STABLE ISOTOPE SIGNATURES OF MOOSE IN RELATION TO SEASONAL FORAGE COMPOSITION: A HYPOTHESIS

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ABSTRACT: Forage plants consumed by moose (*Alces alces*) during winter are isotopically divergent from the forage plants potentially eaten during summer. I examined the natural abundance of ^{15}N and ^{13}C in moose from north-central Alaska, USA, to test the hypothesis that seasonal variation (summer versus winter) in the isotopic composition of the diet can be used to make inferences about food habits across seasons through a temporal record in hooves of moose. Moose hooves collected from hunter-killed males during autumn exhibited temporal oscillations in stable isotope signatures for both carbon and nitrogen. Data on hoof growth in cervids and additional isotope data from immature moose strongly suggest that observed variation in mature animals reflected diet, and comprised a time frame of slightly < 2 years. This isotopic technique has the potential for reconstruction of diets in moose throughout the year without repeated sampling over that time interval, and allows for comparisons of diets among regional populations without extensive fieldwork.

ALCES VOL. 37 (2): 329-337 (2001)

Key words: *Alces alces*, browsing, diet composition, forage chemistry, foraging ecology, moose, stable isotopes

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Northern ungulates undergo seasonal physiological changes as a consequence of the nature of their seasonal food supply and climate (Klein 1970). Foraging ecology of moose (*Alces alces gigas*) in Alaska, USA, is characterized by intake of low-protein food in winter, principally the dormant twigs of deciduous shrubs and trees (Wolff 1976, Risenhoover 1989, Kielland and Osborne 1998), and high-protein forage in summer consisting of aquatic plants (such as *Potomogeton* spp. and *Caltha* spp.) and the leaves of deciduous shrubs and trees (Renecker and Schwartz 1998). In addition to large differences in nutritive value between winter and summer forage, there are other significant chemical differences among these species as well. Recent advances in the ecology of taiga plants have revealed substantial variation in the stable isotope signature of nitrogen (δ^{15}N) across plant functional types in these ecosystems (Schulze et al. 1994, Kielland et al. 1998, Kielland 2001). Although the mechanism for such chemical variation could be attributable to several edaphic and physiological factors (Michelsen et al. 1996, Nadelhoffer et al. 1996, Kielland et al. 1998), the pattern of variation among plant functional types is predictable: deciduous trees and shrubs that constitute the winter diet of moose are depleted in ^{15}N (Kielland and Bryant 1998), whereas forage species that compose diets in summer are more enriched in ^{15}N (Barnett 1994, Kielland 2001). Insofar as isotopic composition of diets influence distribution of isotopes in animals (DeNiro and Epstein 1981, Ben-David et al. 1997a), the marked seasonal dichotomy in isotopic composition in the forage of Alaskan moose might be recorded in their tissues. Inspired by Klein (1964), I attempt to decipher some foraging characteristics in moose using, instead of skeletal ratios, isotopic ratios within the hoof.

Previous studies of time-dependent variation in isotope signatures have relied mostly on recapture for repeated measurements of a particular tissue type such as blood. The constraint that such a sampling schedule may entail has prompted the inquiry of how questions of dietary history may be elucidated through sampling of alternative tissues. One tissue type that lends itself to an identifiable time line is the keratinaceous tissue in hair, nails, or baleen (Schell et al. 1989). Herein I examine the isotopic composition ($\delta^{15}N$, $\delta^{13}C$) of known forage plants of moose and moose hooves in an attempt to: (1) examine the inter-specific variation in isotopic signatures of forage species constituting the major food plants for moose in summer and winter; and (2) juxtapose this forage chemistry with similar parameters in hoof chemistry from a known time interval. Under the assumption that the trophic enrichment in ^{15}N natural abundance is ≈2-3‰ (Steele and Daniel 1978, Minagawa and Wada 1984), the hoof signature was hypothesized to be enriched by at least that much relative to the most depleted item in the diet. Moreover, the potential isotopic variation among forage species available in the summer versus winter should cause an isotopic oscillation along the hoof, from the hairline to the distal end, with a potential amplitude of approximately 7‰ between summer and winter (Fig. 1).

