



# Using Bayesian Stable Isotope Mixing Models to Estimate Wolf Diet in a Multi-Prey Ecosystem

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**ABSTRACT** Stable isotope analysis (SIA) of wolf (*Canis lupus*) tissues can be used to estimate diet and intra-population diet variability when potential prey have distinct  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. We tested this technique using guard hairs collected from 44 wolves in 12 northwestern Montana packs, summer 2009. We used hierarchical Bayesian stable isotope mixing models to determine diet and scales of diet variation from  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of wolves and potential prey, white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), elk (*Cervus canadensis*), moose (*Alces alces*), snowshoe hare (*Lepus americanus*), and other prey. As a check on SIA results, we conducted a separate diet analysis with temporally matched scats (i.e., collected in summer 2008) from 4 of the same packs. Wolves were centered on the ungulate prey in the isotope mixing space. Both methods revealed differences among pack diets and that wolves may consume moose in greater proportions than predicted by available biomass. Stable isotope analysis, and scat results were not entirely concordant; assumptions related to tissues of use in SIA, hair growth period in wolves, and scat sampling may have contributed to a mismatch between methods. Incorrect fractionation values, insufficient separation of prey in the isotope mixing space, choice of prior information in the Bayesian mixing models, and unexplained factors may have distorted diet estimates. However, the consistently high proportion of moose in pack diets suggests that increased population monitoring would benefit management of moose and wolves. Our results also support suggestions of other researchers that species-specific fractionation values should be used whenever possible, and that SIA may sometimes only provide indices of use for general groups of prey (e.g., large ungulates). © 2012 The Wildlife Society.

**KEY WORDS** Bayesian, *Canis lupus*, diet, fractionation, Montana, stable isotopes, wolf.

Diets of wolves (*Canis lupus*) and other large carnivores have traditionally been estimated from kills (Burkholder 1959, Boyd et al. 1994, Ballard et al. 1997, Kunkel et al. 1999) and scats (Putman 1984, Leopold and Krausman 1986, Merkle et al. 2009), or through a combination of these methods (Potvin and Jolicoeur 1988, Huggard 1993, Arjo et al. 2002). Stable isotope analysis (SIA) is an increasingly common technique that may provide more comprehensive diet information (Szepanski et al. 1999), which could be particularly useful for managers where wolves are common predators of wild ungulates and, occasionally, livestock.

Stable isotope analysis determines the relative proportions of each food source in the diet of consumers by measuring changes that occur in isotope ratios as tissues are consumed, metabolized, and reorganized during trophic steps (Peterson

and Fry 1987). Isotopic compositions of carnivore tissues, thus, reflect those of their prey, and because all nutrients assimilated into tissues during growth can be measured, a comprehensive diet record can be derived from direct evidence (DeNiro and Epstein 1978, 1981), in contrast to diet records that must be assembled from scat contents or evidence at kills.

Stable isotope analysis can estimate these comprehensive diet records over a range of temporal scales and across multiple population levels. Depending on the turnover rates of the available sample tissue, short- or long-term diet records can be determined (Peterson and Fry 1987). For example, blood contains isotopic values of food sources metabolized over the preceding 10–14 days (Hilderbrand et al. 1996), hair reflects diet over a period of months (Darimont and Reimchen 2002), and bone tissue stores a lifetime's diet history (Tieszen et al. 1983). Because the tissue samples used for SIA can be attributed to individuals, dietary variability can be estimated at multiple population levels (Urton and Hobson 2005, Darimont et al. 2008). This could have particular utility for studies of wolf diet, which may vary among individuals, packs, and regions (Semmens et al. 2009).

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For any diet study that uses SIA, 3 fundamental assumptions must be met. First, the technique requires a priori knowledge of prey availability to the consumers, and only the contribution of prey selected as potential diet sources can be measured. Second, the specific contribution of each dietary source can only be determined if sources are isotopically distinct (Ben-David et al. 1997). The third assumption is that isotope values change predictably during trophic steps. The relative retention of the heavier isotope (i.e., enrichment or depletion) as prey tissues are metabolized and assimilated into consumer tissues is termed trophic fractionation. When SIA is used to determine diet, appropriate fractionation values are applied to stable isotope values of prey before comparison with the isotopic composition of a consumer's tissues. Experimental data are absent for wolves, and the convention in SIA is to use available fractionation values of the closest relative to the taxon of interest. The most commonly used fractionation values in wolf diet studies were estimated from controlled feeding studies on red foxes (*Vulpes vulpes*; Roth and Hobson 2000).

Estimating wolf diet using SIA would provide the first methodological evaluation of this technique in the United States portion of the Northern Rockies, and could yield insight into the dietary ecology of a recolonizing predator of great management interest. The first reproducing wolf pack was documented within Glacier National Park, Montana in 1986 (Ream et al. 1989), and the northwestern Montana wolf population increased from  $\geq 23$  in 1995 (Pletscher et al. 1997) to  $>300$  in 2009 (Sime et al. 2010). Examination of wolf diet can provide information about how wolves use common and rare prey, and how this use varies over space and time. New data on what wolves consume, and the scales at which diet variation might occur could be useful to managers in 2 ways. First, they will be able to provide current information to the public. Second, such data might assist managers in setting hunting seasons for ungulates and wolves (J. S. Williams, Montana Fish, Wildlife and Parks [MFWP], personal communication).

We used stable isotope values of wolves and their prey to estimate the summer diet and intra-population diet variation of wolves from 12 packs in northwestern Montana. Variation among packs was reported from kills in northwestern Montana (Kunkel et al. 2004) and elsewhere from summer scats (Van Ballenberghe et al. 1975, Tremblay et al. 2001), and stable isotope data have been used to determine summer diet variability between individuals (Urton and Hobson 2005). Therefore, we predicted that pack and individual variability would explain most of the variation in diet among all wolves. As a check on our SIA results, we conducted a separate diet analysis on temporally matched scats from 4 of the 12 packs, and predicted no significant differences in proportions of prey biomass consumed between the 2 sets of data.

## STUDY AREA

The study area encompassed approximately 10,000 km<sup>2</sup> of northwestern Montana, a largely rugged mountainous landscape, interspersed with heavily forested valleys that included

portions of Kootenai National Forest, Flathead National Forest, and Glacier National Park. Forests were dominated by Douglas fir (*Pseudotsuga menziesii*), lodgepole pine (*Pinus contorta*), spruce (*Picea* spp.), western larch (*Larix occidentalis*), and ponderosa pine (*Pinus ponderosa*). Other conifers were western red cedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), and subalpine fir (*Abies lasiocarpa*). Black cottonwood (*Populus trichocarpa*), willow (*Salix* spp.), and alder (*Alnus* spp.) were the common riparian species (Pfister et al. 1977). Potential wolf prey in the study area included bighorn sheep (*Ovis canadensis*), mountain goat (*Oreamnos americanus*), elk (*Cervus canadensis*), white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), moose (*Alces alces*), beaver (*Castor canadensis*), snowshoe hare (*Lepus americanus*), mountain cottontail (*Sylvilagus nuttallii*), and small mammals. Other predators were mountain lion (*Puma concolor*), black bear (*Ursus americanus*), grizzly bear (*Ursus arctos*), lynx (*Lynx canadensis*), bobcat (*Lynx rufus*), coyote (*Canis latrans*), red fox, and wolverine (*Gulo gulo*).

