

Diet reconstruction of wolves following a decline in Manitoba moose populations

by

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A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

In partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Department of Biological Sciences

University of Manitoba

Winnipeg

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Abstract

Moose populations in southern Manitoba have declined. Although the primary cause of this decline is unclear, wolf predation is considered a main contributor because wolves have limited moose populations elsewhere. To help understand the role of wolf predation in limiting moose populations we reconstructed the proportional diet of wolves using stable isotope analysis and a Bayesian stable isotope mixing model (MixSIAR). We tested the assumptions of MixSIAR by running sensitivity analyses on trophic discrimination and prior information.

We observed differences in wolf diet in areas where moose and deer coexist and are spatially separated, with changes both seasonally and annually. Our results suggest white-tailed deer were preyed on during winter, even in areas where deer are locally rare, suggesting prey catchability is more important than local prey density. Seasonal changes in prey accessibility strongly affect wolf predation rates, and manipulating alternative prey populations could mitigate predation impacts on moose.

Acknowledgements

I would like to thank my advisor, Jim Roth, for his support and guidance throughout this project as well as my graduate committee members Rick Baydack, Jane Waterman, and Jillian Detwiler for their advice and expertise. I would also like to acknowledge all of the invaluable laboratory technicians that contributed to this project: Ian Burron, Jessica McGraw, Nicole Kaminski, Jillian St. George, Dana Kowalsky, Arielle Nagy, Erin Ciwko, Ana Maria Pereira Antunes, Tera Edkins, Alyssa Eby, Anghela Sivananthan, and Ryan Sigurdson. Hank Hristienko, Kelly Leavesley, Daniel Dupont, Rich Davis, Brian Kiss, and Gerald Shelemy at Manitoba Conservation all helped immensely by collecting samples, sharing their expertise, as well as providing data and field opportunities. Tim Sallows, Roxanne Grzela, and others with Parks Canada provided additional support by collecting samples and sharing data. Manitoba Conservation, Canada Foundation for Innovation (CFI), Natural Sciences and Engineering Research Council of Canada (NSERC), Manitoba Big Game Trophy Association, the Faculty of Graduate Studies and the Faculty of Science at the University of Manitoba all helped to fund this project. I would like to express my upmost appreciation for the support and encouragement of my fellow graduate students who were always there when I needed them, whether it was to learn Bayesian statistics or to provide a much needed laugh. Without them graduate school would have been much harder and far less exciting—thank you: Michelle Ewacha, Christa Szumski, Tazarve Gharajehdaghipoor, Jen Sojka, Dylan Pond, Dawn Wood, Andy Coulson, Kelsey O'Brien, Jackie Verstege, Kyle Ritchie, Elaine Anjos, Annemarie Van Der Marel, Marci Trana, Ryan McDonald, Bonnie McCullagh, and Chris Deduke. Thank you to my family and friends back home in “the States” for your love and support. A special thank you to my husband whom despite his constant battle with border control still visits me, and as always is the most supportive and caring person in the world.

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Thesis Format

This thesis is in manuscript format. I wrote chapters two and three as individual manuscripts each with their own abstract, introduction, methods, results, discussion, references, and figures. The first and last chapters include the overall introduction and conclusion of my thesis, which contain a summary of the background and conclusions of my research, and implications beyond what is included in the manuscripts. At the end of my thesis is an appendix that consists of additional analyses relevant to chapter three.

I collected hair and muscle samples of wolves and their prey alongside Manitoba Conservation for stable isotope analysis. I prepared samples and trained technicians to prepare samples for stable isotope analysis. Additional prey information was provided by Manitoba Conservation or collected by myself in collaboration with Manitoba Conservation and Parks Canada. I performed all data analyses and wrote all chapters with guidance from my committee.

