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Patterns of $\delta^{13}C$ and $\delta^{15}N$ in wolverine *Gulo gulo* tissues from the Brooks Range, Alaska

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Abstract Knowledge of carnivore diets is essential to understand how carnivore populations respond demographically to variations in prey abundance. Analysis of stable isotopes is a useful complement to traditional methods of analyzing carnivore diets. We used data on $\delta^{13}C$ and $\delta^{15}N$ in wolverine tissues to investigate patterns of seasonal and annual diet variation in a wolverine *Gulo gulo* population in the western Brooks Range, Alaska, USA. The stable isotope ratios in wolverine tissues generally reflected that of terrestrial carnivores, corroborating previous diet studies on wolverines. We also found variation in $\delta^{13}C$ and $\delta^{15}N$ both between muscle samples collected over several years and between tissues with different assimilation rates, even after correcting for isotopic fractionation. This suggests both annual and seasonal diet variation. Our results indicate that data on $\delta^{13}C$ and $\delta^{15}N$ holds promise for qualitative assessments of wolverine diet changes over time. Such temporal variation may be important indicators of ecological responses to environmental perturbations, and we suggest that more refined studies of stable isotopes may be an important tool when studying temporal change in diets of wolverines and similar carnivores [*Current Zoology* 55 (3): 188 – 192, 2009].

Key words Stable isotopes, Nitrogen, Carbon, Arctic, Carnivore, Caribou

Knowledge of carnivore diets is essential to understand how carnivore populations respond demographically to variations in prey abundance. While traditional methods of analyzing carnivore diets, i. e. analyses of content in stomachs and feces, may be prone to shortcomings associated with non-random samples with inherent pseudoreplication (Reynolds and Aebisher, 1991; Deb, 1997; Darimont and Reimchen, 2002), analyses of stable isotopes have shown to be a powerful complement (Hobson, 1999; Kelly, 2000). Moreover, in boreal and arctic areas it is often difficult to obtain observation of predation events (either direct or through snow-tracking), carcasses for stomach contents or fecal droppings year round for large carnivores, so that that temporal diet change often becomes difficult to measure with these techniques.

Dalerum and Angerbjörn (2005) suggested several different approaches to use stable isotopes to resolve both long-term and seasonal variation in diets. Similar to traditional dietary analyses using fecal or stomach content, stable isotope measures compared across samples collected over time might reveal either short or long-term dietary variation, depending on intervals between samples. An interesting alternative is also to analyze combinations of tissues with different metabolic rates. Since each tissue reflects the average dietary isotope signature for the specific time period under which that tissue has been assimilating, one can track dietary records accumulated over different time periods and hence qualitatively assess seasonal changes in diets (Tiezen et al., 1983; Hobson and Clark, 1992). For instance, muscle, a tissue with a relatively high metabolic rate and consequently high molecular turnover, will reflect the diet only for the season in which it is collected, while collagen, with its low metabolic rate and slow molecular turnover, will reflect the diet over several years, including several seasons (Dalerum and Angerbjörn, 2005).

The wolverine *Gulo gulo* is a terrestrial mustelid with a circumpolar distribution, which primarily inhabits tundra and taiga of northern latitudes (Wilson, 1982). Wolverines primarily rely on ungulates, particularly as food during winter (Haglund, 1965; Rausch and Pearson, 1972; Gardner, 1985; Magoun, 1987; Persson, 2003; Dalerum et al., 2009). The diet of wolverines during summer is less well understood, but there are indications that other prey such as microtine rodents may be important (Landa et al., 1997). In the western Brooks Range, north-western Alaska, wolverines rely heavily on migratory

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caribou *Rangifer tarandus* as food during winter (Magoun, 1987; Dalerum et al., 2009). However, previous studies in this area have failed to fully resolve temporal variation in wolverine diets, particularly seasonal diet variation. To improve our ecological understanding of this wolverine population, we here used variation in $\delta^{13}C$ and $\delta^{15}N$ within and between wolverine tissues to investigate patterns of temporal diet variation as a complement to previous diet studies based on stomach and fecal content (Magoun, 1987; Dalerum et al., 2009).

