UTILITY OF STABLE ISOTOPE ANALYSIS IN STUDYING FORAGING ECOLOGY OF HERBIVORES: EXAMPLES FROM MOOSE AND CARIBOU

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ABSTRACT: Recently, researchers emphasized that patterns of stable isotope ratios observed at the individual level are a result of an interaction between ecological, physiological, and biochemical processes. Isotopic models for herbivores provide additional complications because those mammals consume foods that have high variability in nitrogen concentrations. In addition, distribution of amino acids in plants may differ greatly from that required by a herbivore. Thus, substantial amounts of amino acids would be synthesized or recycled by herbivores. At northern latitudes, where the growing season of vegetation is short, isotope ratios in herbivore tissues are expected to differ between seasons. Summer ratios likely reflect diet composition, whereas winter ratios would reflect diet and nutrient recycling by the animals. We tested this hypothesis using data collected from blood samples of caribou (*Rangifer tarandus*) and moose (*Alces alces*) in Denali National Park and Preserve, Alaska, USA. Stable isotope ratios of moose and caribou were significantly different from each other in late summer-autumn and winter. Also, late summer-autumn and winter ratios differed significantly between seasons in both species. Nonetheless, we were unable to evaluate whether differences in seasonal isotopic ratios were a result of diet selection or a response to nutrient recycling. We believe that additional studies on plant isotopic ratios as related to ecological factors in conjunction with investigations of diet selection by the herbivores will enhance our understanding of those interactions. Also, controlled studies investigating the relation between diet and physiological responses in herbivores will increase the utility of isotope analysis in studying foraging ecology of herbivores.

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In nature, carbon (C) and nitrogen (N) each occur in 2 stable isotopes: ^{12}C and ^{13}C, ^{14}N and ^{15}N. Ratios of the 2 isotopes as compared with standards are noted as $\delta^{13}C$ for carbon, and $\delta^{15}N$ for nitrogen, and are measured in parts per thousand (‰). Analysis of food webs with natural abundance of stable isotope ratios compares the $\delta^{13}C$ and $\delta^{15}N$ values of consumer and food tissues. Values of $\delta^{13}C$ differ between terrestrial, freshwater, and marine food, as well as between C_3 Crassulacean acid metabolism (CAM), and C_4 plant communities (Gearing 1991). This difference in ^{13}C values between different primary producers enables researchers to trace food sources (Fry and Sherr 1988, Tieszen and Boutton 1988). Values of $\delta^{15}N$ increase with transfer between trophic levels and therefore reflect both diet and trophic position (i.e., trophic fractionation; DeNiro and Epstein 1981). The specific combination of values of $\delta^{13}C$ and $\delta^{15}N$ results from diet composition of individuals (Schoninger and DeNiro 1984, Ambrose and DeNiro 1986, Schell et al. 1988, Gearing 1991, Hobson 1999). Apply-

ing isotopic analysis to tissues such as blood allows repeated sampling of known individuals or populations throughout the year (Hobson and Clark 1993; Ben-David et al. 1997a, 1997b), enabling researchers to investigate some of the factors underlying feeding habits in different seasons and years, and gain a better understanding of the process of diet selection and foraging ecology (Ben-David et al. 1997a, 1997b; Hobson 1999; Wolf and Martinez del Rio 2000).

Recently, researchers emphasized that patterns of stable isotope ratios observed at the individual level are a result of an interaction between ecological, physiological, and biochemical processes (Gannes et al. 1997). Precipitation, temperature, soil characteristics, nutrient availability, and other factors determine plant community composition and also result in high variability in forage availability and quality for primary consumers (Fig. 1; Begon et al. 1990, Klein 1990). Those factors are also responsible for large variation in stable isotope ratios within and between plant species, through effects of nutrient and carbon dioxide (CO_2) supply, and biochemical pathways (Fig. 1).