Chapter 1: Thesis Introduction

Historical wolf (*Canis lupus*) range has decreased as human populations expanded across North America (Figure 1.1), changing the predator-prey landscape. Currently, wolf populations are considered stable in Canada (Mech and Boitani 2010), but moose populations in parts of Canada and the United States have declined (Lenarz et al. 2009, Broders et al. 2012, DeCesare et al. 2014). Wolves have been implicated as a regulating or limiting factor for moose populations in multi-ungulate and single ungulate communities (Messier 1994, Zimmermann et al. 2015). Regulating factors cause a decline in a prey's population numbers and limiting factors prevent a prey population from increasing, but do not cause a decline in prey numbers (Sinclair 1991). Wolves can be an influential limiting factor on moose and other prey (i.e. deer, caribou, or elk), but prey populations in some areas such as Isle Royale, Michigan (Peterson et al. 1998) have increased despite the presence of wolves (Mech 1970b). Many factors can affect wolf predation impacts on prey such as moose, thus greater insight into these predator-prey interactions is needed.

Wolves live and hunt in cooperative packs consisting on average between 3-4 individuals (Ballard et al. 1987). There are typically two breeding individuals per pack as well as other subordinates that do not breed (Borg et al. 2015). Breeding occurs in winter and pups are born in spring, which coincides with the pulse in ungulate populations, allowing adults to meet the nutritional requirements of the pups (Mech 1970). By fall, wolf pups are large enough to hunt larger animals like moose (*Alces americanus*) that are difficult to kill (Mech 1970). Wolves are considered opportunistic generalists because they consume a variety of food items ranging from amphibians to large ungulates (Naughton 2012). As generalists wolves are able to switch their diet whenever a preferred or primary prey source becomes limited or inaccessible.

Prey switching is an alternative foraging strategy that can have various impacts on a predator-prey community. If a predator's primary prey population declines or if the predator population grows

above foraging capacity, then a predator can maintain its population numbers if it switches its diet accordingly. For instance, rabbits (*Oryctolagus cuniculus*) in Scotland sustain mink (*Neovison vison*) population numbers, subsequently maintaining predation pressure by minks on water voles (*Arvicola amphibius*) (Holt 1977). In this example one prey species negatively influences another prey species through indirect interactions involving a shared predator, a phenomenon known as apparent competition (Holt and Lawton 1994). Apparent competition is evident in some wolf-ungulate communities where invasive deer act as supplemental prey to wolves, thus increasing predation pressure on other prey species as wolf populations grow (Latham et al. 2011). Wolf predation and apparent competition may have serious implications for a declining prey population.

In accessible regions of southern Manitoba, moose populations have declined in recent years to an extent that moose could no longer be sustainably harvested (Figure 1.2, Leavesley 2010). Moose are important to Manitoba because of their cultural significance to aboriginal communities as well as the revenue generated from moose hunting and tourism (Crichton et al. 1979). The impact of wolf predation on declining moose populations in Manitoba is unclear. The objective of my thesis is to reconstruct wolf diet using stable isotope analysis and Bayesian mixing models and estimate the proportion of wolf diet composed of moose.

Stable isotope ratios are the ratio of heavy to light isotopes of an element compared to a standard. Carbon and nitrogen are the elements most commonly used to study consumer diet. Carbon is linked to the environment where an animal lives through biogeochemical processes, thus carbon can be traced to the origin of a consumer's diet (Hobson 1999). Nitrogen reflects the trophic position of a consumer (Ben-David and Flaherty 2012). The international standards used for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are Vienna Pee Dee Belemnite carbonate and atmospheric nitrogen, respectively (Ben-David and Flaherty 2012). When food is consumed those tissues that have been digested are assimilated into the consumer's tissues, which are then reflected in the stable isotope ratios of those tissues (Bearhop et al. 2013). To

derive $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from tissues, stable isotope ratios of prepared samples are measured with an isotope ratio mass spectrometer and are denoted using delta notation (δ) and are expressed as per mil (‰) (Ben-David and Flaherty 2012). Traditionally, scat and stomach content analysis were solely used to estimate consumer diet, but these techniques only estimate the diet of an individual hours or days after a feeding event (Hobson and Wassenaar 1999). Stable isotope analysis measures the diet of a consumer by comparing stable isotope ratios in tissues of consumers and their prey (Ben-David and Flaherty 1997), providing a holistic view of consumer diet. Isotopic signatures of animals may also vary depending on the rate of nutrient assimilation, physiology, geographical location, tissue turnover, and type of tissue (Bearhop et al. 2004; Layman et al. 2007; Martinez del Rio et al. 2009; Ben-David and Flaherty 2012).