Elevations ranged from 568 m to 2,663 m in the Cabinet Mountains Wilderness, the Bitterroot Range, Purcell Mountains, and Salish Mountains to the west, and the Whitefish Range to the east (Pfister et al. 1977). Three major rivers occurred in the study area: the Kootenai, the Clark Fork, and the North Fork Flathead. The climate was moderated by the Pacific Ocean, and characterized by warm dry summers and cool wet winters (Caprio and Nielson 1992). Land use included commercial timber harvest, mineral and energy development, federal grazing allotments, hunting, recreational fishing, and off-road vehicle use.

## METHODS

Wolves molt annually beginning in late spring (Mech 1974), with new growth continuing until late autumn (Young and Goldman 1944). Fully grown guard hairs, thus, contain individual summer diet records from the year of growth (Darimont and Reimchen 2002). Because hair samples reflect diet of individual wolves, we collected  $\geq 2$  hair samples/pack in order to estimate pack diet. We assumed that common prey (i.e., white-tailed deer, mule deer, elk, and moose) would comprise the majority of diet, and that approximately 5% of the diet could be composed of beaver, Colombian ground squirrel (*Spermophilus columbianus*), and snowshoe hare (Boyd et al. 1994, Kunkel et al. 1999, Arjo et al. 2002, Urton and Hobson 2005). Accordingly, we selected these 7 species as potential dietary sources in our SIA.

Wolves consume mostly muscle and internal organs of their prey; however, these samples are difficult to obtain for studies of wild wolves, and hair is typically used as the source of prey species isotope values (Darimont and Reimchen 2002, Urton and Hobson 2005). We collected 100 whole hairs/sample from harvested white-tailed deer ( $n = 31$ ), mule deer ( $n = 30$ ), elk ( $n = 25$ ), and moose ( $n = 9$ ) at 4 hunter check stations within the study area in November and December 2008. We collected hairs from beavers ( $n = 3$ ) trapped in damage control operations in September 2009. We collected 44 wolf hair samples from 12 packs ( $\bar{x} = 3.7$  samples/pack,

range = 2–8), May to August 2009. We collected guard hairs from individual day beds (i.e., circular substrate depressions  $\leq 1 \text{ m}^2$ ) at home sites (i.e., dens and rendezvous sites [ $n = 36$ ]) and kills ( $n = 3$ ), assuming shed hairs to be the previous year's growth and, therefore, representative of diet during summer 2008. Because beds may include hairs from multiple wolves (Stenglein et al. 2010), we only sampled from beds  $> 1 \text{ m}^2$  if we found sufficient hairs in a single clump for a complete sample (i.e.,  $\geq 30$  hairs, Darimont et al. 2007). We also collected hairs from wolves captured for population monitoring ( $n = 3$ ), and wolves killed on roads ( $n = 2$ ). We placed all samples in 118 mL Whirl-Pak<sup>®</sup> bags (Nasco, Fort Atkinson, WI) labeled with date, pack, and location.

We sonicated hair samples in glass vials of deionized water using a Branson Tabletop Ultrasonic Cleaner, Model 3510 (Branson Ultrasonic Corporation, Danbury, CT) to remove coarse debris from hairs, and dried samples for 24 hours. We rinsed samples under a ventilation hood in a 2:1 chloroform/methanol solution to remove fine debris and oils (Darimont et al. 2007). We ground dried hairs to powder in a Wig-L-Bug<sup>®</sup> DS-80 amalgamator (Crescent Dental Co., Chicago, IL). We placed 1 mg of ground hair into 5 mm  $\times$  7 mm pre-combusted tin cups, and sent samples in 96-well plates to the University of California, Davis, Stable Isotope Facility for continuous-flow mass spectrometry analysis.

Samples were analyzed for stable isotopes of carbon and nitrogen using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope mass spectrometer (Sercon Ltd., Cheshire, United Kingdom). During mass spectrometry, samples are combusted, resulting in separation of  $\text{CO}_2$  and  $\text{N}_2$ , which are then measured to calculate isotope ratios (Fry 2006). Isotope values are expressed in delta notation ( $\delta$ ) as

$$\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) 1000,$$

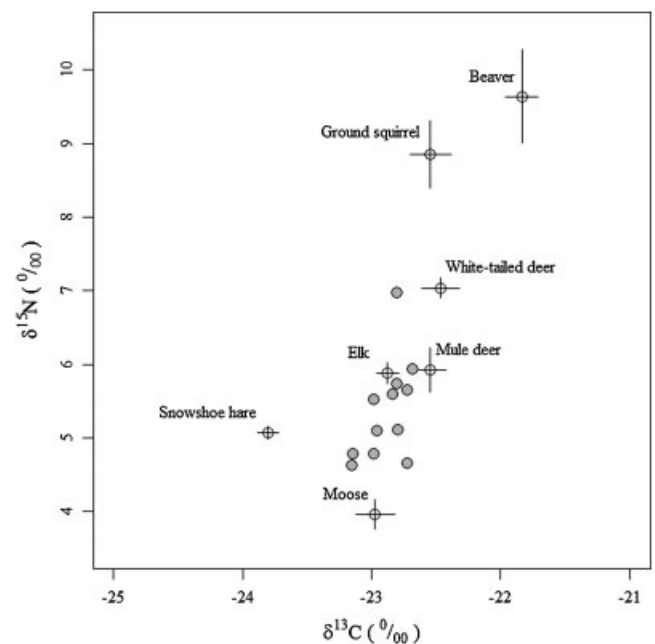
where  $X$  is  $^{13}\text{C}$  or  $^{15}\text{N}$ , and  $R$  is  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ . The standards used in SIA are PeeDee Belemnite limestone for carbon, and atmospheric  $\text{N}_2$  for nitrogen (DeNiro and Epstein 1978, 1981).

We calculated mean and standard deviation from individual  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of all prey species, and used non-parametric Mann–Whitney  $U$  tests to determine if species were isotopically distinct at the 0.05 significance level. We report means and standard deviations of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for wolves and diet sources from our samples, and literature values (Roth et al. 2007) for Columbian ground squirrel (ground squirrel) and snowshoe hare (Table 1). Beaver and ground squirrel could not be isotopically separated (Fig. 1), and because these species would share the same region of the stable isotope mixing space with other potential rodent and lagomorph diet sources (e.g., deer mouse [*Peromyscus maniculatus*], mountain cottontail; Roth et al. 2007), we removed ground squirrel, and used our beaver stable isotope values to represent 1 group of uncommon prey

**Table 1.** Means and standard deviations of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values estimated from hairs for wolves and diet sources in northwestern Montana, 2008 and 2009.

Species	$n$	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		$\bar{x}$	SD	$\bar{x}$	SD
Wolf	44	-22.91	0.32	5.29	0.67
White-tailed deer	31	-25.07	0.8	3.64	0.75
Mule deer	30	-25.15	0.67	2.52	1.63
Elk	25	-25.51	0.41	2.38	0.49
Moose	9	-25.57	0.44	0.56	0.61
Beaver	3	-24.44	0.22	6.24	1.09
Snowshoe hare <sup>a</sup>	207	-26.64	1.15	1.7	1.29
Ground squirrel <sup>a</sup>	16	-25.30	0.56	5.9	2.24

<sup>a</sup> Snowshoe hare and Columbian ground squirrel stable isotope values are from Roth et al. (2007).



**Figure 1.** The mixing space with mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  (+3.4‰ and +2.6‰, respectively for fractionation) of potential wolf prey ( $\pm$ SE), and mean values for 12 wolf packs (gray circles) in northwestern Montana, 2008. Snowshoe hare and Columbian ground squirrel values are from Roth et al. (2007).

(i.e., other). All remaining wolf diet sources were isotopically distinct for  $\geq 1$  isotope value (Table 2).