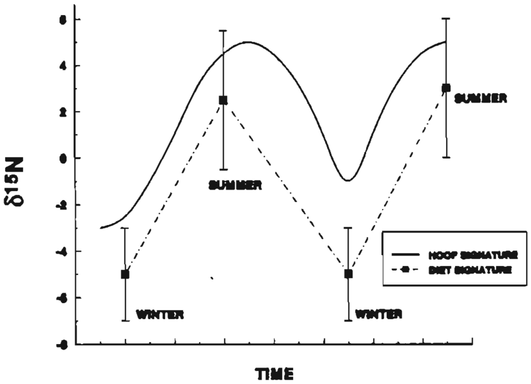

Fig. 1. Hypothesized temporal variation in the $\delta^{15}N$ values of moose hooves in relation to presumed composition of seasonal forage.

METHODS

Forage samples from winter and summer were obtained by sampling vegetation on permanent plots along the Tanana River and upland forests at the Bonanza Creek Long Term Experimental Research site, 20 km SW of Fairbanks, Alaska, USA (64° 51'N, 147° 43'W, elevation 305 m). Additional samples of feltleaf (*Salix alaxensis*) and diamondleaf willow (*S. pulchra*) were obtained from the Koyukuk River drainage in the vicinity of Wiseman (67° 25' N, 150° 06' W, elevation 358 m), Denali National Park (60° 40' N, 149° 20' W, elevation 600 m), and the Colville River near Umiat (69° 22' N, 150° 8' W, elevation 86 m). Vegetation samples were collected in early September 1995-1997 along 120-m transects in each locale. Composite samples of 10 green leaves and twigs of current-annual-growth were collected at 6-12 random points along each transect, depending on location. Samples were dried at 60° C, ground in a Wiley mill (40 mesh) and analyzed by mass spectrometry (Kielland et al. 1998).

Hoof samples of mature male moose were obtained from animals killed in the upper Koyukuk drainage near Wiseman during autumn (1-25 September 1995-2000). Hoof samples from young (< 1 year old) and male yearlings were obtained from wolf (*Canis lupus*) kills collected opportunistically during winter 1997-1998 near Fairbanks, Alaska, USA. Hooves were brushed and thoroughly washed with distilled water to remove possible contaminants from the surface, then stored at -18° C until further processing.

I obtained samples from each hoof using a high-speed Dremel™ rotary tool (Dremel Corporation, Racine, Wisconsin) with a 4 mm-diameter drill bit to carve fine shavings in the hoof to a depth of 2 mm. For each cut, the shavings were collected on a clean sheet of weighing paper and then transferred to glass vials prior to analysis. Cuts were made every 1 cm from the hairline to the distal tip of the hoof, yielding 8-12 samples depending on length (Fig. 2). These samples represent a retrospective, temporal record of nitrogen allocation to the hoof. Therefore, the relative patterns of enrichment and depletion from the hairline are dependent on the date (season) samples were obtained. Fecal samples were collected from each adult male killed in autumn, and rumen contents were inspected visually as to the predominant forage item.

The stable isotope ratio of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) was determined on a Europa 20-20 mass spectrometer at the Institute of Marine Science, University of Alaska, Fairbanks, Alaska, USA. Results are expressed as ratios relative to PeeDee limestone (^{13}C) or atmospheric nitrogen (^{15}N) (Peterson and Fry 1987). Ages of moose were determined from cementum layers in the incisors (Gasaway et al. 1978), performed at the Alaska Department of Fish and Game in Fairbanks, Alaska, USA.