Consumer diet can be measured over multiple periods of an animal's life depending on the tissue that is used, as different tissues have different turnover rates. For example blood serum turnover is rapid and so the stable isotope signatures from blood serum reflect consumer diet from hours or days prior to sample collection (Urton and Hobson 2005). Inert tissues such as feathers, hooves, and hair do not turn over, and thus reflect the diet of the individual during the period of tissue growth (Roth and Hobson 2000). By using multiple tissues from the same individuals we can piece together consumer diet over a long period of time, which is how we reconstructed the winter and summer diet of wolves.

We assumed that muscle tissue, which has a continuous turnover rate, represents the diet of the consumer during the two months prior to sampling (Tieszen et al. 1983, Hobson and Clark 1993). Therefore, muscle tissue collected from wolves trapped in the winter trapping season (mostly December – February) reflects diet during late fall and winter. Wolves molt their fur annually beginning in spring; the new coat simultaneously grows as the old coat is shed during summer and throughout the fall (Darimont and Reimchen 2002). As the hair grows, nutrients from the consumed food are assimilated into the hair, thus the carbon and nitrogen signatures of guard hair represents the summer diet of wolves.

MixSIAR is a comprehensive hierarchical multivariate Bayesian stable isotope mixing model that allows researchers to estimate the proportional diet of a consumer including variability from diet-tissue discrimination, consumers, and prey; as well as incorporates several prey species, concentration dependencies, and prior information attained from expert knowledge (Stock and Semmens 2013). MixSIAR differs from other mixing models because it includes a multivariate hierarchical component allowing researchers to estimate the probable diet of a consumer and accounts for a greater amount of variability from sources, trophic discrimination, covariates (e.g., age, region, or time), and individual consumers within a population (Stock and Semmens 2013).

The results from MixSIAR are heavily influenced by the position of prey in relation to consumers in iso-space (Parnell et al. 2013). Iso-space is the bivariate, 2-dimensional space between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ where a consumer and its prey are contained (Yeakel et al. 2011). Outliers in the consumer data that are outside the source iso-space hypervolume do not accurately represent the diet of the population (Ward et al. 2010) and should be excluded from the analysis. If mean prey signatures overlap in iso-space, MixSIAR may not be able to differentiate which source is contributing to the consumer's diet (Ward et al. 2010). Overlapping prey sources can be combined, but only if there is a biological reason (Phillips et al. 2005). To assess whether our prey sources were isotopically distinct from one another, we compared the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of each prey species.

MixSIAR is not only sensitive to the configuration of consumers and prey within iso-space, but also by the trophic-discrimination values and prior information chosen by the researcher (Parnell et al. 2013). Trophic discrimination is the difference between the stable isotope ratios of a consumer and its prey (Martinez del Rio and Anderson-Sprecher 2008). Trophic discrimination occurs as a consumer metabolizes the nutritional components of its diet, during which isotopes of the same element are processed in different ways (Kelly et al. 2012). Trophic discrimination may vary by species, tissue type, rate of growth, tissue turnover, isotopic routing, body size of the animal, protein quality and quantity,

type of nitrogen excretion, and consumer diet (Roth and Hobson 2000; McCutchan et al. 2003; De Smet et al. 2004; Mill et al. 2007; Halley et al. 2010; Kelly and Martinez del Rio 2010). To account for trophic discrimination in stable isotope mixing models, a diet-tissue discrimination correction factor is applied to prey stable isotope ratios (Parnell et al. 2013). Using accurate discrimination factors is imperative to estimate consumer diets using stable isotope analysis (Martinez del Rio et al. 2009); controlled feeding studies with captive animals are used to attain accurate discrimination factors (Kelly et al. 2012). However, few experiments measuring trophic discrimination have been conducted for many species, and so proxy discrimination factors from the best available information for a species are used to carry out many field studies (Martinez del Rio et al. 2009). We ran a sensitivity analysis to examine some of the variation in diet estimates that may be associated with different trophic discrimination factors.