We used a hierarchical Bayesian stable isotope mixing model approach recently developed and tested on similar data to determine proportional contributions of prey to wolf diet and to estimate intra-population variation (Moore and Semmens 2008, Semmens et al. 2009). This technique can account for variability in prey species isotope values and trophic fractionation, and it can limit the uncertainty inherent in examining many potential diet sources. The Bayesian approach explicitly incorporates source isotope uncertainty by factoring in mean and variance parameters for each source and isotope (e.g., mean and variance of  $\delta^{15}\text{N}$  for elk), and variance in fractionation values.

In a Bayesian framework, the use of prior information (e.g., prior knowledge of wolf diet) can further help to resolve such

**Table 2.** Mann–Whitney *U* test scores for tests of difference between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of wolf diet sources from hairs collected in northwestern Montana, 2008 and 2009.

Prey		White-tailed deer	Mule deer	Elk	Moose	Beaver	Snowshoe hare <sup>a</sup>	Ground squirrel <sup>a</sup>
White-tailed deer ( <i>n</i> = 31)	$\delta^{13}\text{C}$		435	253*	86	24	1,082***	229
	$\delta^{15}\text{N}$		166***	64***	0***	0***	535***	75***
Mule deer ( <i>n</i> = 30)	$\delta^{13}\text{C}$	435		239*	82	13*	1,086***	238
	$\delta^{15}\text{N}$	166***		285	7***	6*	2,325***	62***
Elk ( <i>n</i> = 25)	$\delta^{13}\text{C}$	253*	239*		92	0**	1,251***	122*
	$\delta^{15}\text{N}$	64***	285		0***	0**	1,536**	27***
Moose ( <i>n</i> = 9)	$\delta^{13}\text{C}$	86	82	92		1*	486*	37*
	$\delta^{15}\text{N}$	0***	7***	0***		0*	376**	0***
Beaver ( <i>n</i> = 3)	$\delta^{13}\text{C}$	24	13*	0**	1*		29**	9
	$\delta^{15}\text{N}$	0***	6*	0**	0*		0**	18
Snowshoe hare ( <i>n</i> = 207)	$\delta^{13}\text{C}$	1,082***	1,086***	1,251***	486*	29**		574***
	$\delta^{15}\text{N}$	535***	2,325***	1,536**	376**	0**		156***
Ground squirrel ( <i>n</i> = 16)	$\delta^{13}\text{C}$	229	238	122*	37*	9	574***	
	$\delta^{15}\text{N}$	75***	62**	27***	0***	18	156***	

\*, \*\*, and \*\*\* indicate  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values are statistically different at the 0.05, 0.01, and 0.001 significance levels, respectively.

<sup>a</sup> Snowshoe hare and Columbian ground squirrel stable isotope values are from Roth et al. (2007).

uncertainties, and refine estimates. To calculate informative priors, we used MFWP population data (<http://fwp.mt.gov/hunting/planahunt/>) to estimate total available biomass of each large ungulate prey species within the 8 hunting districts potentially overlapped by the 12 sampled wolf packs (Sime et al. 2010). For each hunting district, we multiplied species population estimates by literature estimates of consumable biomass (i.e., kg/individual adult) for white-tailed deer, mule deer (Dusek et al. 1989), elk (Quimby and Johnson 1951), and moose (Schladweiler and Stevens 1973). We used a population estimation method from the literature for snowshoe hares (Murray et al. 2002), and consumable biomass/individual (Fuller and Keith 1980) to estimate their availability to wolves in our study area. Because snowshoe hare tend to comprise a small portion of wolf diet relative to availability (Messier and Crête 1985, Fuller 1989, Thurber and Peterson 1993, Arjo et al. 2002), we reduced the resulting estimate by a third to limit a potentially unrealistic influence in the model. We used literature estimates on density of lodges (i.e., lodges/km<sup>2</sup>), colony size, home range, and consumable biomass (Fuller and Keith 1980), to calculate beaver availability. We multiplied lodge density by 10,000 (i.e., the approximate km<sup>2</sup> size of our study area) to estimate the number of lodges in our study area, and multiplied this number by the colony size estimate, and the result by estimated consumable biomass. Because we also used beaver as a SIA proxy for isotopically similar prey species, we did not adjust this estimate to reflect any discrepancy between availability and use.

The total estimated consumable biomass for beaver and the adjusted estimate for snowshoe hare were split evenly among the 8 hunting districts. For each hunting district, we calculated percent consumable biomass of the 6 prey categories, and used these data to calculate a Dirichlet prior distribution of alpha values (i.e., a single value for each prey category) for models with informative priors in the program R (R version 2.13.0, [www.r-project.org](http://www.r-project.org), accessed 24 Jun 2011). We used +2.6‰ and +3.4‰ as our diet-hair trophic fractionation values of  $\delta^{13}\text{C}$  and

$\delta^{15}\text{N}$ , respectively (Roth and Hobson 2000), and variance values (J. D. Roth, University of Manitoba, personal communication) from the same experimental feeding study on isotopic fractionation in red foxes.

We estimated 8 hierarchically structured models to determine the scale at which most wolf diet variability occurred within our study area using R code adapted from Semmens et al. (2009). To explore the sensitivity of the choice of prior, we estimated all models with informative and non-informative priors. We estimated all models with and without residual error terms to incorporate variability in individual isotope values unrelated to diet: 2 models assumed a single invariant diet for all wolves and incorporated random effects at the group level (i.e., packs had a shared global mean diet, and varied around that), 2 models assumed a shared mean diet for all packs and incorporated random effects at the individual level (i.e., diet was allowed to vary among individuals but not packs), 2 models allowed diet to vary among packs but not individuals, and 2 models allowed variation among packs and individuals. We evaluated data support for each model using the Deviance Information Criterion (DIC).

Scats produced by wolves during summer 2008 contained undigested remains of the same prey whose digested flesh contributed to tissue growth (i.e., including guard hairs used for SIA) of wolves in that period. We collected 204 scats at home sites (*n* = 114) and opportunistically from roads (*n* = 90) within the home ranges of 4 of the SIA packs between June and August 2008 to check our SIA results with a separate diet analysis. We only collected scats  $\geq 32$  mm in diameter to minimize the probability of collecting coyote scats (Weaver and Fritts 1979, Arjo et al. 2002, Reed et al. 2006). Assuming coyotes would be unlikely to venture into den areas or rendezvous sites, we collected all adult canid scats <250 m from the center of home sites. We assumed all scats <15 mm diameter to be pup scats and did not collect them. We placed individual scats in brown paper bags labeled with date, pack, and location.

We sterilized scats in a 533LS Getinge/Castle steam sterilizer (Getinge/Castle, Rochester, NY), hand-separated them, and used macro and microscopic characteristics of hair and bone to identify contents to species (Putman 1984, Leopold and Krausman 1986, Spaulding et al. 1997). We recorded frequency of occurrence (FO) of each prey species in scats for each pack, and calculated biomass consumed of each species/scat using the regression equation

$$y = 0.439 + 0.008x,$$

where  $y$  was the mass (kg) of prey consumed/scat and  $x$  was the mean adult mass of the prey species (Floyd et al. 1978, Weaver 1993). We generated 5,000 bootstrapped samples to estimate mean and variance of prey biomass consumed by each pack using FO weighted by biomass from the regression equation using R. We tested for differences in diet among packs using Pearson's chi-squared tests of proportions on FO counts weighted by total FO (i.e., all prey occurrences for a given pack) and mean proportion of prey biomass consumed from bootstrapped samples (SPSS, Inc., Chicago, IL).