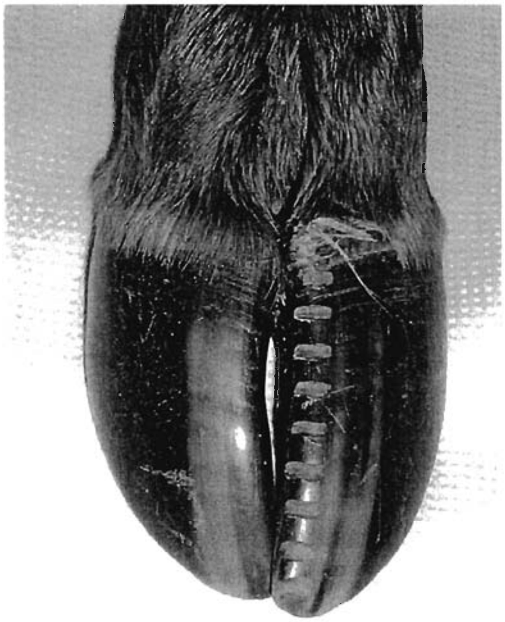

Fig. 2. Front hoof of 6-year-old male moose from which samples were collected for isotope analysis, Alaska, USA, 1999. Cuts in the hoof are at 1-cm intervals.

RESULTS

Plant Chemistry

Forage species potentially available to moose included forbs and aquatic plants during parts of summer, and twigs of deciduous trees in winter, showed strong divergence in $\delta^{15}N$ values. Current annual growth in twigs of deciduous shrubs and trees, such as willows (*Salix alaxensis, S. pulchra*), birch (*Betula papyrifera*), and aspen (*Populus tremuloides*) exhibit depleted $\delta^{15}N$ signatures, whereas aquatic plants were enriched in ^{15}N (Fig. 3). Leaves of deciduous browse were not significantly enriched compared with current annual growth.

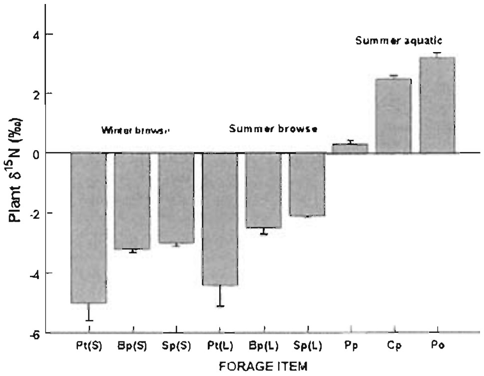

Fig. 3. Stable isotope signatures ($\delta^{15}N$) of moose forage species available in the winter and summer. The species are: Pt - *Populus tremuloides*, Bp – *Betula papyrifera*, Sp – *Salix pulchra*, Pp – *Potentilla palustris*, Cp – *Caltha palustris*, Po – *Potomogeton* spp. In woody browse species S and L refer to current annual stems and leaves, respectively. Samples were collected in the upper Koyukuk River drainage, Alaska, USA, in September 1995 – 2000. Mean ± SE, $n = 8$.

Fecal Chemistry

Average isotopic enrichment of feces relative to the diet of moose from the upper Koyukuk was +1.4°/oo, somewhat lower than in other regions of Alaska (Denali and Colville River) (Table 1). The average enrichment among those populations (+2°/oo), however, was similar to previously published data from domesticated animals (Table 1).

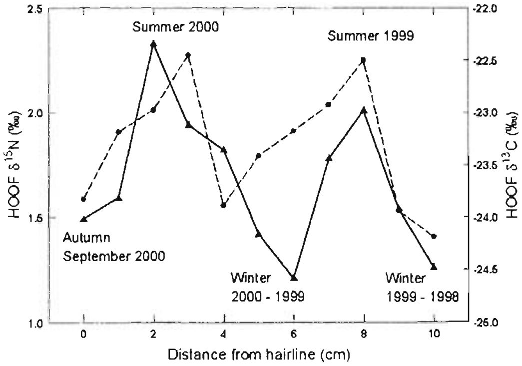

Fig. 4. Temporal variation of $\delta^{15}N$ and $\delta^{13}C$ in hoof of an 8-year-old male moose killed in the upper Koyukuk River drainage, Alaska, USA, in September 2000. The time frame is relative to distance from hairline from present (0 cm) back approximately 2 years (10 cm).