Prior information is used in Bayesian statistics to produce a model that more appropriately fits the data that were collected by giving the model a starting point based on empirical information (Kruschke 2013). MixSIAR uses Markov Chain Monte Carlo (MCMC) methods to run multiple iterations resulting in a posterior distribution of solutions to the model, or in this case, the distribution of the proportion of wolf diet belonging to a prey species. MixSIAR updates these dietary proportions by using the posterior distribution from the previous iteration as a prior distribution for the next iteration (Parnell et al. 2013). Prior information may strongly influence the resulting posterior distribution, depending on sample size of the consumers and the precision of the priors (Kruschke 2013). We conducted a sensitivity analysis to describe the variation in wolf diet estimates using different priors.

The main objective of this thesis was to estimate predator diet using stable isotopes and Bayesian mixing models, which could be used in strategic regional management plans for the conservation of moose populations in Manitoba. In chapter two, we tested the assumptions of our model and applied it to wolves in one region of Manitoba where moose have declined and two of the most important wolf prey, moose and deer, are spatially segregated. In chapter three, we applied the

same methods as chapter two, but estimated predator diet in an area with a different suite of prey that existed sympatrically, and compared these estimates to our results from the previous chapter, where primary prey existed allopatrically. The final chapter provides an overall summary and conclusion and discusses some implications of this research for predator-prey management. In sum, this thesis provides information on predator-prey interactions in a human-influenced community, and suggests how proportional diet analysis can be useful for management of large game species.

Literature cited

- Ballard, W.B., Whitman, J.S., and C.L. Gardner. 1987. Ecology of an exploited wolf population in south-central Alaska. *Wildlife Monographs* 98:3-54.
- Bearhop, S., Adams, C. E., Waldron, S., Fuller, R.A., and H. Macleod. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology* 73:1007–1012.
- Bearhop, S., Waldron, S., Votier, S.C., and R.W. Furness. 2013. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiological and Biochemical Zoology* 75:451–8.
- Ben-David, M., and E. Flaherty. 1997. Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. *Oecologia* 111:280-291.
- Ben-David, M., and E. Flaherty. 2012. Stable isotopes in mammalian research: a beginner's guide. *Journal of Mammalogy* 93:312–328.
- Borg, B.L., Brainerd, S.M., Meier, T.J., and L. R. Prugh. 2015. Impacts of breeder loss on social structure, reproduction and population growth in a social canid. *Journal of Animal Ecology* 84:177-187.
- Broders, H.G., Coombs, A.B., and J.R. McCarron. 2012. Ecothermic responses of moose to thermoregulatory stress on mainland Nova Scotia. *Alces* 48:53-61.
- Crichton, V. 1979. An experimental moose hunt on Hecla Island, Manitoba. *Alces* 15: 245-279.
- Darimont, C.T., and T.E. Reimchen. 2002. Intra-hair stable isotope analysis implies seasonal shift to salmon in gray wolf diet. *Canadian Journal of Zoology* 80:1638-1642.
- DeCesare, N.J., Smucker, T.D., Garrott, R.A., and J.A. Gude. 2014. Moose status and Management in Montana. *Alces* 50:35-51.
- De Smet, S., Balcaen, A., Claeys, E., Boeckx, P., and O. Van Cleemput. 2004. Stable carbon isotope analysis of different tissues of beef animals in relation to their diet. *Rapid Communications in Mass Spectrometry* 18:1227–1232.
- Halley, D. J., Minagawa, M., Nieminen, M., and E. Gaare. 2010. Diet: tissue stable isotope fractionation of carbon and nitrogen in blood plasma and whole blood of male reindeer. *Polar Biology* 33:1303–1309.
- Hobson, K.A., and R.G. Clark. 1993. Turnover of ¹³C in cellular and plasma fractions of blood: implications for non-destructive sampling in avian dietary studies. *Auk* 110:638-641.
- Hobson, K.A., and L.I. Wassenaar. 1999. Stable isotope ecology: an introduction. *Oecologia* 120:312-313.
- Hobson, K.A. 1999. Tracing diets and origins of migratory birds using stable isotope techniques. *Society of Canadian Ornithologists, Special Publication* 1:21-41.

