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Intra-hair stable isotope analysis implies seasonal shift to salmon in gray wolf diet

C.T. Darimont and T.E. Reimchen

Abstract: Seasonal shifts in diet are widespread, but our ability to detect them can be limited. Comparisons of stable isotope signatures in metabolically inert tissue portions grown at different times are inadequately exploited in dietary reconstructions. We propose that segments of guard hair can index diet to periods of growth (i.e., seasons differing in resource availability). We examined inter-hair $\delta^{13}C$ and $\delta^{15}N$ signatures from gray wolves (*Canis lupus*) of British Columbia to test whether the bulk of enriched (marine-derived) nutrients was assimilated during fall, the peak of salmon (*Onchorynchus* spp.) migration. In five animals, we detected a seasonal dietary shift: relatively more ^{13}C and ^{15}N was assimilated during fall than during summer, suggesting use of salmon during fall. Twelve wolves and both controls showed no seasonal shift in diet. Using salmon when available may be adaptive, given its predictability, spatial constraint, caloric content, and lower potential to inflict injury relative to that imposed by large mammals. Our study complements others that also used novel and fine-scale isotope approaches and may permit the identification of otherwise undetectable niche differentiation among conspecifics or heterospecifics.

Résumé : Les changements saisonniers dans le régime alimentaire sont chose commune, mais notre capacité à les détecter est parfois limitée. La comparaison des signatures des isotopes stables dans des portions de tissus métaboliquement inertes dont la croissance se produit à différents moments est sous-utilisée dans la reconstruction des régimes alimentaires. Nous croyons que des segments des poils de garde peuvent servir à retracer le régime alimentaire durant les périodes de croissance (i.e. les saisons qui offrent des ressources différentes). Nous avons examiné les signatures de $\delta^{13}C$ et de $\delta^{15}N$ dans les poils de garde chez des loups (*Canis lupus*) de Colombie-Britannique pour déterminer si la majorité des nutriments enrichis (d'origine marine) sont assimilés à l'automne, période où la migration des saumons (*Onchorynchus* spp.) bat son plein. Chez cinq loups, nous avons noté des modifications saisonnières du régime alimentaire; des quantités relativement plus élevées de ^{13}C et de ^{15}N étaient assimilées à l'automne, ce qui semble indiquer une consommation accrue de saumons à cette saison. Aucun changement saisonnier dans le régime alimentaire n'a été enregistré chez 12 autres loups, ni chez les 2 loups témoins. L'utilisation de saumons, lorsqu'ils sont présents, peut être une habitude adaptative, étant donné leur prévisibilité, leur restriction spatiale, leur contenu calorifique et la fréquence beaucoup moins grande des blessures reliée à cette chasse par comparaison à la chasse aux gros mammifères. Les résultats de notre étude viennent s'ajouter à ceux d'autres recherches basées sur l'utilisation de techniques originales et précises d'examen des signatures d'isotopes stables; ces techniques pourront éventuellement servir à repérer des différences de niches chez des individus de la même espèce ou d'espèces distinctes, différences qui ne sont pas décelables autrement.

[Traduit par la Rédaction]

Introduction

Dietary shifts among seasons are widespread, but our ability to detect them can be limited. Continued direct observation of feeding may not be feasible if subject animals are elusive or expensive to monitor. Examination of stomachs may be inappropriate if the species is rare or of conservation concern. Scat analyses can represent small, nonrandom samples of single meals by unknown individuals, and prey items often differ in digestibility.

The ratios of carbon and nitrogen stable isotopes in animal tissue are related to diet and provide an alternative method to estimate food use. Whole-body $\delta^{13}C$ signatures are enriched relative to diet only negligibly (DeNiro and Epstein 1978) and have been used to indicate relative contribution of isotopically distinct carbon sources such as relatively enriched marine versus relatively depleted terrestrial food-

Received 5 March 2002. Accepted 14 August 2002. Published on the NRC Research Press Web site at http://cjz.nrc.ca on 17 October 2002.

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DOI: 10.1139/Z02-149

webs (e.g., Chisholm et al. 1982). Marine foods also are elevated in ^{15}N, but an enrichment of 3–5‰ at each trophic step (DeNiro and Epstein 1981; Minagawa and Wada 1984) obscures differentiation between isotopically distinct food sources. The discrete behaviour of both isotopes allows for a cross-referenced analysis of diet.