Differences in $\delta^{13}C$ among C_3, CAM, and C_4 plants are caused by differences at the diffusion, dissolution, and carboxylation steps of the different photosynthetic pathways (Lajtha and Marshall 1994). Additional variability in $\delta^{13}C$ signatures, within plants of the same species, may result from differences in sources of CO_2 (atmospheric versus respired), light intensity, water availability, and temperature (Lajtha and Marshall 1994). For example, values of $\delta^{13}C$ may increase in plants that suffer from significant water stress (Lajtha and Marshall 1994, Barnett 1995). Alternatively, lower temperatures at higher altitudes can cause increases in values of $\delta^{13}C$. Michelsen et al. (1996) demonstrated an increase in $\delta^{13}C$ values in plants of the same species from nonwarmed plots compared with those growing in warmed plots.

Several studies demonstrated that values of $\delta^{15}N$ varied between plants with differences in assimilation of nitrogenous compounds. Because soil N (i.e., NH_4^+ and NO_3^-) usually is more enriched in ^{15}N than the atmosphere, plants that rely on soil N would have elevated $\delta^{15}N$ values compared with those that obtain N through symbiotic fixation (Lajtha and Marshall 1994, Högberg 1997). Nonetheless, differences in rates of soil processes (i.e., mineralization, nitrification, and denitrification) may lead to differences in values of $\delta^{15}N$ in different soil pools (Lajtha and Marshall 1994). Other studies described changes in $\delta^{15}N$ in plants, as a result of anoxic soil conditions related to flooding (Klingsmith and Van Cleve 1993, Nadelhoffer and Fry 1994, Högberg 1997, Hedin et al. 1998), increases in $\delta^{15}N$ with increasing rooting depth (Nadelhoffer and Fry 1988, Högberg 1997), as well as changes in $\delta^{15}N$ that accompany plant phenology (Kielland et al. 1998). Additional variability

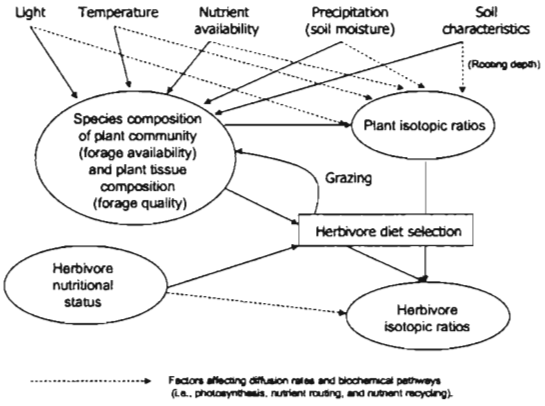

Fig. 1. Schematic representation of the interaction between biochemical, physiological, and ecological processes that may influence stable isotope ratios in an individual herbivore. Solid lines represent ecological interactions; dotted lines represent factors that influence diffusion rates and biochemical pathways that influence stable isotope ratios.

in $\delta^{15}N$ signatures within plants of the same species may result from differences in nutrient inputs. For example, N fertilization by salmon (*Oncorhyncus*) carcasses and activity of piscivorous carnivores resulted in elevated levels of $\delta^{15}N$ in plants growing in riparian and beach-fringe forests compared with the same plant species growing in adjacent areas (Ben-David et al. 1998a, 1998b).

Physiological condition and nutritional requirements of an animal influence diet selection based on forage availability and quality (Robbins 1983, Klein 1990). Diet selection, however, will not necessarily be the sole mechanism determining the isotopic ratios observed at the individual level, because biochemical processes within the animal can result in high variability in fractionation and assimilation of different isotopes (Fig. 1). For example, when a consumer assimilates structural lipids and proteins from one source, but derives most of its energy from another (such as carbohydrates), the latter will be underestimated when the consumer tissues are analyzed (Schwarcz 1991, Tieszen and Fagre 1993). Similarly, different components of the diet (i.e., carbohydrates, lipids, and proteins) usually are routed to different tissues without being first homogenized and repartitioned (Ambrose and Norr 1993, Tieszen and Fagre 1993). Such routing of nutrients could cause biases in dietary estimates when only one tissue is analyzed. In addition, animals feeding on diets with low protein contents (e.g., herbivores), or nutritionally stressed animals, often reserve dietary protein for tissue maintenance as well as recycle endogenous nitrogen (Castellini and Rea 1992). Hobson et al. (1993) demonstrated that enrichment in values of $\delta^{15}N$ occurred in nutritionally stressed birds, suggesting that recycling of dietary and endogenous N could result in elevation in $\delta^{15}N$.