To check our SIA results with scat data, we used R to estimate confidence intervals (CIs) of difference between the CIs estimated in the Markov Chain Monte Carlo simulations from the best Bayesian model and CIs from bootstrapped scat data for the 4 packs with matched samples. Because white-tailed deer and mule deer could not be distinguished using remains in scats, we used combined CIs weighted according to SIA results for this comparison. We also used weighted CIs combining beaver and snowshoe hare data for comparison with the "other" prey CIs from scats. We determined the level of similarity between techniques by identifying whether or not CIs of difference contained 0, and report statistical significance at the 0.05, 0.01, and 0.001 levels for 32 comparisons (i.e., 4 for each pack's scat data compared to SIA data from Bayesian models with informative and non-informative priors).

We ranked the 4 most common prey groups in diet for the 4 matched samples packs from SIA results of the best model with informative and non-informative priors (i.e., using the combined deer, and combined beaver and snowshoe hare groups), and from scat analysis, giving scores of 4 to 1 in descending order. We summed ranks for each prey group and analysis method across the 4 packs, and divided each prey group total by the total number of ranks to determine a rank sum percent and facilitate a graphical comparison of rank order of contribution of prey to diet.

Because we collected all our noninvasively sampled hairs at wolf home sites, and because we followed literature protocols to ensure against collecting coyote scats, we were confident that our samples came from wolves. As an additional check, we used DNA analysis to verify this assumption. We randomly selected 5 SIA packs and randomly sampled a hair sample from each of those. We randomly sampled 3 scats from the Candy Mountain pack and 2 each from the other 3 scat packs. We sent hairs and scats to the University of California, Los Angeles, where the samples were analyzed

for species verification using primers designed to only amplify canid specific mitochondrial DNA (Munoz-Fuentes et al. 2009).

## RESULTS

Mean isotope values for wolf packs were centered on the ungulate prey species in the mixing space (Fig. 1). From models with informative priors, moose was the most common diet item for 6 packs, and white-tailed deer and elk were the most common prey item for 3 packs each. These 3 prey were variously the second and third most common diet items for 10 and 12 packs, respectively, with snowshoe hare being the second most common for the other 2 packs. From models with non-informative priors, moose was the most common diet item for 11 packs, with beaver the most common prey for 1 pack (i.e., Ksanka). Elk was the second most common prey for all packs, and moose, beaver, and snowshoe hare were variously the third most common diet item (Table 3).

The model that included pack variation alone with no residual error term was the only model that received strong support (i.e., for models including informative and non-informative priors). We found weak support for the model that included pack variation with the residual error term (i.e., differences in DIC scores of 9.3 and 8.8 between this and the top model with informative and non-informative priors, respectively). No other models were supported, and for both sets of priors, 3 of the 4 models that included individual variation did not converge (Table 4).

From scats, ungulate prey comprised 96% of biomass consumed by wolves. Deer (42%) contributed the largest proportion to wolf diet, followed by elk (36%), and moose (18%). The remaining 4% of biomass consumed consisted of ground squirrel and unidentified mammals (Table 5). Deer was the most common prey item for the Candy Mountain and Pulpit Mountain packs, elk the most common for Bearfite, and moose the most common for Twilight (Fig. 2).

Pack diets differed based on scat analysis. Bearfite was different from Candy Mountain ( $\chi^2 = 21.142$ ,  $P < 0.001$ ) and Pulpit Mountain ( $\chi^2 = 18.61$ ,  $P < 0.001$ ), but not Twilight ( $\chi^2 = 1.233$ ,  $P = 0.54$ ). Candy Mountain was different from Twilight ( $\chi^2 = 30.685$ ,  $P < 0.001$ ), but not Pulpit Mountain ( $\chi^2 = 4.073$ ,  $P = 0.13$ ). Pulpit Mountain was different from Bearfite ( $\chi^2 = 18.61$ ,  $P < 0.001$ ) and Twilight ( $\chi^2 = 22.801$ ,  $P < 0.001$ ).

Confidence intervals of difference from SIA and scat data revealed no differences in comparisons of moose consumption by the Bearfite and Twilight packs with the informative priors model. We did not find differences between SIA and scat estimates in all comparisons of the "other" prey group, and only 1 difference in comparisons of elk consumed (i.e., Bearfite pack, informative priors model). All except 1 comparison of deer consumed (i.e., Twilight) were different using the informative priors model. All comparisons of moose and deer consumed were different using the non-informative priors model (Table 6).

Additional comparisons detected differences among consumption estimates for the 2 techniques. In our non-

**Table 3.** Posterior median estimates and 95% confidence intervals of summer 2008 diet proportions for 12 northwestern Montana wolf packs, from Bayesian stable isotope mixing models using informative (I) and non-informative (N) priors.

