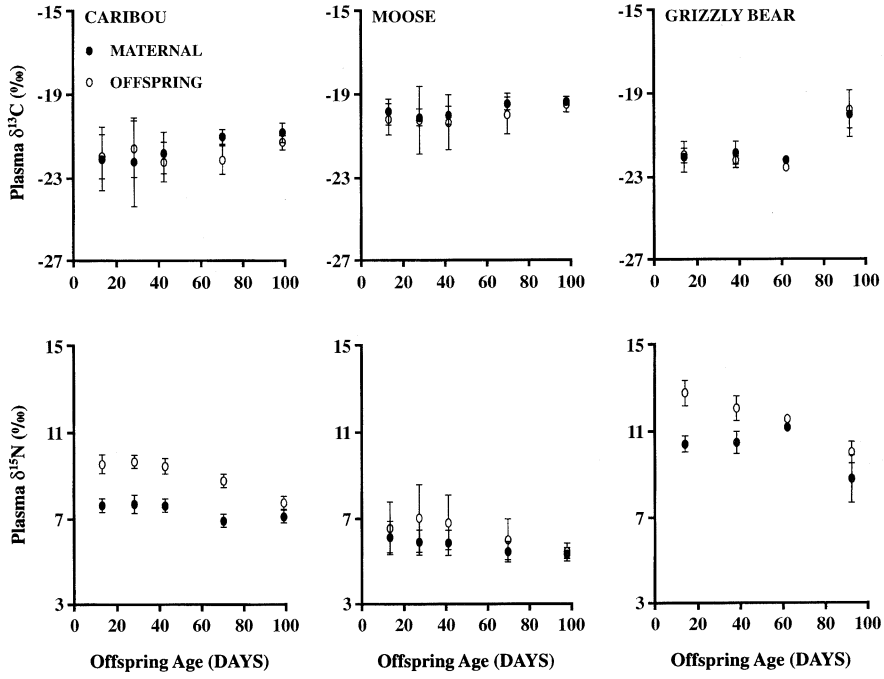

Fig. 6 Isotope signatures of plasma from caribou, moose, and grizzly bear mothers and their nursing offspring for the first 3 months of lactation

18 samples having a decreasing $\delta^{15}N$ pattern over time that would be supportive of an enrichment due to milk consumption, although not necessarily a complete trophic level effect. However, four sea lions had an increasing pattern that was the opposite of what should have occurred, and three were either constant or showed no consistent age effect.

Hobson et al. (2000) recently reported a mean $\delta^{15}N$ enrichment between the hair of lactating, hibernating black bears (*U. americanus*) and their cubs of 2.5±1.2‰, but a very minimal C enrichment (0.7±1.1‰) that is opposite of the depletion reported for the cave bear. While the above $\delta^{15}N$ enrichment in black bears approaches that reported for cave bears, Hobson et al. (2000) pointed out perhaps the major weakness (decoupling of the parent-offspring isotope signatures) in using whole bones or hair in these studies. Although Fogel et al. (1989) were careful to temporally match the nails of mothers and offspring, the isotope signatures of whole bone or hair from adult female bears represents the diet during the periods of their growth which may not correspond either temporally or biochemically to the nutrients stored and ultimately transferred to the offspring in the milk several months later during hibernation (Hobson et al. 2000). Samples from archaeological sites will be even more problematic in that mother/offspring remains are unlikely to be paired and the timing of death is largely unknown and may even be from different years.

In summary, interspecific comparisons indicated no general pattern of a trophic level enrichment between the signatures of mothers and their offspring during gestation and lactation. Even two closely related species had different $\delta^{15}N$ relationships during lactation (caribou mothers and offspring with significantly different signatures and moose with no difference), while two markedly different species had similar patterns (both caribou and grizzly bears with significant differences between mothers and offspring). The species-specific differences may be useful in some ecological studies, but the error in previously published dietary estimates of omnivores and carnivores that used only the adult $\delta^{15}N$ signature for animals consuming all age classes of prey is relatively small in most cases as: (1) milk-dependent offspring are available for a relatively short period of time, and (2) the isotope difference between mother and offspring is generally not a trophic level increase. However, future investigators should not assume that nursing offspring and adults have either the same signature or a trophic level enrichment and should first establish the parameters that apply to a particular species/environment/diet combination.

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