Hoof Chemistry

Adult male moose exhibited distinct oscillations between enrichment and depletion of both $\delta^{15}N$ and $\delta^{13}C$ between samples taken from the hairline to the distal end of the hoof (Fig. 4). That oscillation comprised approximately 2 (apparent) cycles. All moose exhibited the same general pattern of temporal isotopic composition. Samples showed increased enrichment relative to the hairline for the first few centimeters, corresponding to apparent summer conditions (because they were killed in autumn), and did not reach a minimum of the first oscillation until about 6-8 cm (Fig. 4). Nevertheless, some older moose (6-8 years), purported to spend the entire year in the timbered slopes of the Brooks Range and largely eating diamond-leaf willow throughout the year (J. Reakoff, personal communication), exhibited less variation in isotope signatures (Fig.5; stippled line – 8-year-old male). Those oscillations had approximately the same period, indicating they represented cycles over the same time interval. Even though these male moose were all mature individuals (3-8 years old) from the same geographic location, there was considerable variation among individuals. The largest isotopic amplitude was approximately 2.75°/oo, whereas the lowest was only 0.7°/oo.

Rumen contents of all males, by way of

Table 1. Stable isotope values in moose feces relative to the forage species consumed in different regions in Alaska compared to domesticated cattle. Data on moose diets were based on visual observation and literature[1] (DP), rumen analyses (KR), and browse inventories (CR). (Mean ± SE of 6-12 composite samples).

Species or locale	Forage $\delta^{15}N$ (°/oo)	Feces $\delta^{15}N$ (°/oo)	Enrichment (°/oo)
Angus steers[2]	+0.6 (0.00)	+2.6 (0.07)	+2.0 (0.26)
Moose			
Denali Park	-3.0 (0.30)	-0.5 (0.01)	+2.5 (0.31)
Koyukuk River	-2.0 (0.10)	-0.6 (0.10)	+1.4 (0.14)
Colville River	-5.7 (0.40)	-3.4 (0.01)	+2.3 (0.37)

[1] Van Ballenberghe et al. (1989).
[2] Steele and Daniel (1978).

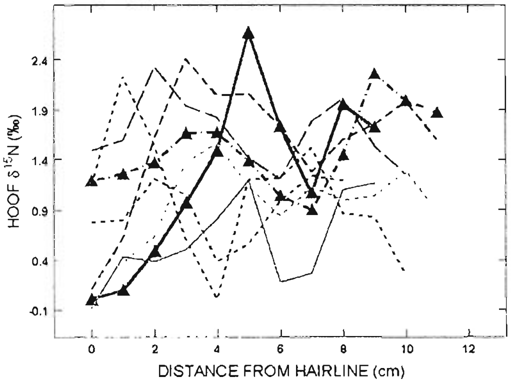

Fig. 5. Hoof values of δ^{15}N and δ^{13}C in adult male moose from the Koyukuk River, Alaska, USA, high-lighting the most (solid line) and least (dashed line) variation among animals.

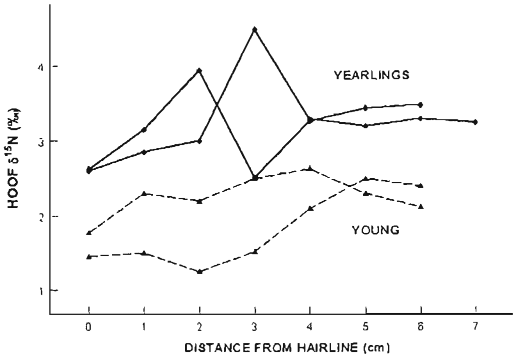

Fig. 6. Nitrogen isotope values in hooves of yearling (solid lines) and young (stippled lines) moose from the Bonanza Creek Experimental Forest, near Fairbanks, Alaska, USA, during winter 1997-1998.

visual estimation, largely consisted of the leaves and stems of diamondleaf willow (*Salix pulchra*), with a minor contribution of feltleaf willow (*S. alaxensis*), birch (*Betula papyrifera*, *B. nana*), aspen (*Populus tremuloides*), cottonwood (*Populus balsamifera*), and aquatic plants (*Pedicularis palustris* and *Potomogeton* spp.). This observation is consistent with the δ^{15}N signatures adjacent to the hairline, which were all enriched 2-3°/oo relative to diamondleaf willow.