1 Materials and methods

1.1 Study area

We collected wolverine tissues from the Noatak and Kobuk River drainages in the western Brooks Range, Alaska (68°35 N – 65°15 N; 162°55 W – 159°15 W). The area lies within the migratory range of the Western Arctic Caribou Heard (WACH), which passes through the study area during the spring and fall migrations each year (Dau, 2003). However, caribou groups may remain in the study area even between migration periods. Moose *Alces alces*, Dall's sheep *Ovis dalli* and occasionally muskoxen *Ovibos moschatus* are other ungulates occurring in the area. The area also host a range of smaller mammals, such as beaver *Castor canadensis*, porcupine *Erethizon dorsatum*, snowshoe hare *Lepus americanus*, arctic ground squirrels *Spermophilus parryii* and microtine rodents, as well as potential avian prey species including ptarmigan *Lagopus* spp. and several species of migratory geese and waterfowl. Sheefish *Stendous leucichthys nelma*, dolly varden *Salvelinus malma* and chum salmon *Oncorynchus keta* spawn in the river systems.

1.2 Collection of tissue samples

Between 1996 and 2002, we purchased skinned out wolverine carcasses from local hunters as part of a study on the ecology of wolverines in the area (Dalerum et al., 2005, 2007b, 2009). We also obtained a limited number of samples of caribou, moose and ptarmigan as reference material. We recorded approximate date and location of harvest for each carcass. Most wolverines were from the lower Kobuk and Noatak rivers and were harvested during February and March, although we collected animals harvested from November through to April. From each wolverine, we collected a muscle sample from the quadriceps muscle, and from wolverines harvested the winters 2000/2001 and 2001/2002 we also collected a femur, and from wolverines harvested the winter 2000/2001 we additionally collected a liver sample.

1.3 Sample preparation and isotope analyses

We dried and pulverized muscle and liver samples and removed lipids according to Bligh and Dyer (1959). We dried the samples after lipid extraction before final analysis of stable isotope ratios. We obtained bone powder from femur bones using a hand-held electric drill and extracted collagen with the modified Longing method (Brown et al., 1988). We removed lipids using the same protocol as for the muscle samples and lyophilized the samples before final analysis. We conducted analysis of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios on a Carlo Erba elemental analyzer (E1108 CHNS-O) connected to a Fison Optima isotope ratio mass spectrometer, with an accuracy of $\leqslant$ 0.1‰. Isotope values are presented as δX values, which represent the proportional deviation in parts per thousand (‰) from a standard:

$$\delta X = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 100$$

where X is either ^{13}C or ^{15}N, and R is either $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. The accepted standard for carbon is Pee Dee Belemnite (PDB) and the standard for nitrogen is atmospheric nitrogen.

We analyzed $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in liver from 24 wolverines harvested during the winter 2000/2001, in muscle from 71 wolverines harvested from the winters 1995/1996 to 2001/2002, and in femur collagen from 37 wolverines harvested during the winters 2000/2001 and 2001/2002. We also analyzed $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in muscle from 2 moose, 10 caribou and 4 ptarmigan (Appendix 1).