The herbivore model provides additional complications for interpretation of isotopic ratios, because herbivores consume foods that have high variability in N concentrations (Bryant 1987, Ben-David et al. 1998a, Kielland et al. 1998). In addition, the distribution of individual amino acids in plants may differ greatly from that required by the herbivore (Robbins 1983). In ruminants, endogenous secretion and recycling of N (via saliva, pancreatic secretion, and urea) are affected by food intake, protein and fiber contents of the feed, and presence of antinutritive factors (such as tannins; Robbins et al. 1987a, 1987b; Moen and DelGuidice 1997; Schwartz and Renecker 1998; Zebrowska and Kowlcyzyk 2000). Thus, substantial amounts of amino acids would be synthesized or recycled by ruminants (Robbins 1983). Such recycling and internal synthesis of amino acids may result in further fractionation of stable isotopes in the herbivore yielding values different from those expected based on diet alone. In this study, we evaluate the usefulness of stable isotope analysis in investigating foraging ecology of 2 northern herbivores, moose (*Alces alces*) and caribou (*Rangifer tarandus*), based on predictions derived from the interaction between plant isotopic ecology (Table 1), diet selection of the 2 herbivores, and seasonal changes in nutritional conditions of these 2 species (Figs. 2 and 3).

At northern latitudes, where the growing season of vegetation is short (Rachlow and Bowyer 1991), herbivores encounter radically different foraging environments in summer and winter. Summer is characterized by high availability of high-quality forage. In contrast, in winter herbivores would encounter forage that is low in N concentrations (Klein 1990). In addition, although only C_3 plants occur at northern latitudes, changes in temperatures and water availability may lead to seasonal changes in values of $\delta^{13}C$. Thus, we expect that iso-

Table 1: Isotopic ratios (range of values in ‰) of potential forage plant species for moose and caribou from northern Alaska and the Yukon. Values were assembled from the literature (Barnett 1995, Kielland et al. 1998, Kielland 2001, Ben-David and Hik unpublished data).

Plant Type	Plant species	$\delta^{13}C$	$\delta^{15}N$
Shrubs and trees	*Betula*	(-31.5)–(-27.7)	(-6.1)–(0.0)
	Populus		(-8.2)–(-0.8)
	Vaccinium	(-29.9)–(-25.9)	(-8.0)–(-0.8)
	Salix	(-28.7)–(-24.0)	(-6.3)–(2.3)
Herbs	*Artemesia*	(-28.3)–(-26.6)	(-3.2)–(-1.3)
	Carex	(-26.8)–(-23.8)	(-1.8)–(5.3)
	Eriophorum	(-26.3)–(-24.7)	(2.1)–(5.2)
	Festuca	(-28.3)–(-25.3)	(-3.7)–(-0.1)
	Petasites	(-26.0)–(-25.0)	(-0.3)–(10.1)
Aquatic plants[1]	*Potentilla*		0.3
	Caltha		2.5
	Potomogeton		3.2
Mushrooms		(-27.3)–(-20.5)	(-3.6)–(9.1)
Lichens		(-25.6)–(-21.3)	(-4.9)–(1.7)

[1]Average values (Kielland 2001).

tope ratios in herbivore tissues would significantly differ between seasons. Because winter forage likely would be low in N concentrations, high rates of N recycling would be expected, resulting in elevated $\delta^{15}N$ values in tissues collected during that season (i.e., additional fractionation in addition to the trophic effect). In contrast, we expect that isotopic ratios in summer would more closely reflect diet composition.