In contrast to mature moose (> 2 years old), isotopic signatures in young and yearlings (killed by wolves during winter) did not exhibit the cyclic pattern observed in adults (Fig. 6) and there was no close correspondence between δ^{15}N and δ^{13}C (data not shown). Rather, young showed a gradual enrichment with time in the hoof, but with no distinct period of enrichment or depletion. Yearlings exhibited a single period of enrichment-depletion, corresponding to their single life experience of a full summer and winter (Fig. 6). Although the sample sizes for these data are small and somewhat circumstantial, the pattern of isotopic fluctuations among age groups is consistent with the hypothesis that isotopic oscillations, or lack thereof in animals < 2 years old, represent temporal variations related to diet.

DISCUSSION

Even though all mature moose I examined exhibited temporal variation in stable isotope signatures of hooves, the absolute observed variation (range 2-3°/oo) was substantially less than the predicted maximum variation (range ~7°/oo; Fig. 1). This result is likely a consequence of both biochemical and behavioral attributes of moose-forage relations. First, maximum enrichment in hoof isotope values were depleted relative to that of aquatic plants and forbs, suggesting that these forage classes constitute a minor portion of the diet in summer. Second, moose are apt to mix their diet (Weixelman et al. 1998), so the potential amplitude in hoof signatures may not be realized in a manner that the extremes in diet isotope composition would otherwise predict (e.g., -8.7°/oo for *Salix alaxensis* during winter [Kielland and Bryant 1998] and +5°/oo for forbs during summer [Barnett 1994]). Lastly, adult moose in this region of the upper Koyukuk may rely heavily on willows throughout the year, as has been observed for other montane populations (Van Ballenberghe et al. 1989). Thus, the usefulness of this technique is sensitive to the biological attributes of the systems to which it is applied, suggesting that the technique must be used with caution and preferably augmented by site-specific information regarding vegetation chemistry and habitat use by moose.

Compared with other biological samples (e.g., blood, muscle, or feces) from which stable isotope signatures have been obtained, hooves represent a sink for carbon and nitrogen. Thus, variation in isotope values, because of recycling, sink strength, and other metabolic processes, is minimized for this particular tissue. In view of this condition and the values for vegetation data, I suggest that the 2 cycles each represent annual variation in isotope values of hooves. Consequently, the apparent annual cycle represents the hoof length between the first 2 minima, which was at 6-8 cm. This interpretation is consistent with observations of annual growth rates in other cervids (7.8 cm in caribou; Barnett 1994). Nevertheless, in the absence of direct measurements, this interpretation remains hypothetical.

Implications for Management

Application of stable isotope techniques offers a potentially powerful tool to decipher biological information that is seminal to the management of wildlife (Hilderbrand et al. 1999). For example, the isotopic variation among potentially available forages can be used to calculate proportions of each item in the diet (Ben-David et al. 1997b). With this approach, the manager can estimate, for example, the use of forbs and aquatic plants versus willows in a particular area. Conversely, incompatibility between animal isotope values and that of the local forages could yield information regarding animal movements. Use of isotopic signatures in hooves has several advantages over other tissues such as muscle or hair. Hooves do not break down in response to temperature and moisture the way muscle tissue does, and thus storage is easier. Further, the long temporal record of the hoof obviates frequent re-sampling; it is simple to obtain enough material for analyses, and samples can be provided to the researcher by the general public. Although the present study relied on samples collected from animals killed by hunters or predators, samples can be obtained from live animals as well, as has been done with bears (Kielland, unpublished data).