- Holt, R.D. 1977. Predation, apparent competition, and the structure of prey communities. *Theoretical Population Biology* 12:197-229.
- Holt, R.D., and J.H. Lawton. 1994. The ecological consequences of shared natural enemies. *Annual Review of Ecology and Systematics* 25:495-520.
- Kelly, L. J., and C. Martínez del Rio. 2010. The fate of carbon in growing fish: an experimental study of isotopic routing. *Physiological and Biochemical Zoology* 83:473-480.
- Kelly, D. J., Robertson, A., Murphy, D., Fitzsimons, T., Costello, E., Gormley, E., Corner, L.A.L., and N.M. Marples. 2012. Trophic enrichment factors for blood serum in the European badger (*Meles meles*). *PLoS ONE* 7:e53071.
- Kruschke, J.K. 2013. Bayesian estimation supersedes the t-test. *Journal of Experimental Psychology*. 142:573-603.
- Latham, A.D.M., Latham, M.C., McCutchen, N.A., and S. Boutin. 2011. Invading white-tailed deer change wolf-caribou dynamics in northeastern Alberta. *Journal of Wildlife Management* 75:204-212.
- Layman, C., Arrington, D., Montana, C., and D. Post. 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology* 88:42-48.
- Leavesley, K. 2010. Big game aerial survey report moose survey GHA 26: winter 2009-2010. Manitoba Conservation, Winnipeg, Manitoba, CA.
- Lenarz, M.S., Fieberg, J., Schrage, M.W., and A.J. Edwards. 2010. Living on the edge: viability of moose in northeastern Minnesota. *Journal of Wildlife Management* 74:1013-1023.
- Martinez del Rio, C., and R. Anderson-Sprecher. 2008. Beyond the reaction progress variable: the meaning and significance of isotopic incorporation data. *Oecologia* 156:765-772.
- Martinez del Rio, C., Wolf, N., Carleton, S.A., and L. Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews of the Cambridge Philosophical Society* 84:91-111.
- McCutchan, J., Lewis, W., Kendall, C., and C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 2:378-390.
- Mech, L.D. 1970. Pup birth and stages of development. Pages 46-47 in L.D. Mech and L. Boitani, editors. *Wolves: behavior, ecology, and conservation*. University of Chicago Press, Chicago, USA.
- Mech, L. D., editor. 1970. *The wolf: the ecology and behavior of an endangered species*. Natural History Press, New York, USA.
- Mech, L.D., and L. Boitani. 2010. *Canis lupus* (IUCN SSC Wolf Specialist Group). The IUCN Red List of Threatened Species. Version 2014.3. <www.iucnredlist.org>.
- Messier, F. 1991. On the concepts of population limitation and population regulation as applied to caribou demography. In *Proceedings of 4th North American Caribou Workshop*, St. John's, NFLD.,

October 31 to November 3, 1989. *Edited by* C.E. Butler and S.P. Mahoney. Newfoundland and Labrador Wildlife Division, St. John's. pp. 260-277.

Messier, F. 1994. Ungulate population models with predation: a case study with the North American moose. *Ecology* 75:478-488.

Mill, A.C., Pinnegar, J.K., and N.V.C. Polunin. 2007. Explaining isotope trophic-step fractionation: why herbivorous fish are different. *Functional Ecology* 21:1137-1145.

Naughton, D. 2012. The natural history of Canadian mammals. Canadian Museum of Nature and University of Toronto Press, Toronto, Ontario, CA.

Parnell, A. C., Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W., Jackson, A.L., Grey, J., Kelly, D.J., and R. Inger. 2013. Bayesian stable isotope mixing models. *Environmetrics* 24:387-399.

Peterson, R.O., Thomas, N.J., Thurber, J.M., Vucetich, J.A., and T.A. Waite. 1998. Population limitation and the wolves of Isle Royale. *Journal of Mammalogy* 79:828-841.