We tested these predictions on data collected from moose and caribou in Denali National Park and Preserve, Alaska, USA. We hypothesized that isotope ratios of moose and caribou would be significantly different from each other during late summer-autumn as well as in winter, representing differences in diet selection by the herbivores (Fig. 2). Because both herbivores enter a negative energy balance in winter (Renecker and Hudson 1986, Schwartz et al. 1988, Gerhart et al. 1996, Schwartz and Renecker 1998), both likely would exhibit high rates of N recycling in that season. Thus, we hypothesized that both herbivores would exhibit elevated levels of $\delta^{15}N$ in winter compared with those exhibited in summer (Fig. 3). In addition, moose maintain a diet based on shrubs in winter (Holleman and Luick 1977, White and Trudell 1980, Bowyer et al. 1997, Renecker and Schwartz 1998), whereas caribou consume large amounts of lichens during that period (Boertje 1984). We hypothesized, therefore, that during winter, values of $\delta^{13}C$ in tissues of caribou would represent consumption of lichens and would be significantly enriched compared with values from late summer-autumn (Fig. 3), but no such enrichment would occur in moose.

STUDY AREA

Denali National Park and Preserve is composed of mountain peaks > 3,000 m flanked by lower mountains and broad, lowland flats. Higher mountains are covered with permanent ice and snow, whereas slopes < 2,400 m are vegetated by alpine sedge (*Carex* spp.) and low shrub (*Salix* spp. and *Betula* spp.) tundras. Treeline

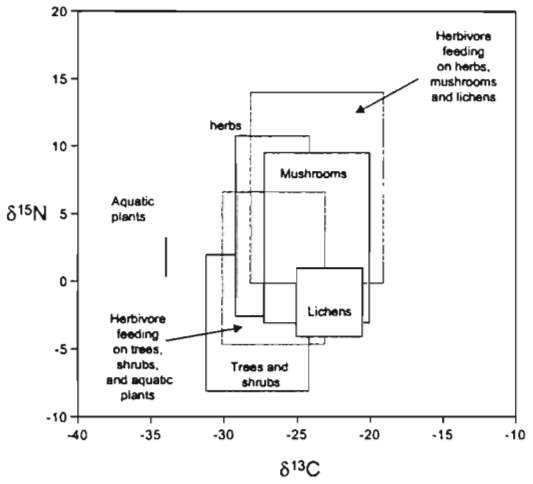

Fig. 2. Isotopic ratios (range of values in ‰) of potential forage plants for moose and caribou from northern Alaska, USA, and the Yukon, Canada (Table 1), and the range of predicted isotopic ratios for herbivores feeding on those plants. Predicted range was calculated based on extreme values of the range of each category. A trophic fractionation of 1‰ for carbon and 3‰ for nitrogen were applied in creating the herbivore ranges (DeNiro and Epstein 1981, Fry and Sherr 1988). Because data on $\delta^{13}C$ of aquatic plants are lacking, calculation of range of isotope ratios for herbivores feeding on those plants was based on the assumption that $\delta^{13}C$ of aquatic plants was within the range of trees and shrubs.

occurs at about 800 m, and is characterized by spruce (*Picea* spp.) forests, tussock (*Eriophorum* spp.) tundra, and riparian spruce-willow communities. Adams and Dale (1998) provide a thorough description of the study area.

METHODS

Collection of Samples

Female caribou and moose were immobilized and captured via darts fired from a helicopter (Adams and Dale 1998). Caribou were captured during 22-27 September 1993, 14-21 March 1993, and 13-22 March 1998. Moose were captured 2-6 November and 9-14 March 1998. Blood samples were collected from immobilized individuals in sterile, plain-blood collection tubes, protected from freezing while in the field, and centrifuged within 12 hr of collection. Sera and clots were separated, and clots were stored frozen until prepared for further analyses. Based on allometric relation between body size and longevity of blood cells (Berlin 1964), we estimated that blood cells collected from caribou in late September represent late summer-autumn diets (based on longevity of 90 days and a half-life of 45 days). Similarly, we estimated that blood cells collected in early November represent late summer-autumn diets for moose (based on longevity of 120 days and a half-life of 60 days). For both species, these estimates represent the end of the growing season, prior to rut and associated hypophagia (Miquelle 1990) and before the animals enter a negative energy balance. Samples collected in mid-March represent winter conditions and diets for both species.