Stable isotope analysis of metabolically active tissues can access dietary "windows" that span weeks (e.g., blood or muscle; Hilderbrand et al. 1996) to lifetimes (e.g., bone collagen; Chisholm et al. 1982), depending on metabolic turnover rate of tissue. Unless animals are resampled or different animals from different periods are sampled, no information can be provided regarding temporal changes in diet.

Metabolically inert tissue, however, reflects diet only during growth and retains this information in a chronological manner. Hobson et al. (1996) found negligible variation in δ^{13}C and δ^{15}N values among 5-mm whisker segments from captive harp seals (*Pagophilus groenlandicus*) kept on a constant diet. They suggested that any departure from baseline variation along the whisker shafts of wild marine mammals could be interpreted as evidence for dietary change.

The annual migration of spawning salmon (*Onchorynchus* spp.) presents to consumers a predictable and nutritious seasonal resource in the Pacific Northwest (Willson and Halupka 1995), yet how this pulse of nutrients affects consumer and community ecology remains poorly understood. The diet of gray wolves (*Canis lupus*) in this area, determined from identification of hair and bone in scats, is thought to consist predominantly of ungulates (Scott and Shackleton 1980; Milne et al. 1989; Kohira and Rextad 1997). Salmon are potentially important, but evidence for their consumption has been limited (only detected in Kohira and Rextad 1997), possibly owing to full digestion of salmon bones in scats.

In contrast, stable isotope analysis of bone collagen suggested that marine resources, presumably salmon, contributed considerable lifetime protein to coastal and interior Alaskan wolves (Szepanski et al. 1999). However, the analytical model used could not discriminate between consumption of seasonally present salmon and other isotopically enriched marine foods available to wolves throughout the year. In this paper, we examined intra-hair δ^{13}C and δ^{15}N signatures from wolves of adjacent British Columbia (B.C.). When combined with knowledge of moult pattern, we propose that segments of guard hair can index diet to periods of growth (i.e., seasons differing in resource availability). We tested whether wolves from B.C. used marine resources, and if so, whether the bulk of enriched (marine-derived) nutrients was assimilated during fall, when migrating salmon are most abundant.

Materials and methods

We sampled hair from the mid-ventral region of 15 museum and taxidermist pelts and one field-collected sample of shed hair (bedding site) from a broad geographic range within B.C. (Fig. 1). We limited our samples to pelts from winter-killed wolves, which ensured fully grown guard hairs. Taxidermied samples were from the 1990s and museum specimens ranged from the 1930s to the 1970s. Sex was not determined, and only general origins of taxidermist samples were known. Two samples from Jasper Park in Alberta served as controls, originating far from access to spawning salmon. We also included a sample from Wood Buffalo Park in northern Alberta – Northwest Territories. Although it initially was to serve as a control, later we learned that wolves from Wood Buffalo may have access to spawning salmon from the Arctic drainage.

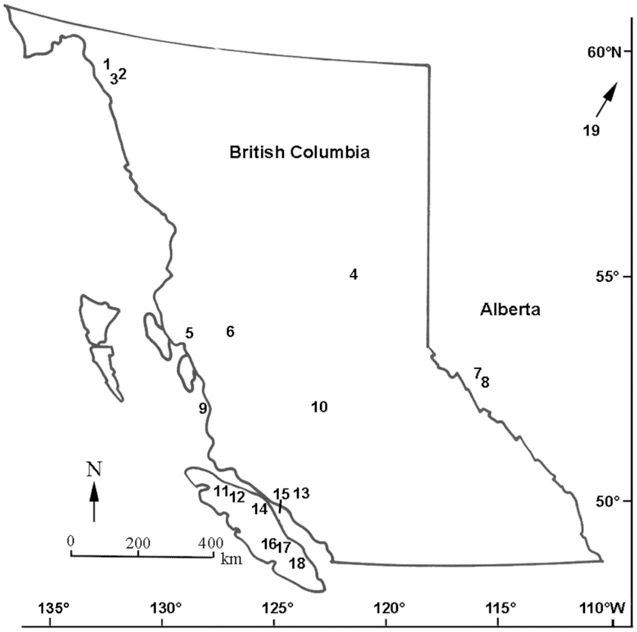


Fig. 1. Study area showing approximate origins of sample gray wolves (*Canis lupus*) from British Columbia and control wolves (samples 7, 8) from Alberta. Sample 19 originates from Wood Buffalo Park in northern Alberta – Northwest Territories.