Phillips, D.L., Newsome, S.D., and J.W. Gregg. 2005. Combining sources in stable isotope mixing models: alternative methods. *Oecologia* 144:520-527.

Roth, J.D., and K.A. Hobson. 2000. Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction. *Canadian Journal of Zoology* 78:848-852.

Rubenstein, J.R., and K.A. Hobson. 2004. From birds to butterflies: animal movement patterns and stable isotopes. *TRENDS in Ecology and Evolution* 19: 256-263.

Sinclair, A.R.E. 1989. Population regulation in animals. *In* *Ecological concepts: the contribution of ecology to an understanding of the natural world. Edited by* J.M. Cherrett. Blackwell Scientific Publications, Oxford. pp. 197-241.

Stock, B.C., and B.X. Semmens. 2013. MixSIAR GUI User Manual, version 1.0. <http://conserver.iugocafe.org/user/brice.semmens/MixSIAR>.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., and N.A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues : implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* 57:32-37.

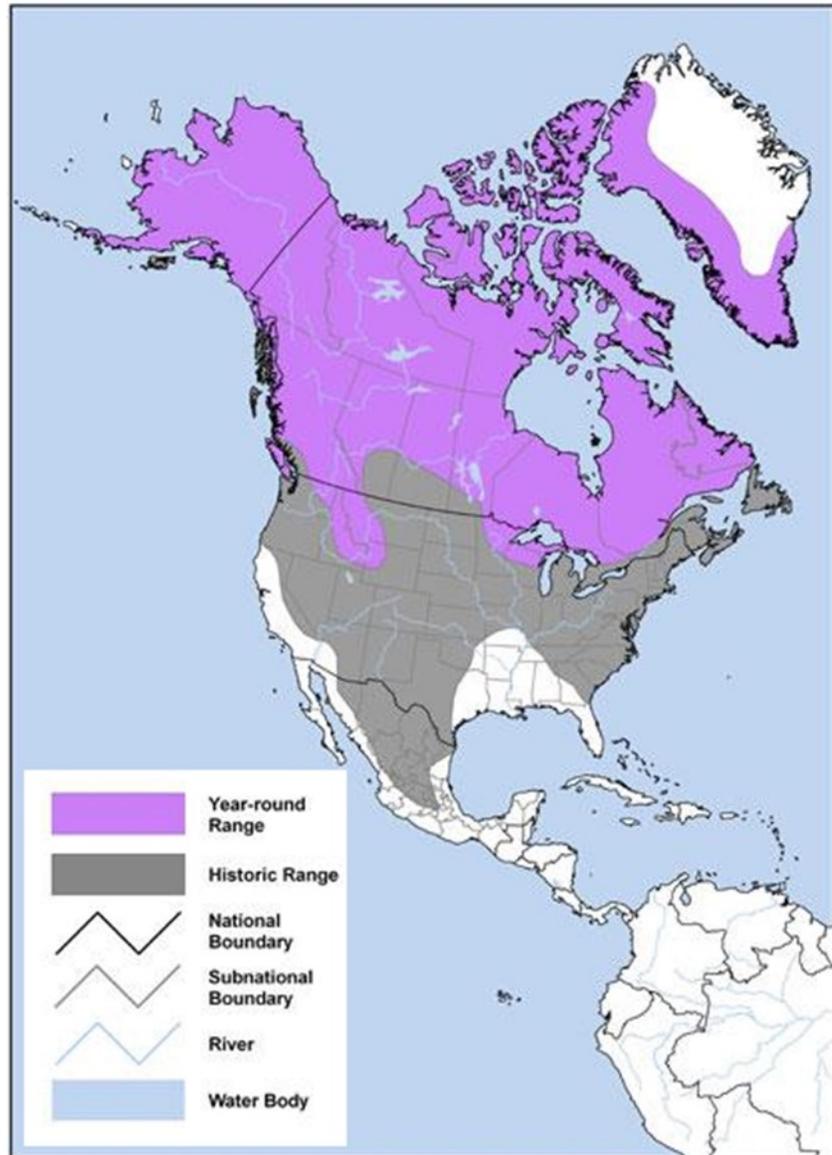
Tremblay, J.P., Jolicoeur, H., and R. Lemieux. 2001. Summer food habits of gray wolves in the boreal forest of the Lac Jacques-Cartier Highlands, Quebec. *Alces* 37:1-12.