1.4 Estimation of isotopic fractionation

The stable isotope ratios in an animals' tissue are a function of the source of the element in question and the fractionation of heavy versus light isotopes in the metabolic pathways that lead to each respective tissue (Peterson and Fry, 1987; Ponsard and Averbuch, 1999). Therefore, we estimated the isotope signatures of the diet by correcting each tissue using tissue specific fractionation coefficients. For liver and muscle, we used fractionation coefficients derived from captive red foxes (Roth and Hobson, 2000; liver: 0.4 for $\delta^{13}C$ and 3.6 for $\delta^{15}N$, muscle: 1.2 for $\delta^{13}C$ and 3.7 for $\delta^{15}N$). There are no published diet to tissue fractionation values from controlled field experiments on carnivores for either $\delta^{13}C$ or $\delta^{15}N$ in collagen. Therefore, we used fractionation values derived from controlled feeding experiments on domestic pigs as a collagen fractionation coefficient for ^{13}C (Howland et al., 2003: 2.9), and collagen to collagen enrichment between wolves and herbivores as a crude estimator of collagen fractionation coefficient for $\delta^{15}N$ (Fox-Dobbs et al., 2007: 4.6). Although not species specific, with inherent uncertainties due to different metabolism between species, these are the fractionation values published that are most likely to mimic wolverines diet to tissue fractionation.

1.5 Data analysis

We used one-way ANOVA's with $\delta^{13}C$ and $\delta^{15}N$ as response variables and year as an independent factor to test for annual variation of $\delta^{13}C$ and $\delta^{15}N$ in wolverine muscle and nested ANOVA's to test for differences in $\delta^{13}C$ and $\delta^{15}N$ between wolverine tissues. In the analyses, we included year of harvest and used estimated diet isotope values, i.

e. values corrected for isotopic fractionation. We ran one analysis for each element.

Average values are given with standard errors. Statistical significance was set at $P = 0.05$, and all tests were two-tailed. Analyses were conducted with the statistical software R release 1.9.1 for Linux (http://www.r-project.org).

2 Results

The stable isotope ratios in wolverine tissues generally reflected that of terrestrial carnivores. Average $\delta^{13}C$ in wolverine liver was -22.68 (± 0.10) and average $\delta^{15}N$ was 6.56 (± 0.11). Average $\delta^{13}C$ in wolverine muscle was -22.68‰ ($\pm$ 0.07), with yearly averages ranging from -22.21‰ to -22.95‰. Average $\delta^{15}N$ in muscle was 6.50‰ ($\pm$ 0.10), with yearly averages ranging from 6.13‰ to 7.26‰ (Fig.1). In collagen, average $\delta^{13}C$ was -20.49 ($\pm$ 0.10, annual averages -20.46 and -20.47) and average $\delta^{15}N$ was 6.74 ($\pm$ 0.11, annual averages 6.57 and 7.19).

There was a significant annual variation in both $\delta^{13}C$ ($F_{1,69} = 14.58$, $P < 0.001$) and $\delta^{15}N$ ($F_{1,69} = 5.07$, $P = 0.028$) in wolverine muscle (Fig.1). There was further within individual variation in both $\delta^{13}C$ and $\delta^{15}N$ between the three tissues after correcting for isotopic fractionation ($\delta^{13}C$: $F_{2,61} = 33.64$, $P < 0.001$; $\delta^{15}N$: $F_{2,61} = 13.84$, $P < 0.001$), although the differences between tissues were quite small (Fig.2). Both $\delta^{13}C$ and $\delta^{15}N$ seemed to reflect a diet of terrestrial herbivores (Fig.2B). The isotope values directly contradicted that any marine protein, such as anadromous salmon, had been ingested (Fig.2B).

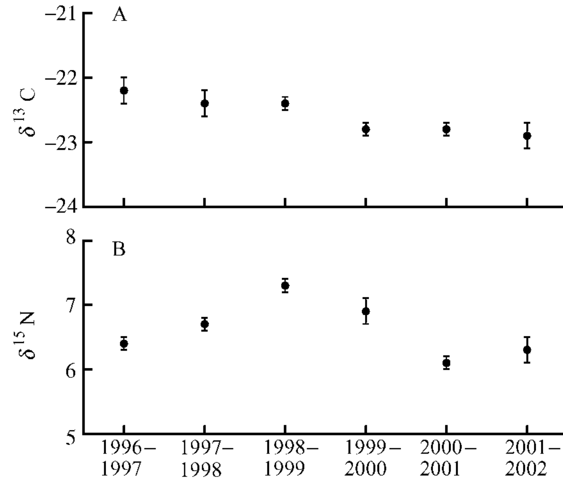

Fig.1 Annual variation in $\delta^{13}C$ (A) and $\delta^{15}N$ (B) in wolverine muscle samples from 1996 through 2002

Figure presents Means ± 1 *SE*.