Stable Isotope Analysis

Clotted blood cells were freeze-dried and ground by hand to fine powder with a mortar and pestle. Subsequently, a subsample (1.8-2.2 mg) was weighed into a miniature tin cup (4 x 6 mm) for combustion. We used a Europa 20/20 continuous flow, isotope ratio, mass spectrometer system to obtain the stable isotope ratios. Several samples chosen at random were analyzed in duplicates and 1 glycine standard was analyzed with every 20 samples. Results were accepted only if the variance between the duplicates did not exceed that of the standard ($\delta^{13}C_{std}$ = -37.2, $\delta^{15}N_{std}$ = 10.08). Isotopic data for forage plant species from Alaska and the Yukon were obtained from the literature (Barnett 1995, Kielland et al. 1998, Kielland 2001, Ben-David and Hik unpublished data).

Statistical Analysis

We employed the K nearest-neighbor

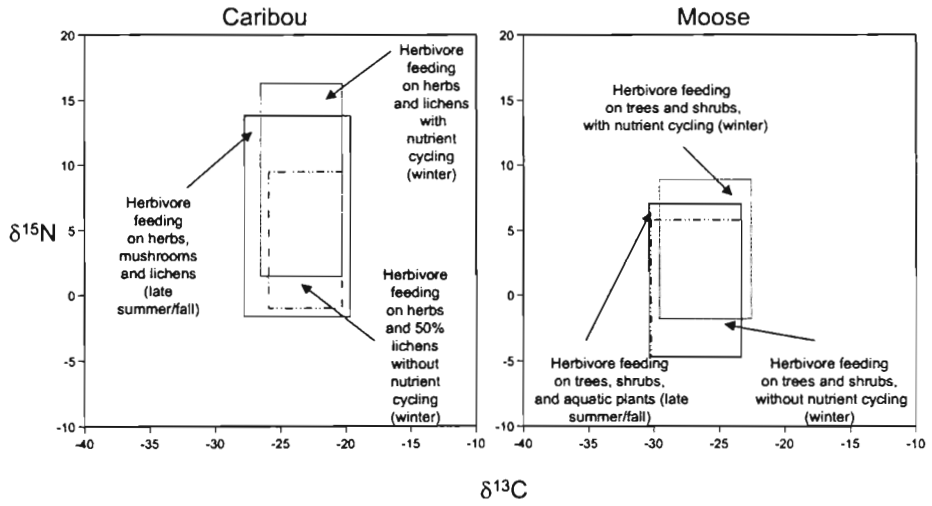

Fig. 3. Predicted isotopic ratios (range of values in ‰) of caribou and moose based on isotopic values of plants, and assumptions on diet selection by the herbivores. Nutrient cycling was assumed to create fractionation values of 1‰ for carbon and 3‰ for nitrogen. A predicted range of values with consumption of 50% lichens for caribou was selected based on data from Boertje (1984).

randomization test (Rosing et al. 1998) to investigate whether isotope ratios of moose and caribou were significantly different from each other during late summer-autumn and winter. To test whether the 2 herbivores exhibited elevated levels of $\delta^{15}N$ in winter compared with those recorded in summer, we used an analysis of variance (ANOVA) with $\delta^{15}N$ as the dependent variable and species and season as the independent ones (Zar 1984; SPSS for Windows, SPSS Incorporated, Chicago, Illinois, USA). Similarly, to test whether values of $\delta^{13}C$ in tissues of caribou during winter would represent consumption of lichens and would be significantly different from the values recorded in tissues of moose, we used an ANOVA with $\delta^{13}C$ as the dependent variable and species and season as the main effects (Zar 1984; SPSS for Windows, SPSS Incorporated, Chicago, Illinois, USA).

RESULTS

Isotopic ratios from blood cells of moose and caribou exhibited high individual variability (Figs. 4 and 5). Nonetheless, values for each species were significantly different from each other in all seasons (Fig. 4; K nearest neighbor randomization test, $P < 0.001$). Similarly, isotopic ratios of blood cells from moose and caribou in late summer-autumn were significantly different from those in winter (Fig. 4; K nearest neighbor randomization test, moose $P = 0.05$, caribou $P < 0.01$). Subsequent analysis revealed that although isotopic ratios of blood cells from caribou were significantly different between late summer-autumn 1993 and winter 1993 (Fig. 4; K nearest-neighbor randomization test, $P = 0.002$), no such difference was detected between late summer-autumn 1993 samples and winter 1998, or between winter 1993 and winter 1998 (Fig. 4; K nearest-neighbor randomization test, $P = 0.17$ and $P = 0.1$, respectively).