Van Ballenberghe, V., and W.B. Ballard. 1994. Limitation and regulation of moose populations: the role of predation. *Canadian Journal of Zoology* 72:2071-2077.

Ward, E.J., Semmens, B.X., and D.E. Schindler. 2010. Including source uncertainty and prior information in the analysis of stable isotope mixing models. *Environmental Science and Technology* 44:4645-4650.

Yeakel, J.D., Novak, M., Guimaraes, P.R., Jr., Dominy, N.J., Koch, P.L., et al. 2011. Merging resource availability with isotope mixing models: the role of neutral interaction assumptions. *PloS ONE* 6: e22015.

Zimmermann, B., Sand, H., Wabakken, P., Liberg, O., and H.P. Andreassen. 2015. Predator-dependent functional response in wolves: from food limitation to surplus killing. *Journal of Animal Ecology* 84:102-112.



Created February 2010; Adapted from Mech and Boitani 2008 and Oregon State University, 2003.Natureserve.org



Figure 1.1. Map of current and historic range of gray wolves (*Canis lupus*). Historic wolf range size has decreased by 75% since European settlers began colonizing North America (Mech and Boitani 2010).

Manitoba, Canada

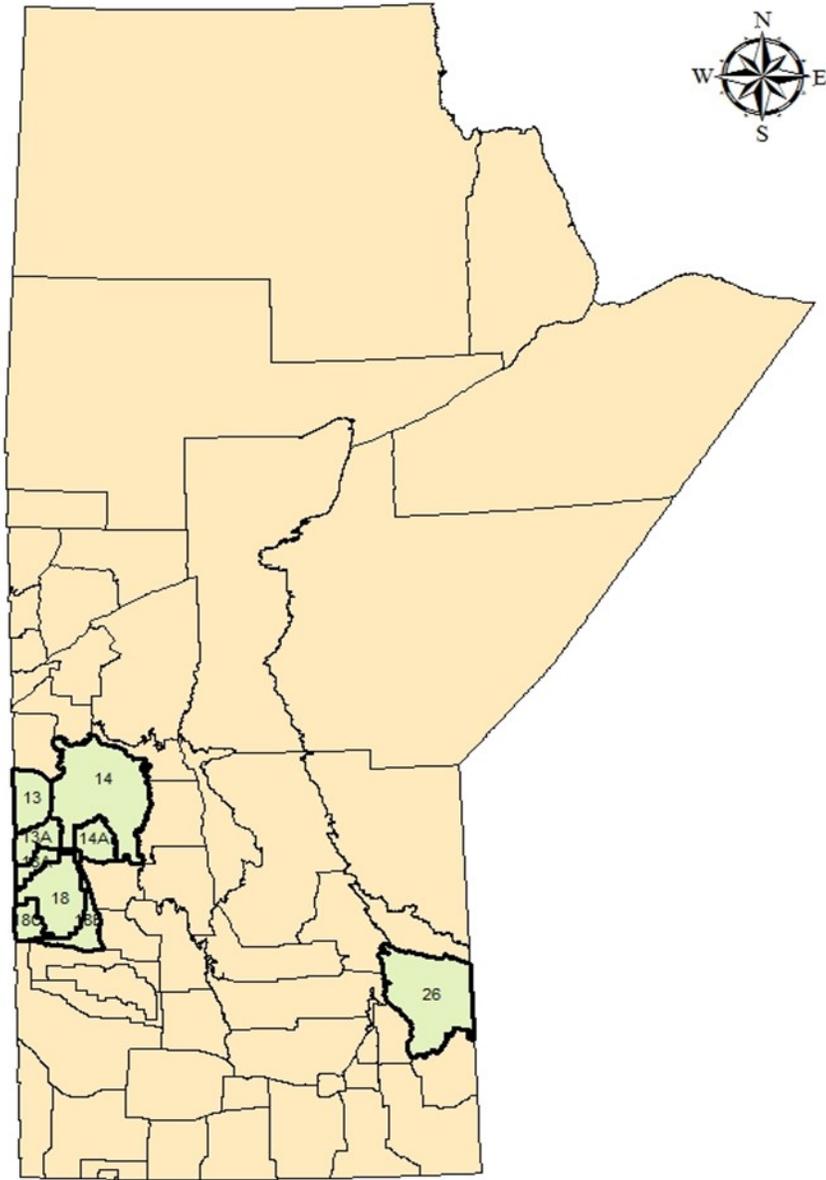


Figure 1.2. Game hunting areas in Manitoba where moose have declined.

Chapter 2: Diet reconstruction of wolves following a decline in moose populations

Abstract

In southern Manitoba, moose (*Alces americanus*) populations have declined in recent years. Although the primary cause of this decline has been unexplained, wolf (*Canis lupus*) predation has been suggested as a main contributor because wolves have been known to limit moose populations elsewhere. To understand the importance of moose in wolf diets we estimated annual and seasonal variation in diet as well as spatial differences related to prey availability, using a comprehensive hierarchical multivariate Bayesian stable isotope mixing model (MixSIAR). We tested the assumptions of MixSIAR before reconstructing wolf diet by running several sensitivity analyses on trophic discrimination and the influence of prior information. Trophic discrimination values measured in red fox (*Vulpes vulpes*) produced the most reasonable diet estimates compared to wolf and arctic fox (*Vulpes lagopus*) values. Informative priors provided a clearer delineation of important prey consumed, so all subsequent analyses used informative priors estimated by Manitoba Conservation.