Pack	Median proportion of diet (95% CI) from diet source					
	White-tailed deer	Mule deer	Elk	Moose	Beaver	Snowshoe hare
Fishtrap						
I	0.26 (0.02, 0.56)	0.05 (0.00, 0.34)	0.31 (0.01, 0.86)	0.19 (0.00, 0.47)	0.00 (0.00, 0.11)	0.07 (0.00, 0.33)
N	0.06 (0.00, 0.33)	0.05 (0.00, 0.25)	0.29 (0.04, 0.74)	0.33 (0.09, 0.57)	0.11 (0.01, 0.24)	0.06 (0.00, 0.25)
Bearfite						
I	0.10 (0.01, 0.26)	0.03 (0.00, 0.18)	0.09 (0.00, 0.37)	0.57 (0.31, 0.78)	0.00 (0.00, 0.05)	0.14 (0.00, 0.40)
N	0.03 (0.00, 0.15)	0.03 (0.00, 0.14)	0.14 (0.02, 0.38)	0.63 (0.40, 0.80)	0.04 (0.00, 0.10)	0.07 (0.00, 0.31)
Thirsty						
I	0.12 (0.01, 0.30)	0.03 (0.00, 0.25)	0.09 (0.00, 0.42)	0.59 (0.26, 0.81)	0.00 (0.00, 0.05)	0.07 (0.00, 0.38)
N	0.04 (0.00, 0.17)	0.03 (0.00, 0.18)	0.15 (0.02, 0.42)	0.62 (0.39, 0.81)	0.04 (0.01, 0.12)	0.05 (0.00, 0.26)
Candy Mt.						
I	0.16 (0.01, 0.42)	0.04 (0.00, 0.37)	0.16 (0.01, 0.70)	0.41 (0.03, 0.72)	0.00 (0.00, 0.07)	0.08 (0.00, 0.45)
N	0.05 (0.00, 0.23)	0.04 (0.00, 0.23)	0.22 (0.03, 0.60)	0.50 (0.20, 0.74)	0.06 (0.01, 0.17)	0.06 (0.00, 0.28)
Pulpit Mt.						
I	0.18 (0.02, 0.41)	0.04 (0.00, 0.23)	0.37 (0.03, 0.79)	0.26 (0.03, 0.48)	0.00 (0.00, 0.08)	0.09 (0.00, 0.27)
N	0.06 (0.00, 0.26)	0.04 (0.00, 0.20)	0.33 (0.05, 0.68)	0.36 (0.15, 0.57)	0.08 (0.01, 0.19)	0.07 (0.00, 0.23)
Twilight						
I	0.12 (0.01, 0.29)	0.04 (0.00, 0.27)	0.09 (0.00, 0.39)	0.62 (0.38, 0.83)	0.00 (0.00, 0.05)	0.04 (0.00, 0.23)
N	0.04 (0.00, 0.17)	0.03 (0.00, 0.19)	0.15 (0.02, 0.39)	0.64 (0.44, 0.82)	0.04 (0.01, 0.12)	0.04 (0.00, 0.18)
Kootenai S.						
I	0.28 (0.04, 0.53)	0.06 (0.00, 0.26)	0.37 (0.04, 0.78)	0.16 (0.01, 0.39)	0.00 (0.00, 0.17)	0.05 (0.00, 0.20)
N	0.08 (0.00, 0.33)	0.05 (0.00, 0.21)	0.30 (0.05, 0.63)	0.31 (0.11, 0.50)	0.16 (0.02, 0.27)	0.06 (0.00, 0.18)
Ksanka						
I	0.66 (0.08, 0.95)	0.03 (0.00, 0.47)	0.08 (0.00, 0.45)	0.05 (0.00, 0.22)	0.00 (0.00, 0.29)	0.05 (0.00, 0.29)
N	0.07 (0.00, 0.55)	0.04 (0.00, 0.36)	0.21 (0.03, 0.56)	0.20 (0.04, 0.42)	0.29 (0.03, 0.44)	0.07 (0.00, 0.26)
Lazy Ck.						
I	0.08 (0.01, 0.24)	0.03 (0.00, 0.32)	0.05 (0.00, 0.27)	0.64 (0.07, 0.90)	0.00 (0.00, 0.04)	0.10 (0.00, 0.64)
N	0.03 (0.00, 0.14)	0.02 (0.00, 0.18)	0.10 (0.01, 0.29)	0.70 (0.36, 0.88)	0.04 (0.00, 0.10)	0.05 (0.00, 0.37)
Lydia						
I	0.17 (0.01, 0.44)	0.05 (0.00, 0.40)	0.16 (0.01, 0.71)	0.41 (0.03, 0.71)	0.00 (0.00, 0.07)	0.07 (0.00, 0.43)
N	0.05 (0.00, 0.24)	0.04 (0.00, 0.25)	0.21 (0.03, 0.60)	0.50 (0.20, 0.74)	0.06 (0.01, 0.18)	0.06 (0.00, 0.26)
Murphy Lk.						
I	0.25 (0.01, 0.56)	0.05 (0.00, 0.42)	0.22 (0.01, 0.84)	0.22 (0.01, 0.51)	0.00 (0.00, 0.09)	0.08 (0.00, 0.42)
N	0.05 (0.00, 0.32)	0.04 (0.00, 0.29)	0.26 (0.03, 0.77)	0.36 (0.08, 0.62)	0.09 (0.01, 0.23)	0.07 (0.00, 0.30)
Kintla						
I	0.26 (0.02, 0.06)	0.05 (0.00, 0.40)	0.25 (0.01, 0.84)	0.22 (0.01, 0.52)	0.00 (0.00, 0.11)	0.07 (0.00, 0.37)
N	0.06 (0.00, 0.32)	0.05 (0.00, 0.27)	0.27 (0.03, 0.72)	0.36 (0.10, 0.62)	0.10 (0.01, 0.25)	0.06 (0.00, 0.25)

parametric tests, moose was ranked first by SIA from models with both types of priors, deer and elk were second from models with informative and non-informative priors, respectively. Other prey and deer were the least

common prey with informative and non-informative priors, respectively. Deer and elk ranked as the most common prey items from scats, followed by moose and other prey items (Fig. 3).

**Table 4.** Summary of 8 stable isotope mixing models explaining summer diet variation among 44 wolves and 12 packs in northwestern Montana, 2008. Models could include variation among packs, individuals, or residual error, as indicated by Y or N (i.e., yes, the model included this source of variation, or no, the model did not), and are ranked according to data support.

Rank	Informative priors <sup>a</sup>				Non-informative priors <sup>b</sup>			
	Pack	Individual	Residual	DIC <sup>c</sup>	Pack	Individual	Residual	DIC
1	Y	N	N	93.8	Y	N	N	76.8
2	Y	N	Y	103.1	Y	N	Y	85.6
3	N	N	Y	136.8	N	N	N	120.7
4	N	Y	N	147.0	N	N	Y	121.4
5	N	N	N	151.1	N	Y	N	141.5
6	Y	Y	N	NA <sup>d</sup>	Y	Y	N	NA
7	Y	Y	Y	NA	Y	Y	Y	NA
8	N	Y	Y	NA	N	Y	Y	NA

<sup>a</sup> We calculated prior information on summer wolf diet by estimating available biomass from Montana Fish, Wildlife and Parks population data for 8 hunting districts in northwestern Montana (<http://fwp.mt.gov/hunting/planahunt/>) overlapped by the estimated home ranges of sampled wolf packs (Sime et al. 2010), and available biomass of beaver and snowshoe hare from the literature (Fuller and Keith 1980, Murray et al. 2002).

<sup>b</sup> Models with non-informative prior information assumed all diet source contributions were equally likely.

<sup>c</sup> The Deviance Information Criterion is used to evaluate data support. Smaller values indicate greater support for a model.

<sup>d</sup> NA = models did not converge.

**Table 5.** Diet estimated from scats of 4 northwestern Montana wolf packs between June and August 2008.

Prey	Mass (kg)	kg/scat <sup>a</sup>	FO <sup>b</sup>	Weighted FO <sup>c</sup>	% biomass <sup>d</sup>
Deer	60 <sup>e</sup>	0.92	136	96.17	0.42
Elk	260 <sup>f</sup>	2.52	47	81.72	0.36
Moose	318 <sup>g</sup>	2.98	22	40.26	0.18
Other	14 <sup>h</sup>	0.55	22	8.85	0.04
Total			227	227.00	1.00

<sup>a</sup> We calculated biomass consumed/scat from regression equations (Floyd et al. 1978, Weaver 1993).

<sup>b</sup> Frequency of occurrence of prey items from all scats.

<sup>c</sup> We calculated mean proportions of biomass consumed by each pack with bootstrapped data from FO of each prey item weighted by values from the regression equation. Weighted FO for each pack was the product of bootstrapped mean values for each species and total FO of all species. This column represents weighted FO totals for each species across all packs.

<sup>d</sup> Percent biomass consumed of each prey item calculated as weighted FO/total FO.

<sup>e</sup> Assumed from Dusek et al. (1989).

<sup>f</sup> From Quimby and Johnson (1951).

<sup>g</sup> From Schladweiler and Stevens (1973).

<sup>h</sup> From Fuller and Keith (1980).

From our DNA tests, all 5 hair samples were identified as wolf. Of the 9 scats tested, only 3 amplified, each of which was identified as wolf.

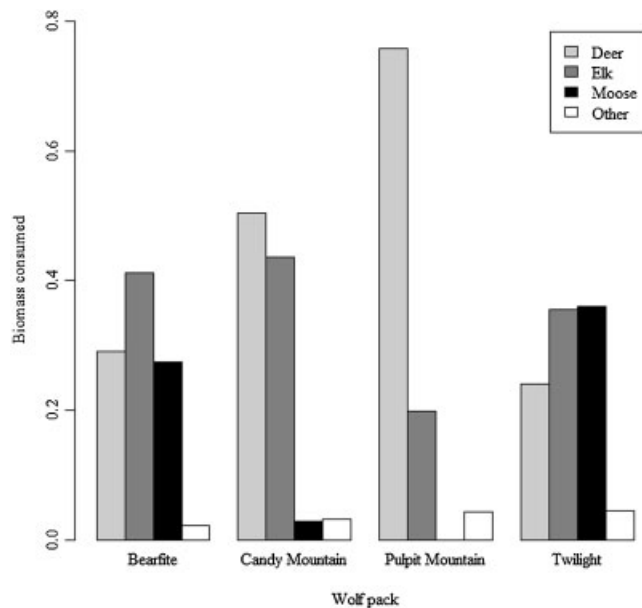
## DISCUSSION

Our results comprise a mix of methodological validation and inconsistency, and include the first indication that moose is a large component of wolf diet in northwestern Montana. The mean isotope values for 12 wolf packs were centered on the ungulate prey in the mixing space (Fig. 1), and differences in summer diet among packs explained most of the variation when models were estimated with informative or non-informative priors (Table 4). In our check using scat data from 4 of these packs, ungulates comprised a similarly

large proportion of summer wolf diet, and chi-squared tests revealed differences among pack diets. In the direct comparisons between informative priors SIA and scat results, 6 of 16 were significantly different, but of those that matched (i.e., were not significantly different), 2 were moose comparisons (Table 6). The lack of concordance between methods necessitates cautious interpretation, but the surprising prominence of moose in wolf diet suggests that management of this species would benefit from increased monitoring.