Biological information obtained through analyses of stable isotopes, however, is limited by some critical, and largely untested, assumptions regarding the biochemistry of stable isotopes in animals and plants (Gannes et al. 1997). Moreover, temporal and spatial variation in isotope signatures, which is at the heart of herbivore diet analyses, can complicate higher-up trophic interpretations if not part of the initial investigation. For example, apparent seasonal variation in isotope values of moose hooves, and presumably in other tissues, indicate that isotopic signatures in predators such as wolves could vary independently of their prey base. Also, significant enrichment in $\delta^{15}N$ over the growing season in moose and caribou, both within individuals and across latitudinal gradients among populations (Kielland and Finstad 2000), offers a cautionary note regarding inferences of prey selection based on "generic" values for those species. Moreover, average isotopic values might vary among sex or age groups. In my study, young and yearling moose exhibited more enriched isotopic signatures compared with mature males, indicating that predation on different age classes could explain isotopic variation among predators that could confound inferences regarding prey species (e.g., caribou versus moose).

Furthermore, intensity by which animals use a particular habitat for feeding or social purposes can yield feedback to the characteristics of the habitat itself (Molvar et al. 1993, Frank and Evans 1997, Ben-David et al. 1998, Kielland and Bryant 1998). These animal-induced modifications of the habitat (e.g., changes in soil fertility or isotopic composition of the vegetation) can cascade through the trophic web and confer different isotopic distributions among animals than would occur in the absence of such habitat-animal feedback.

I conclude that analyses of the stable isotope composition of mature male moose and their food supply were consistent with the hypothesis that variation in hoof $\delta^{15}N$ is a function of variation in the $\delta^{15}N$ signature of forage. The oscillating pattern of hoof isotopic composition appears to represent annual cycles, and indicates that the temporal record in the hoof comprises approximately 2 years (equal to the turnover time of the hoof). This interpretation is corroborated by isotopic analysis of hooves in young and yearlings, as well as by independent estimates of hoof growth rates in other cervids. The application of stable isotope analysis in animal ecology, however elegant and technologically simple, is nevertheless fraught with several heuristic difficulties, primarily stemming from uncertain variation in isotopic discrimination based on many physiological processes. Until these uncertainties are removed, the application of stable isotope analysis should be done with caution and preferably in conjunction with additional methods of analysis.

ACKNOWLEDGEMENTS

This research was supported by the Bonanza Creek LTER program, the National Science Foundation, and the Alaska Department of Fish and Game. I thank M. Ben-David for constructive criticism of the manuscript, B. Finney for many stimulating conversations regarding the use of stable isotopes in biology, and J. Reakoff of Wiseman, Alaska, USA, for providing samples and insights into many aspects of moose ecology. I am much appreciative of N. Haubenstock and P. Rivera for their analytical expertise and cheerful support of the study. Lastly, I thank D. R. Klein, whose friendship over 20 years, professional accomplishments, and curiosity about Alaskan natural history, has inspired so many young biologists like myself, both in Alaska and around the world.

REFERENCES

BARNETT, B. 1994. Carbon and nitrogen isotopes in caribou tissue, vascular plants, and lichens from northern Alaska. M.S. Thesis, University of Alaska, Fairbanks, Alaska, USA.

BEN-DAVID, M., R. T. BOWYER, L. K. DUFFY, D. D. ROBY, and D. M. SCHELL. 1998. Social behavior and ecosystem processes: river otter latrines and nutrient dynamics of terrestrial vegetation. Ecology 79:2567-2571.

_______, R. W. FLYNN, and D. M. SCHELL. 1997b. Annual and seasonal variation in diets of martens: evidence from stable isotope analysis. Oecologia 111:281-290.

_______, T. A. HANLEY, D. R. KLEIN, and D. M. SCHELL. 1997a. Seasonal changes in diets of coastal and riverine mink: the role of spawning Pacific salmon. Canadian Journal of Zoology 75:803-811.

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.

FRANK, D. A., and R. D. EVANS. 1997. Effects of native grazers on grassland N cycling in Yellowstone National Park. Ecology 78:2238-2248.

GANNES, L. Z., D. M. O'BRIAN, and C. MARTINEZ DEL RIO. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78:1271-1276.

Gasaway, W.C., D.B. Harkness, and R.A. Rausch. 1978. Accuracy of moose age determinations from incisor cementum layers. Journal of Wildlife Management 42:558-563.