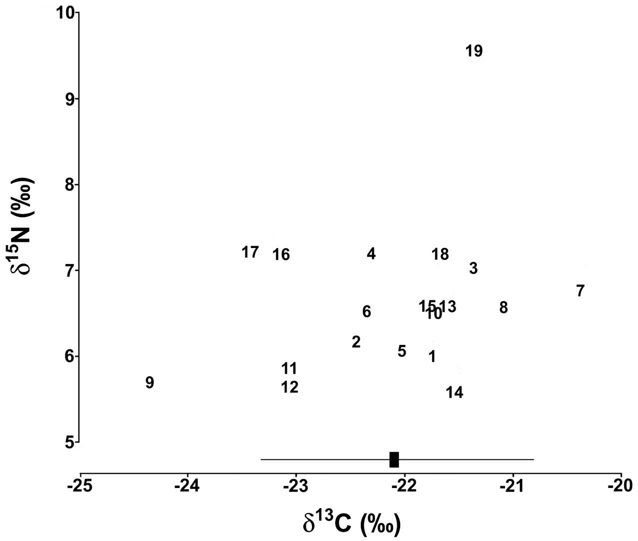
Guard hairs were severed into equal base and tip segments and washed and rinsed three times with 95% ethanol to remove surface debris and oils (Macko et al. 1999). After drying at 60°C for at least 48 h, we powdered the hair in a Wig-L-Bug grinder (Crescent Dental Co., Chicago, Ill.). Approximately 1 mg was subsampled for continuous-flow isotope ratio mass spectrometry analysis at the stable isotope facility, University of Saskatchewan, Saskatoon, using a Europa Scientific ANCA-NT gas–solid–liquid preparation module coupled to a Europa Scientific Tracer 20-20 mass spectrometer (PDZ Europa, Cheshire, England). Isotope signatures are expressed in δ notation as ratios relative to Pee Dee limestone (carbon) and atmospheric N_2 (nitrogen) standards as follows:

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

where X is ^{13}C or ^{15}N and R is the corresponding ratio ^{13}C/^{12}C or ^{15}N/^{14}N. We report average δ^{13}C and δ^{15}N values in guard hairs ((base + tip)/2), as well as δ^{13}C and δ^{15}N values in base and tip segments.

Experimental data on diet to consumer fractionation values are absent for many taxa, including wolves. Whole body δ^{13}C values of consumers are typically enriched 1‰ relative to prey (DeNiro and Epstein 1978). Based on captive studies with mink (*Mustela vison*) and mice (*Mus* spp.), hair from wolves is likely further enriched by another 1‰ for a total diet to consumer hair fractionation value of approximately 2‰ (M. Ben-David, personal communication). To assess use of marine resources, we compared the average guard hair

Fig. 2. Values of δ^{13}C and δ^{15}N in whole guard hairs ((base + tip values)/2) from sample wolves of British Columbia and Wood Buffalo Park (northern Alberta – Northwest Territories) and controls from Jasper Park, Alberta. Solid rectangle and horizontal bar denote mean δ^{13}C value and standard deviation, respectively, for temperate zone terrestrial protein (from Hilderbrand et al. 1996), plus 2‰ for diet to consumer hair fractionation.



δ^{13}C values in individual wolves (plus 2‰) with the mean and standard deviation predicted for consumers of temperate zone terrestrial protein (Hilderbrand et al. 1996).