Values of $\delta^{15}N$ were not significantly different between winter and late summer-autumn for moose (Fig.4; ANOVA, $P = 0.40$). In contrast, a trend for higher values of $\delta^{15}N$ in late summer-autumn was observed in caribou (Fig.4; ANOVA, $P = 0.058$). In both species, a significant en-

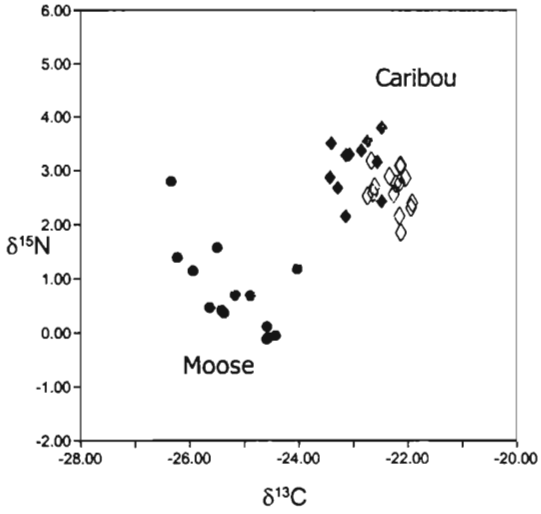

Fig. 4. Stable isotope ratios of blood cells from moose and caribou collected in Denali National Park and Preserve, Alaska, USA, in autumn and spring 1993 and 1998. Solid symbols represent late summer-autumn values and open symbols are winter values. For caribou, dark open symbols represent winter 1993 and light symbols winter 1998. Isotopic ratios of blood cells from moose and caribou were significantly different from each other in all seasons (K nearest neighbor randomization test, $P < 0.001$). Values of $\delta^{15}N$ were not significantly different between winter and summer for moose (ANOVA, $P = 0.4$) or caribou (ANOVA, $P = 0.06$). In both species, a significant enrichment of 0.5-0.6‰ in $\delta^{13}C$ occurred in winter (ANOVA, $P = 0.03$ for moose and $P < 0.001$ for caribou).

richment of 0.5 – 0.6‰ in $\delta^{13}C$ occurred in winter (Fig.4; ANOVA, $P = 0.028$ for moose, and $P < 0.001$ for caribou).

DISCUSSION

Stable isotope ratios of moose and caribou in Denali National Park and Preserve were significantly different from each other in both late summer-autumn and winter. Although we had no available isotopic data for forage plants from the Park, data from plants collected at several northern locations in Alaska and the Yukon indicated that moose concentrated on shrub diet in both seasons, whereas caribou fed largely on herbaceous vegetation (Table 1, Fig. 5). In all locations, samples of herbaceous vegetation had enriched values of both $\delta^{13}C$ and $\delta^{15}N$, which is consistent with the enriched values we observed in caribou blood cells (Table 1; Figs. 2 and 5). In addition, caribou may have consumed mushrooms and lichens in late summer-autumn (Boertje 1984, Barnett 1995). Both types of food exhibit enriched $\delta^{13}C$, especially mushrooms, which also were enriched in $\delta^{15}N$ (Table 1, Fig. 2). Stable isotope ratios of moose and caribou were within the predicted range of values for each herbivore based on plant isotope ratios and trophic fractionation (Fig. 5). In addition, values for moose registered inside the area of overlap of predicted isotopic