Our assumption that white-tailed deer would comprise the largest proportion of wolf diet was based on their numerical dominance among the cervids in the study area, the fact that wolves commonly consume these prey, and the absence of any existing information on prey preference. Reliable population estimates for white-tailed deer, mule deer, and elk are generated each year by MFWP from hunter harvest and survey data, but relatively little about the moose population can be determined because so few are harvested (e.g., in the 3 hunting seasons from 2008 to 2010, 160 moose were harvested from 6 of our study area's 8 hunting districts [i.e., those for which data were available]; <http://fwp.mt.gov/hunting/planahunt/harvestReports.html>). Without more intensive monitoring, we cannot know whether moose population estimates are low or high, but our results suggest summer wolf diet includes more moose and less deer than would be expected from availability of ungulate prey biomass.

Underlying any conclusions that can be drawn from this study is the fact that neither SIA nor scat data may accurately represent the sampled packs' diets. Sampling biases beyond those inherited from MFWP population estimates likely combined with intrinsic analysis biases to place our estimates at unknowable points along the reality continuum, but we can assess the relative impact of each bias to discern what wolves in northwestern Montana are likely consuming, and what could have improved this and future studies. Several factors related to data collection could have resulted in mismatched stable isotope and scat data. First, in reporting summer biomass consumed by wolves, we assumed that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of prey hairs taken from harvested animals in November and December would be similar to those of prey consumed by wolves during the summer of that year, and that



**Figure 2.** Percent biomass consumed of each diet source estimated from scats of 4 wolf packs in northwestern Montana between June and August 2008. We weighted proportions by scat sample size for each pack. We used frequency of occurrence of species weighted by biomass consumed/scat for each pack and 5,000 bootstrapped samples to estimate means and variance. We used mean adult mass of identified species from the literature, and used beaver as the representative diet source for the "Other" category.

**Table 6.** Ninety-five percent confidence intervals of difference between estimates of diet source contributions from stable isotope analysis (SIA) and bootstrapped scat data. We estimated stable isotope mixing models using informative (I) and non-informative (N) priors. We collected matched wolf scat and hair samples in 2008 and 2009, respectively, from 4 packs in northwestern Montana.

Pack	95% CIs of difference between SIA and scat analysis estimates			
	Deer <sup>a</sup>	Elk	Moose	Other <sup>b</sup>
Bearfite				
I	0.014, 0.358*	0.006, 0.502*	-0.554, 0.007	-0.375, 0.035
N	0.114, 0.390**	-0.005, 0.472	-0.585, -0.086**	-0.244, 0.029
Candy Mt.				
I	0.100, 0.615*	-0.341, 0.437	-0.695, -0.002*	-0.371, 0.063
N	0.281, 0.646**	-0.242, 0.405	-0.712, -0.169**	-0.182, 0.055
Pulpit Mt.				
I	0.285, 0.890***	-0.638, 0.232	-0.484, -0.029***	-0.222, 0.088
N	0.415, 0.940***	-0.552, 0.245	-0.569, -0.152***	-0.177, 0.076
Twilight				
I	-0.061, 0.296	-0.063, 0.437	-0.518, 0.024	-0.170, 0.068
N	0.043, 0.319*	-0.071, 0.410	-0.515, -0.032*	-0.111, 0.059

\*, \*\*, and \*\*\* indicate statistical differences between diet estimates from SIA and scat data at the 0.05, 0.01, and 0.001 significance levels, respectively.

<sup>a</sup> We combined white-tailed deer and mule deer and used CIs weighted according to SIA results for comparison with the “deer” CIs from scats.

<sup>b</sup> We used weighted CIs combining beaver and snowshoe hare data according to SIA results for comparison with the “other” prey CIs from scats.

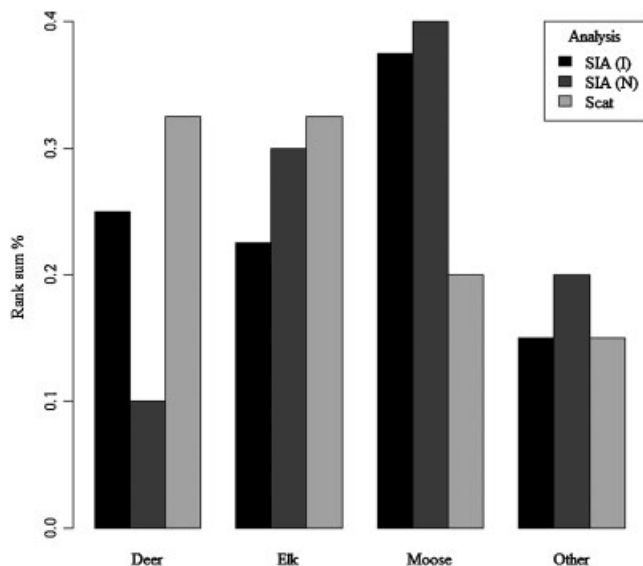
hair values would not differ from those of muscle and internal organs (i.e., the tissues wolves consume). Consistent differences between hair and actual tissues consumed among prey species would likely distort results and cause a mismatch if scat data were accurate. However, a recent study on wolf diet in British Columbia that reported  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from summer hair and winter muscle values of elk and moose suggests such differences are unlikely in these species. Moose  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and elk  $\delta^{13}\text{C}$  were within 1 standard deviation (i.e., from our data for these species) of each other, and the difference between elk hair and muscle  $\delta^{15}\text{N}$  was well inside the range of our values for hair (Milakovic and Parker 2011). Because white-tailed deer was a diet item that may have been underestimated by the SIA in our study, determining if they share this similarity in values between tissues

would be instructive, but the evidence suggests this is not an influential source of error.

Our assumption of time period represented by wolf hairs may also be questionable. We assumed that guard hairs of wolves grow only during the middle 6 months of the year, and therefore, represent diet for that period. We cited a commonly used, but aging anecdotal reference (i.e., Young and Goldman 1944), but recent reports suggest wolves may continue adding guard hairs to their pelage throughout the winter months (K. Loveless, Trent University, unpublished data). Any inadvertently sampled winter-grown hairs would have limited our ability to determine summer diet, and would have rendered summer scats meaningless as checks. Such data collection errors seem unlikely to have played a part in our study, however, because even where wolves are known to rely on moose, they consume proportionately less moose in winter when alternative prey are available (Fritts and Mech 1981, Potvin and Jolicoeur 1988, Milakovic and Parker 2011), suggesting that  $\delta^{15}\text{N}$  of any winter-grown hairs would have placed wolves closer to elk and deer than moose. Until experimental work is conducted on hair growth, we cannot speculate further on this bias.