3 Discussion

These are the first stable isotope data presented for wild wolverines. Wolverine stable isotope signatures generally portrayed a diet relying on terrestrial food sources. However, we have refrained from using our stable isotope data to quantify dietary composition using mixed source models (e. g., Phillips, 2001), since they rely on a number of assumptions regarding animal physiology that have not yet been empirically tested (Dalerum and Angerbjörn, 2005). For instance, although it is now possible to include linear effects of elemental concentration on isotope fractionation (Phillips and Koch, 2002), some data indicate non-linearity in such relationships (e. g.

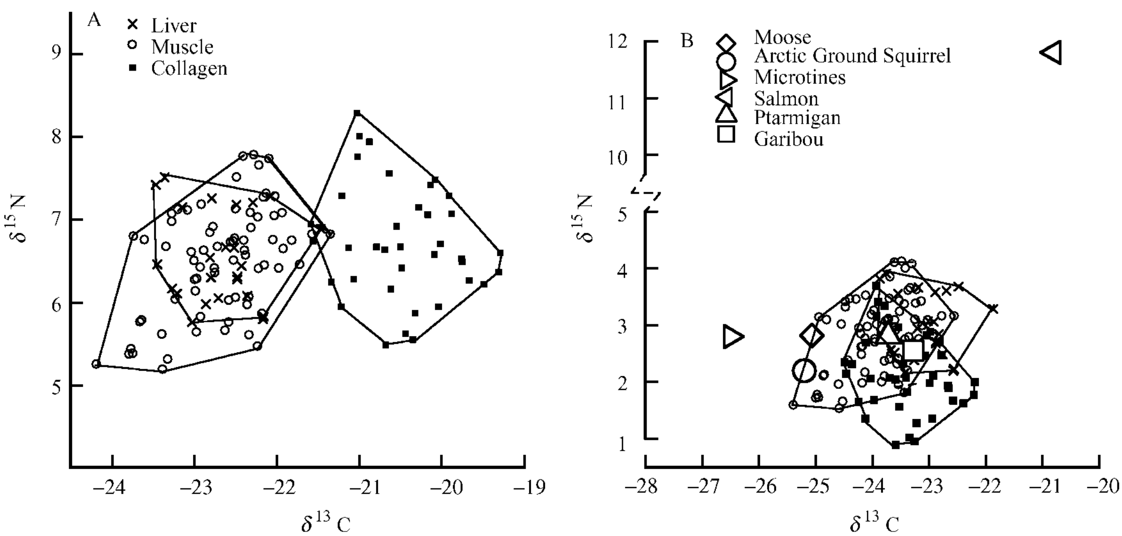

Fig.2 $\delta^{13}C$ and $\delta^{15}N$ in wolverine liver, muscle and collagen samples from 2000/2001 and 2001/2002 (A), and corresponding values corrected for isotopic fractionation as well as values of potential prey species (B)

Caribou, moose and ptarmigan values reflect muscle samples from animals harvested within the study area (Appendix 1), arctic ground squirrels reflect blood values from Kluane national park, Canada (Ben-David et al., 1999), microtines blood values from SE Alaska (Ben-David et al., 1997) and salmon reflect muscle values for chum salmon from Barrow, Alaska (Hoekstra et al., 2002).

Howland et al., 2003). Moreover, experimental data on invertebrates suggest that isotope ratios alone can have gross effects on isotope fractionation (Overmeyer et al., 2008). If such effects are common, it would be virtually impossible to reliably use mixed source models to reconstruct diets from stable isotope data. However, despite our basic approach to analyze stable isotpe data, our results showed important indications of temporal diet variation, both on annual and seasonal scales.