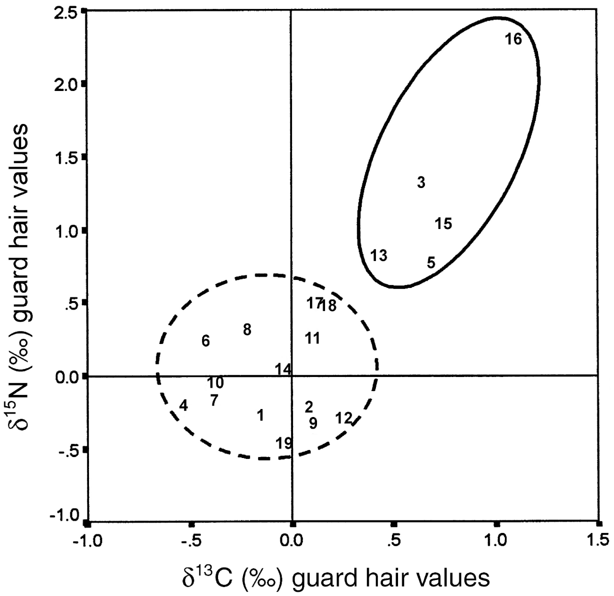
Wolves have one annual moult beginning in late spring when the old coat sheds and a new coat grows until late fall (Young and Goldman 1944). Assuming the coat grows at a relatively constant rate, the base portion of guard hairs would reflect most recent dietary assimilation (fall), whereas the tip portion would reflect summer diet. We examined differences in base and tip signatures for both isotopes. In wolves that received ^{13}C and ^{15}N enrichment primarily from salmon, one would expect coupled enrichments of these isotopes in hair that grew during fall salmon runs (base) relative to hair that grew during summer (tip). Alternatively, if wolves consumed other resources with enriched signatures throughout their moult, this pulse may not be detectable. We compared sample values with controls using *t* tests (SPSS version 10.0).

Results and discussion

Average guard hair isotope values varied little among samples, providing limited insight into the use of marine resources. Mean δ^{13}C and δ^{15}N values (± standard error) for sampled wolves (n = 17) were –22.29 ± 0.24 and 6.58 ± 0.25‰, respectively. Most samples had δ^{13}C values included in the mean and standard deviations predicted (with 2‰ diet to consumer hair fractionation) for consumers of temperate zone terrestrial protein (Fig. 2; Hilderbrand et al. 1996), suggesting a limited use of marine resources over the entire moult.

Surprisingly, δ^{13}C values of both Jasper control wolves (samples 7 and 8) were higher than other samples (t = –2.51, n = 19, df = 17, P = 0.02), but δ^{15}N values were comparable (t = –0.142, n = 19, df = 17, P = 0.89). The sample from Wood Buffalo Park (sample 19) had a highly enriched δ^{15}N value (Fig. 2). Increased information about nonmarine influences on isotope pools (below) in Rocky Mountain and Arctic ecosystems may provide insight into these deviations.

Fig. 3. Differential (fall minus summer) δ^{13}C and δ^{15}N values in guard hair portions (base, tip) of sample wolves from British Columbia and Wood Buffalo Park (northern Alberta – Northwest Territories) and controls from Jasper Park, Alberta. Base portions of guard hair index most recent dietary assimilation (fall); tip portions index summer diet. Broken oval contains samples that showed low interseasonal variability in δ^{13}C and δ^{15}N. Solid oval contains samples with relatively enriched δ^{13}C and δ^{15}N during fall, suggesting the consumption of salmon during fall when they are most abundant. Reference lines are drawn at zero, representing no interseasonal variability in isotope values.



Variation in isotope values may be due to nonmarine influences. Enriched δ^{13}C signatures in controls (and samples) may reflect inclusion of C_4 or water-stressed plants in the diet of prey (Peterson and Fry 1987). Enriched δ^{15}N signals may be due to predation on neonates, which may have increased ^{15}N (Hobson and Sease 1998), nutritional stress (Hobson and Clark 1992; Hobson et al. 1993, but see Ben-David et al. 1999) in wolves, or trophic level of prey species (DeNiro and Epstein 1981; Minagawa and Wada 1984).

An examination of isotope signatures in base and tip hair segments provides finer resolution than analysis of whole guard hair values. Seasonal differences for both Jasper controls showed low interseasonal variability for both isotopes (Fig. 3; within broken oval). In contrast, five samples showed relatively high interseasonal variability by departing exclusively to one direction in isotope space; relatively more ^{13}C and ^{15}N was assimilated during fall than during summer (Fig. 3; within solid oval). This suggests the consumption of enriched marine resources during fall, coinciding with the peak of salmon availability. Apex predators like wolves can incorporate salmon nutrients directly through consumption or indirectly through trophic level effects. Shifts to increased δ^{15}N values during fall may be due to foraging on higher trophic level (and piscivorous) consumers such as ursids and mustelids, both of which can contribute substantially to the diets of wolves (Kohira and Rextad 1997; C.T. Darimont and T.E. Reimchen, unpublished data).