Another potential cause of discordance between datasets could have affected both methods. Because the majority of wolf hair samples (i.e., 82%) and scat samples (i.e., 56%) were collected at home sites, the breeding female in each pack had a greater than average chance of being represented by both datasets compared to other pack members not as closely tied to home sites (Ballard et al. 1991, Mech 1999). Similarly, the hair samples used for SIA may not have contained pack diet information if the individual wolves from which they came primarily hunted alone or paired with another pack member.

Any of the other well described sources of bias from inadequate sample sizes and data interpretation could have affected our scat analysis (Floyd et al. 1978, Reynolds and Aebischer 1991, Trites and Joy 2005). We only collected scats between June and August 2008, and the relatively modest sample sizes collected in small proportions of each home range may not have been representative. Regardless of



**Figure 3.** Non-parametric rankings, according to analysis method (i.e., stable isotope analysis [SIA] with informative priors (I) and non-informative priors (N), and scat analysis) of prey consumed by wolves from matched SIA and scat samples of 4 packs in northwestern Montana, summer 2008. Bars represent relative position out of 4, not proportions of prey consumed.



scat sample size, scats collected during this period may not represent diet during May, September, and October (i.e., the other months of diet record derived from SIA of hairs) because wolves may vary their prey use throughout the 6-month diet period that hairs were assumed to represent (Fritts and Mech 1981, Fuller 1989). In future studies that use scats collected in a continuous proportion of the assumed hair growth period, researchers could consider using SIA of the section of hair grown during that period; a technique that has been successfully tested (Darimont and Reimchen 2002).

Our primary interest was to determine wolf diet with SIA, and several biases intrinsic to this method may have contributed to the surprisingly low deer and high moose content reported from these data. One explanation is that we used fractionation values that were not appropriate for our study. We used the same fractionation values derived from experimental feeding trials on red foxes that have been used in 5 other wolf diet studies, none of which commented on potential problems with these values (Urton and Hobson 2005, Darimont et al. 2009, Semmens et al. 2009, Adams et al. 2010, Milakovic and Parker 2011), but assuming no other errors, red fox values might work for some studies and not others.

Depending on the prime focus of these studies, they can be divided into groups of differing need for precise fractionation values. Three of these studies were primarily aimed at determining the relative contribution of salmon (*Oncorhynchus* spp.) to wolf diet compared to that of ungulate prey (Darimont et al. 2009, Semmens et al. 2009, Adams et al. 2010). For such a question, as long as the fractionation values place potential wolf prey in approximately the same area of the mixing space as wolves, any real significant use of salmon likely would be detected because their isotope values are so distinct from those of ungulates. In studies that seek to determine the relative consumption of wild ungulate prey, the need for precision is much greater because no groups of interest are likely to occupy distant areas of the mixing space (e.g., Fig. 1). In such cases, including separate diet measures (e.g., scats) as checks can be especially useful, but because truth is still unknown, they may be more useful for indicating problems than confirming accuracy (i.e., uncovering a mismatch is useful, but an apparent match may provide false confidence). The other 2 cited studies and our own used scats either as guides for selecting potential prey for SIA (Urton and Hobson 2005), or as checks on SIA results (i.e., this study, and Milakovic and Parker [2011]). Estimates of elk, moose, and Stone's sheep (*Ovis dalli*) from SIA and scats were generally concordant in Milakovic and Parker (2011), but from 1 summer of data, caribou (*Rangifer tarandus*) contributed 28% of diet across packs according to scats, but the greatest pack mean for caribou from the SIA for the same period was 6%. This and our results illustrate the problem of knowing truth, and the potential value of using species-specific fractionation values in mixing models.

The suitability of red fox fractionation values is possibly a matter of coincidence, especially if fractionation in wolves varies regionally. In a study on wolves of Isle Royale,

Minnesota, where controlled feeding conditions were approximated (i.e., because moose were the only available ungulate prey),  $\delta^{15}\text{N}$  fractionation of 4.6‰ was estimated from SIA of wolf collagen. The authors noted that this value was greater than those estimated for other carnivores, and greater than that estimated for the more omnivorous red foxes in Roth and Hobson (2000), and they suggested the difference was due to the high protein diet of wild wolves (Fox-Dobbs et al. 2007). Substitution of this for the red fox  $\delta^{15}\text{N}$  fractionation of 3.4‰ could have substantial effects on the results of any study where >1 potential prey species are placed in relative proximity within the isotope mixing space. Interestingly, the relative effect of the level of diet protein on  $\delta^{15}\text{N}$  fractionation is subject to disagreement in the literature, with other authors suggesting high protein likely leads to lower  $\delta^{15}\text{N}$  fractionation (Robbins et al. 2010). In any case, the commercial feed used for red foxes in Roth and Hobson (2000) was relatively low in protein compared to wolf diet, and true wolf  $\delta^{15}\text{N}$  fractionation likely differs.

The plausibility of results from other studies does not assure their accurate reflection of reality, and choice of fractionation values has been well-documented as a critical one for SIA (Ben-David and Schell 2001, Phillips and Gregg 2001). As costs of SIA decline, and statistical methods for incorporating uncertainty in stable isotope data become more reliable, the use of this technique will likely increase, but lower cost and improved statistics will only lead to more studies with inaccurate results if the basic ingredients of any SIA are poorly chosen. A comprehensive review of the SIA literature recently concluded that as well as being taxon-, and tissue-specific, fractionation values are dependent on diet isotope ratios (i.e., a negative relationship exists between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of diet items and diet-tissue fractionation values), and that because most studies use single non-taxon-specific values for each isotope, regardless of differences in diet items, many may have reported meaningless results (Caut et al. 2009).

Even with precise fractionation values, if ungulate prey are isotopically indistinguishable, determining relative proportions of each contribution may be impossible, and researchers may have to be content simply with confirming that wolves consume ungulates, but not some other prey group that occupies a remote corner of the mixing space. For example, in our analysis, we used beaver as an isotope label for a group of smaller prey not expected to be major components of wolf diet. Results from the non-informative priors model ranked beaver as the primary prey source for the Ksanka pack (Table 3), but if true, such a result would really mean 28% of this pack's diet consisted of beaver and any other potential prey in this area of the mixing space. As the results from the non-informative model are generally less plausible than those from the informative model, we do not consider this result to be meaningful, but the decision to include this combined group is the kind of choice that can have an impact on the overall results for primary prey items because mixing models apportion prey contributions summing to unity among the total specified sources. This quirk of mixing models means even prey that, in reality, contributed nothing

to a consumer's diet will be present as a proportion of diet in the results, and the inclusion of a rare species may lead to underestimation of a common one (Ben-David et al. 1997). Although we found no evidence in scats, we included snowshoe hare as a potential diet source in our SIA as it was common in our study area, and wolves are known to consume them. However, the range of median snowshoe hare proportions in diet was 4–14% across the 12 packs from the model with informative priors, which easily exceeds scat analysis estimates from other studies (Messier and Crête 1985, Fuller 1989, Thurber and Peterson 1993, Arjo et al. 2002). One solution for overestimation of a relatively insignificant diet source in SIA is to exclude it from the mixture. This approach is valid if any real contribution from the eliminated source can be absorbed by a neighboring diet source in the mixing space without contributing to another overestimation. In our analysis, elimination of snowshoe hare would likely have led to greater exaggeration of the moose contribution. As no other likely sources of wolf diet occur in this lower left region of the mixing space (Fig. 1), incorrect fractionation, at least for  $\delta^{15}\text{N}$ , seems a more plausible explanation for moose overestimation in our analysis. In our case, lower  $\delta^{15}\text{N}$  fractionation, would have placed moose further away and white-tailed deer closer to wolves in the mixing space.