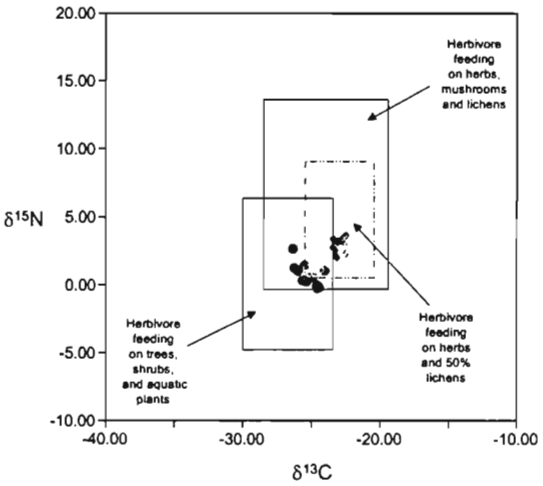

Fig. 5: Stable isotope ratios of blood cells from moose and caribou collected in Denali National Park and Preserve, Alaska, USA, in autumn and spring 1993 and 1998, plotted against the range of predicted isotopic ratios for herbivores. Stable isotope ratios of moose and caribou were within the predicted range of values for each herbivore based on plant isotope ratios and trophic fractionation. Caribou values did not register inside the area of overlap, indicating that trees and shrubs contributed less to the diet of these herbivores than other components of their diet.

values (Fig. 5), whereas caribou values did not, indicating that trees and shrubs contributed less to the diet of those herbivores. These observations agree with information on diets of moose and caribou described by other methods (Holleman and Luick 1977; White and Trudell 1980; Van Ballenberghe et al. 1989; Miquelle et al. 1992; Post and Klein 1996; Bowyer et al. 1997, 1998; Renecker and Schwartz 1998).

Stable isotope ratios of moose and caribou were relatively clustered compared with predicted values (Table 1, Fig. 5). Those predictions, however, were based on range of values rather than means (and standard errors) because sample sizes differed between studies and deriving means from combined data would be inappropriate. Knowledge of the distribution of isotopic ratios in plants in Denali National Park will likely change the boundaries of the predicted herbivore values. Given existing data, whether the degree of clustering in isotopic signatures from moose and caribou is a result of the distribution of isotope ratios in plants in Denali National Park or a consequence of diet selection by the herbivores is unclear (Fig. 1). Additional studies on plant isotopic ratios as related to ecological factors in conjunction with investigations of diet selection by the herbivores in Denali National Park will enhance our understanding of these interactions (Fig. 1) and will increase the utility of isotope analysis.

Determining individual diet selection for both moose and caribou in our sample (Fig. 4) would be impossible without data on isotopic ratios of plants in Denali National Park. Such analysis calls for the use of dual-isotope mixing models, which require that all possible food types (referred to as end-members in the literature) will be significantly different from each other (Ben-David et al. 1997a, 1997b; Rosing et al. 1998). These models are sensitive to the number of potential end-members and the system becomes underdetermined when the number of end-members exceeds 3-4 (Ben-David and Schell 2001, Phillips 2001). Therefore, if the number of forage species or forage classes in Denali National Park that have significantly different isotopic ratios will exceed 3–4, it may be difficult to assess individual diet selection for moose and caribou unless additional stable isotopes of other elements (i.e., H, O, S, Sr) are also analyzed. In addition, the currently available mixing models are sensitive to nutrient routing within the animal (Ben-David and Schell 2001). Development of more appropriate mixing models will depend on conducting controlled studies that will further elucidate relations between diet composition, metabolic pathways, resulting consumer tissue composition, and associated isotopic data.

In contrast to our prediction, values of $\delta^{15}N$ were not significantly different between winter and summer-autumn for either moose or caribou (Fig. 4). This may be a result of an interaction between changes in diet and plant secondary compounds, and physiological changes from gestation, lactation, body growth, tissue catabolism, and N recycling. For example, if caribou selected N fixing herbs or lichens ($\delta^{15}N$ = 0‰) in late summer-autumn, and shrubby vegetation containing low N concentrations ($\delta^{15}N$ = -3‰) in winter, they likely would exhibit similar isotopic signatures ($\delta^{15}N$ = +3‰). In late summer-autumn this signature would represent diet selection, whereas in winter this value could represent diet selection coupled with N recycling. Therefore, our initial expectation for an increase in $\delta^{15}N$ in winter may have been unfounded.

Moreover, Hobson et al. (1993) documented increase in $\delta^{15}N$ values with nutritional stress in several avian species in both field and laboratory studies. Mammals, however, may possess different biochemical pathways from those of birds in which

no additional fractionation of N occurs. In a study on Arctic ground squirrels (*Spermophilus parryii plesius*), no relation between $\delta^{15}N$ and body condition was detected, suggesting that in some mammals, N recycling may not result in enrichment in $\delta^{15}N$ (Ben-David et al. 1999). If so, using values of $\delta^{15}N$ to determine the nutritional status of mammalian herbivores will be impossible. Studies on the influence of ruminant physiology on stable isotope fractionations in animal tissues will enhance the utility of this technique.