The remaining samples ($n = 12$) showed low interseasonal variability (Fig. 3; within broken oval). Differential (fall minus summer) isotope values were constrained within the variation expected for inert tissues of consumers on a constant diet (Hobson et al. 1996). Alternatively, samples near the coast with higher ^{13}C signatures in whole guard hairs may be consuming some salmon during fall, but other marine resources such as pinnipeds, intertidal organisms, and marine carrion consumed during summer may offset fall enrichment from salmon (Szepanski et al. 1999). Interior wolves may have minor seasonal dietary shifts not related to marine influence, like sample wolves from interior B.C. (samples 4, 6, and 10), which had the most relatively depleted δ^{13}C signatures during fall (Figs. 1 and 3).

An even finer reconstruction of trophic processes in wolves and other mammals would benefit from improved information on the timing of hair growth and resource availability. Hair growth may not be uniform among sampled animals, resulting from differing nutritional states, hormonal activity, and thermodynamic demands in different climates (e.g., Maurel et al. 1986). Similarly, although the pulse of salmon migration in B.C. occurs during fall (Groot and Margolis 1991), the timing and availability of runs may differ among areas and seasons. Future studies in a smaller region with good knowledge of salmon availability that use multiple and smaller inter-hair comparisons may show heavy use of enriched resources during smaller time frames. The relatively gross divisions in time represented by the two hair segments in this study, however, did provide a conservative test of a temporal dietary shift, suggesting a significant difference in resource use between seasons in samples showing the shift.

The interpretation of isotopic signals without relevant ecological data can be challenging. Szepanski et al. (1999) related enriched signatures in mainland wolves to lower ungulate densities in mainland areas relative to islands, suggesting a functional response from feeding on deer to consuming salmon. In that study and ours, it is also possible that differences in enrichment among samples relate to variable availability of salmon. Regardless of ungulate density, using salmon when available may be an adaptive strategy owing to its predictability, spatial constraint, high caloric content, and lower potential costs in survival relative to hunting large mammalian prey.[2]

Szepanski et al. (1999) estimated the lifetime assimilation of marine protein (presumably from salmon) to be 18% for coastal wolves and to be 9% for wolves of interior Alaska. This study supports their conclusion. Some of our samples that showed seasonal shifts to enriched foods during fall originated considerable distances from the ocean (Figs. 1 and 3). The ultimate source of enriched nutrients was likely salmon, illustrating the ocean's potential to influence distant terrestrial food webs, similar to that demonstrated in stable isotope analysis of interior North American brown bears (*Ursus arctos*) and black bears (*Ursus americanus*) (Hilderbrand et al. 1996, 1999; Jacoby et al. 1999). Where the two still coexist, migrating salmon can penetrate far into continental western North America and may be an important seasonal resource for wolves. This yearly pulse of salmon biomass, which has been compared with the large ungulate migrations found in African grasslands (Reimchen 2000), may seasonally disrupt wolf–ungulate associations that dominate ecosystems for most of the year.

Comparisons of stable isotope values between or among metabolically inert tissue portions grown during different periods show great promise but are as of yet inadequately exploited in dietary reconstructions. Some notable exceptions include Schell et al. (1989), who demonstrated seasonal changes in isotopically distinct feeding locations of bowhead whales (*Balena mysticus*) recorded along the length of baleen structure. Thompson and Furness (1995) and Klassen et al. (2001) found variation among feathers grown at different times that related to differences in diet. Hobson and Sease (1998) and Wiedemann et al. (1999) analysed tooth annuli to examine interyear variation in diet of pinnipeds and bovids, respectively. The analysis of nitrogen isotopes in tree cores also offers a fine-scale record of salmon uptake over time (Reimchen et al. 2002). Our study complements others that also used novel and fine-scale isotope methods. Examining intra-hair isotope values can yield more informative data than analysis of whole hair values alone. Notably, this approach may permit the identification of otherwise undetectable niche differentiation among conspecifics or heterospecifics.

Acknowledgments

We are thankful to the Raincoast Conservation Society for financial support and to the Royal British Columbia and Cowan Vertebrate Museums for providing access to wolf pelts. While preparing the manuscript, C.T.D. was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Industrial Post-Graduate Scholarship and T.E.R. by a NSERC operating grant (NRC2354) and funds from the David Suzuki Foundation.

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