Hilderbrand, G. V., C. C. Schwartz, C. T. Robbins, M. E. Jacoby, T. A. Hanley, S. M. Arthur, and C. Servheen. 1999. The importance of meat, particularly salmon, to body size, population productivity, and conservation of North American brown bears. Canadian Journal of Zoology 77:132-138.

Kielland, K. 2001. Short-circuiting the nitrogen cycle: strategies of nitrogen uptake in plants from marginal ecosystems. Pages 377-398 *in* N. Ae, K. Okada, and J. Arihara, editors. New concepts in plant nutrient acquisition. Springer-Verlag, Berlin, Germany.

_______, B. Barnett, and D. M. Schell. 1998. Intraseasonal variation in the δ^{15}N signatures of taiga trees and shrubs. Canadian Journal of Forest Research 28:485-488.

_______, and J. P. Bryant. 1998. Moose herbivory in taiga: effects on biogeochemistry and vegetation dynamics in primary succession. Oikos 82:377-383.

_______, and G. Finstad. 2000. Differences in ^{15}N natural abundance reveal seasonal shifts in diet choice of reindeer and caribou. Rangifer 12:145.

_______, and T. Osborne. 1998. Moose browsing on feltleaf willow: optimal foraging in relation to plant morphology and chemistry. Alces 34:149-155.

Klein, D. R. 1964. Range related differences in growth of deer reflected in skeletal ratios. Journal of Mammalogy 45:226-235.

_______ 1970. Tundra ranges north of the boreal forest. Journal of Range Management 23:8-14.

Michelsen, A., I. K. Schmidt, S. Jonasson, C. Quarmby, and D. Sleep. 1996. Leaf ^{15}N abundance of subarctic plants provide field evidence that ericoid, ectomycorrhizal and non- and arbuscular mycorrhizal species access different sources of soil nitrogen. Oecologia 105:53-63.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between δ^{15}N and animal age. Geochimica et Cosmochimica Acta 48:1135-1140.

Molvar, E. M., R. T. Bowyer, and V. Van Ballenberghe. 1993. Moose herbivory, browse quality, and nutrient cycling in an Alaskan treeline community. Oecologia 94:472-479.

Nadelhoffer, K. J, G. R. Shaver, B. Fry, A. Giblin, L. Johnson, and R. Mckane. 1996. ^{15}N natural abundances and N use by tundra plants. Oecologia 107:386-394.

Peterson, B.J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Reviews of Ecology and Systematics 18:293-320.

Renecker, L. A., and C. C. Schwartz. 1998. Food habits and feeding behavior. Pages 403-439 *in* A. W. Franzmann and C.C. Schwartz, editors. Ecology and management of the North American moose. Smithsonian Institution Press, Washington, D.C., USA.

Risenhoover, K. 1989. Composition and quality of moose winter diets in interior Alaska. Journal of Wildlife Management 53:568-577.

Schell, D. M., S. M. Saupe, and N. Haubenstock. 1989. Bowhead whale (*Balaena mysticetus*) growth and feeding as estimated by δ^{13}C techniques. Marine Biology 103:433-443.

Schulze, E-D., G. Gebauer, and F. S. Chapin,

III. 1994. Nitrogen nutrition and isotope differences among life forms at the northern treeline in Alaska. Oecologia 104:406-412.

STEELE, K. W., and R. M. DANIEL. 1978. Fractionation of nitrogen isotopes by animals: a further complication to the use of variation in the natural abundance of ^{15}N for tracer studies. Journal of Agricultural Science (Cambridge) 90:7-9.

VAN BALLENBERGHE, V., D.G. MIQUELLE, and J.M. MACCRACKEN. 1989. Heavy utilization of woody plants by moose during summer at Denali National Park, Alaska. Alces 25:31-35.

WEIXELMAN, D. A., R. T. BOWYER, and V. VAN BALLENBERGHE. 1998. Diet selection by Alaskan moose during winter: effects of fire and forest succession. Alces 34:213-238.

WOLFF, J. O. 1976. Utilization of hardwood browse by moose on the Tanana floodplain of interior Alaska. Research Note PNW-267. U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station, Portland, Oregon, USA.