The observed annual variation in muscle isotope signatures could be caused by three, not mutually exclusive, levels of variation: (i) a dietary variation between years, (ii) no dietary variation but a variation in the isotope signatures of the prey among years, or (iii) variation in source assimilation among years, for instance through state dependent factors among the wolverines such as nutritional status. Although we do not have the ability to distinguish between these possibilities, an annual dietary variation would corroborate a concurrent study based on stomach content from the same area (Dalerum et al., 2009), which similarly found differences in diet compositions between years. While Dalerum et al. (2009) did not find a strong correlation between wolverine diet variation and annual variation in caribou abundance, they did find indications that variation in the dietary importance of caribou was linked to caribou mortality, and that wolverines may have switched from caribou to moose during periods of low availability of caribou, thus retaining its role as a largely ungulate dependent carnivore and scavenger.

We also found isotope variation among tissues with different assimilation rates, both before and after we corrected them for isotopic fractionation. To find differences among tissues prior to applying fractionation coefficients is not surprising, since several studies have highlighted that tissue specific metabolism causes varying degrees of isotope discrimination (e. g. Tiezen et al., 1983). However, differences among tissues remained even after we had corrected the values for different fractionation levels. Although these differences might have been confounded by several factors, including our estimates of diet-tissue fractionation and possible remnant lactation effects in collagen in yearling animals (e. g. Dalerum et al., 2007a), the observed variation provide a valuable indication of seasonal diet variation among wolverines. Although a seasonal diet variation is not an exclusive interpretation of these data, it is corroborated by previous studies that have indicated similar seasonal variation in wolverine diets (Magoun, 1987; Landa et al., 1997; Lofroth et al., 2007), and we suggest that further efforts should be made to elucidate seasonal diet variation in this species.

To conclude, we have shown stable isotope patterns that indicate both annual and seasonal dietary variation in wolverines from the Brooks Range. Such variations may be important indicator of ecological responses to environmental perturbations, and we suggest that seasonal diet variation is further studied in this species. Our data on $\delta^{13}C$ and $\delta^{15}N$ hold promise as a tool for quantifying temporal dietary change in wolverines, and we suggest that more refined studies of stable isotopes, for instance through compound specific analyses of stable isotopes, could be an important tool when doing so.

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Appendix 1 Values of $\delta^{13}C$ and $\delta^{15}N$ (Mean ± *SE*) for liver, muscle and femur collagen and muscle from wolverines harvested during the winters 1996/1997 through 2001/2002, as well as values for potential prey species

Winter	Species	Tissue	*n*	$\delta^{13}C$	$\delta^{15}N$
2000/2001	Wolverine	Liver	24	– 22.7 ± 0.1	6.6 ± 0.1
1996/1997	Wolverine	Muscle	9	– 22.2 ± 0.2	6.4 ± 0.1
1997/1998	Wolverine	Muscle	6	– 22.4 ± 0.2	6.7 ± 0.1
1998/1999	Wolverine	Muscle	10	– 22.4 ± 0.1	7.3 ± 0.1
1999/2000	Wolverine	Muscle	9	– 22.8 ± 0.1	6.9 ± 0.2
2000/2001	Wolverine	Muscle	24	– 22.8 ± 0.1	6.1 ± 0.1
2001/2002	Wolverine	Muscle	13	– 23.0 ± 0.2	6.34 ± 0.2
2000/2001	Wolverine	Collagen	24	– 20.5 ± 0.1	6.6 ± 0.1
2001/2002	Wolverine	Collagen	13	– 20.5 ± 0.1	7.2 ± 0.2
2002/2003	Caribou	Muscle	11	– 23.3 ± 0.2	2.6 ± 0.2
2002/2003	Moose	Muscle	2	– 25.1 ± 0.4	2.8 ± 0.0
2002/2003	Ptarmigan	Muscle	4	– 23.7 ± 0.2	2.8 ± 0.3