An additional factor that may have contributed to low deer and high moose estimates was our choice of prior information in the Bayesian mixing models. The only consistently reliable statement about wolf prey preferences supported by the literature is that wolves may kill whatever vulnerable prey they encounter (Mech and Peterson 2003). Wildlife managers in northwestern Montana implicitly support this assumption in their belief that white-tailed deer are the primary prey of wolves (J. S. Williams, personal communication). With no evidence to the contrary for our study area, we assumed wolf diet could follow availability of biomass, and used MFWP ungulate population data in our estimation of the Dirichlet prior distribution of alpha values used in the mixing models. We extended this assumption in our calculation of the beaver and hare components of this distribution, which we made using population estimates of those taxa from the literature and some appropriate adjustments. Because calculating the prior this way included a total available biomass estimate of >12% for snowshoe hare, this component of our prior distribution may have affected the results by biasing estimates towards that area of the mixing space (i.e., closer to snowshoe hare and moose, and further away from white-tailed deer). However, our priors more likely had the effect of keeping our results more in line with reality because a comparison of results from informative and non-informative priors models revealed high sensitivity to the choice of priors. Because non-informative priors, in fact, inform the model that all included prey sources have an equal chance of being consumed, they may compound the effect of other inaccurate model inputs (e.g., fractionation values). Regardless of priors, wolf values were clustered between elk and moose on the  $\delta^{15}\text{N}$  axis of the mixing space, and with no other potential prey immediately near it, moose was

likely coerced by the mixing model to comprise an inflated component of diet.

Any of these factors could have distorted our results, and it is difficult to assess, without independent testing, which of them was the most influential factor in our study. Unexplained factors could also have affected our analysis. Three of our primary prey species (i.e., the deer and elk) may have been too closely spaced in the isotope mixing space to be considered as distinct dietary end points by the mixing model. In this and other similar cases, we echo the caution of earlier researchers in this field that results from SIA should be considered as indices of prey use rather than accurate diet estimates (Ben-David and Schell 2001).

One factor that could be tested in future is the appropriateness of red fox fractionation values for wolf diet studies. Species-specific fractionation values are rarely available for wildlife studies because few experimental studies have been conducted to derive them. In some cases non-specific values may be adequate (e.g., when SIA is used to distinguish 2 general prey groups centered in distinct areas of the mixing space), but when managers are interested in examining a consumer's use of a particular prey species, the precision of SIA would likely be improved if the consumer's actual fractionation values are different enough from previously used non-specific values. In our study area, precise SIA results could provide more useful information to managers on the level and variation in use of large ungulate prey by wolves within their regions. In northwestern Montana, where the moose is a relatively rare but popular game species, managers could use a combination of accurate data from SIA of wolf diet and monitoring data on moose to inform decisions on adjusting moose and wolf harvest quotas to maintain populations.

Despite the differences in results from each technique, the conclusion that summer wolf diet varied among packs was supported. Summer diet of wolves has previously been examined at the regional, pack, and individual levels, and combinations of these scales. Most studies examined wolf diet through scat analysis in relatively homogeneous landscapes (Fritts and Mech 1981, Peterson et al. 1984, Huggard 1993). More recent studies have used stable isotopes to examine diet variation across heterogeneous landscapes where prey availability may vary seasonally (e.g., where wolves have differential access to spawning Pacific salmon; Darimont and Reimchen 2002, Darimont et al. 2009, Adams et al. 2010). We examined wolf diet using both techniques in a relatively homogeneous landscape where prey availability is relatively constant (i.e., the same prey are available to wolves throughout the year), and our results emphasize the pack as a unit of interest for wolf diet, and the importance of considering social structure of wolves in management decisions (Hebblewhite and Merrill 2008).

Few summer diet studies have been conducted in ecosystems with a diversity of potential prey similar to our study area. In Banff National Park, Alberta, Canada, where 6 wild ungulate species were available to wolves, 2 wolf pack diets were comprised of  $\geq 70\%$  elk (Huggard 1993). One study in the eastern portion of our study area, reported winter diet

from kills to vary between packs with different amounts of deer and elk being consumed (Kunkel et al. 2004), and the only summer diet study in our area did not report pack diets (Arjo et al. 2002). We focused on an area of northwestern Montana where we assumed deer comprised most of the biomass available to wolves, but elk and moose were also present and expected to comprise some proportion of wolf diet. Assuming estimates from the informative priors model were closer to reality, our results confirmed that deer, elk and moose comprise the bulk of diet, but also suggested that information on what wolves eat could be strongly affected by which packs and how many of them are selected for study, and which diet analysis technique is used.

When the prey of interest can be separated in the mixing space, SIA results could provide useful indices for managers concerned about the effects of large carnivores on ungulate populations. Hair samples may be more readily obtainable than scats (e.g., for a similar field effort, we collected hair samples from 12 packs and scat samples from 4 packs), and in some cases, existing sources of wolf hair could be exploited for negligible extra field hours or costs. In Montana, for example, hairs could be collected by MFWP wolf management specialists during annual capture and radio-collaring of wolves for population monitoring ( $n = 17$  in 2009), from wolves killed or radio-collared in control actions by United States Department of Agriculture Wildlife Services agents ( $n = 158$  in 2009), and from wolves harvested by hunters during the regulated hunting season ( $n = 72$  in 2009; Sime et al. 2010). Because hair samples for SIA require no special storage, and take up little space, managers could store hairs indefinitely and conduct SIA at any time. We conducted approximately 200 hours of SIA laboratory work for our study, but  $\geq 50\%$  of this time was spent on preparing prey species hairs, and this will not need to be repeated for northwestern Montana because the isotope values of the region's prey are unlikely to change. Laboratory time must be budgeted, but some stable isotope facilities (e.g., the University of California, Davis Stable Isotope Facility, Davis, CA) offer specimen preparation services. Managers interested in obtaining isotope data would still have to devote time to sample labeling, data recording, and statistical analysis, but the exploratory work detailed here and in previous studies provides step-by-step instructions on how to use stable isotope mixing models to interpret diet data (Moore and Semmens 2008, Semmens et al. 2009).

## MANAGEMENT IMPLICATIONS

Although methodological problems may have produced wolf diet results that over- and underrepresented the true contribution of moose and white-tailed deer, respectively, this study provided evidence that moose require closer management attention. In northwestern Montana, MFWP moose population estimates are imprecise, and if some wolf packs consume greater proportions of moose than expected, managers may need to monitor the moose population more closely, and adjust wolf and moose hunting quotas accordingly. Such recommendations would apply to any ungulate population vulnerable to wolf predation, and our

results suggest that predicting effects on a regional scale would be very difficult because wolf packs may have different diets.

Stable isotope analysis is a relatively low cost method for obtaining an index of what wolves eat in a given area, but a clearer picture may emerge if wolf specific fractionation values become available. When specific packs are of interest to managers,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of potential prey can be distinguished, and multiple samples from a pack can be obtained, SIA has the potential to provide managers with a comprehensive record of how wolves use prey. This may be particularly beneficial when trying to understand how much livestock a wolf pack consumes. Domestic cattle are isotopically distinct from wild ungulates (Stewart et al. 2003, Derbridge and Krausman, University of Montana, unpublished data), and different levels of reliance on livestock could be determined depending on whether hair (i.e., a 6-month diet record) or bone (i.e., a lifetime diet record) is examined (Tieszen et al. 1983, Peterson and Fry 1987, Darimont and Reimchen 2002).

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