Alternatively, our inability to detect seasonal changes in $\delta^{15}N$ may have been a result of the tissue we chose to analyze. Other studies documented significant oscillations in both $\delta^{15}N$ and $\delta^{13}C$ in hooves of caribou representing seasonal changes in isotopic values (Barnett 1995). Similarly, Kielland (2001) observed oscillations of $\delta^{15}N$ in hooves of moose, which may indicate seasonal changes in diet. Hooves, which are a keratinous tissue, will likely act as a sink for sulfur-bearing amino acids such as methionine and cysteine (Robbins 1983). Because many plants have low concentrations of those amino acids (Robbins 1983) cycling of those amino acids may be more prevalent and will be better noted in tissues such as hair and hooves. Thus, to obtain a thorough understanding of diet selection and nutritional status of herbivores with stable isotope analysis, investigating more than 1 tissue may be necessary.

In both caribou and moose, a significant enrichment of 0.5-0.6‰ in $\delta^{13}C$ occurred in winter (Fig. 4). Again, whether this enrichment represents nutrient recycling or seasonal dietary changes is unclear. For caribou, the enrichment in $\delta^{13}C$ may represent increased consumption of lichens. Boertje (1984) observed a significant increase in consumption of lichens from 17% in summer to 43% in autumn, and 62% in winter. For moose this may be a result of shifting from feeding on foliage to feeding on shrub stems (Weixelman et al. 1998) rather than represent recycling of nutrients. Although values of $\delta^{15}N$ do not seem to differ between stems and leaves for moose forage plants (Kielland 2001), data on $\delta^{13}C$ values are lacking. Such information will assist in elucidating the patterns we observed. Again, controlled studies on the relation between diet and physiological responses in these herbivores will improve the utility of stable isotope analysis for studying foraging ecology of herbivores.

Sexual segregation is common among northern ungulates (Miquelle et al. 1992, Kie and Bowyer 1999), and may translate to dietary differences between the genders in winter. Unfortunately, data for this study were obtained through a companion study that targeted females only (Adams and Dale 1998). Therefore, we are unable to investigate the influence of sexual segregation on isotopic ratios of male and female moose and caribou in Denali Park. Detection of gender differences in isotope ratios may provide a tool for investigating the differences in effects of environmental conditions on the 2 genders. Similarly, seasonal changes in isotopic ratios for moose and caribou suggest that stable isotope analysis may be useful in tracking responses of individuals to changing environmental conditions. Relating data on temperature, precipitation, snow depth, and other variables to isotopic ratios in herbivores may assist in developing tools for monitoring environmental changes such as those associated with global warming (Bada et al. 1990).

In conclusion, our data demonstrated that stable isotope analysis could be developed as a useful tool for assessing foraging ecology of herbivores. Studies on plant isotopic ratios as related to ecological factors, in conjunction with investigations of diet selection by the herbivores, will en-

hance our understanding of those interactions (Fig. 1). Also, controlled studies investigating the relation between diet and physiological responses in herbivores will increase the utility of isotope analysis. In this study, we detected both seasonal and species differences, and evaluated the dietary niche of the 2 species with isotope ratios. Such investigations of stable isotope ratios may be exceedingly important for studying less known or endangered species, as well as detection of gender differences in isotope ratios of well-studied species. In addition, the ability to use stable isotopes as markers would allow determining relations in higher trophic positions. For example, that moose and caribou in Denali National Park had significantly different isotopic ratios will assist researchers in determining the importance of moose and caribou to the diet of predators such as wolves (*Canis lupus*) and wolverines (*Gulo gulo*; Szepanski et al. 1999). Stable isotope analysis, therefore, provides a link between ecosystem processes and behavior of predators (Ben-David et al. 1998a, 1998b). This technique also has the potential of providing a reliable monitoring tool of the effects of environmental change on communities and ecosystems through the study of individuals.

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