We estimated wolf diets in winter (from stable isotope ratios of muscle) and summer (using guard hair) over four years. During winter wolf diet alternated between deer (*Odocoileus virginianus*) and moose, but overall deer were the most prominent prey, even for wolves captured in an area where moose dominated and deer were rare, suggesting a preference for deer. In summer, however, wolves consumed less deer, and instead ate mainly moose and beaver (*Castor canadensis*). This seasonal shift could be due to changes in prey accessibility, as in winter deep snow reduces deer movements and ice restricts access to beaver, whereas in summer naïve yearling moose have become independent and are no longer defended by the cow. Thus, catchability of a prey species may override the importance of prey density. These results suggest wolf predation has the greatest impact on moose populations in summer. Management efforts focused on increasing the abundance and accessibility of alternative prey may have the greatest impact on moose conservation during summer.

Introduction

Predators can limit population sizes of their prey (Lindell and Forsman 1996, Keech et al. 2011). However, determining the direct effects of predators on prey populations may be confounded by human disturbances within a community (Van Ballenberghe and Ballard 1994), making it difficult to distinguish the impact of prey mortality factors in a complex ecosystem. In southern Manitoba, moose (*Alces americanus*) populations have declined in recent years (Leavesley 2010), and several potential mortality factors may have affected moose numbers, including predation, harvest by humans, habitat degradation through fire suppression or development, disease, or harsh weather. Thus far, the driving force of moose decline in Manitoba has been unexplained, although some stakeholders suggest that wolf (*Canis lupus*) predation is a main contributor.

Wolves are categorized as opportunistic generalists because they consume a variety of different prey species (e.g., amphibians, birds, small mammals, and large ungulates) (Naughton 2012). Optimal foraging theory suggests that the most easily caught and abundant prey will be the species most consumed by a predator (Charnov 1976). Wolves hunting in packs typically focus on large ungulate prey such as moose because they provide more food per capita (Mech 1970). A generalist predator such as wolves may stabilize one prey population by switching its diet to another prey species (Charnov 1976). Wolves may limit moose populations (Messier and Crête 1985), and so the presence of an alternate prey species may alleviate predation pressure on moose.

We reconstructed the seasonal diet of wolves over four years to examine temporal variation in wolf diet because prey abundance and accessibility vary throughout the year and between years as the environment changes. We estimated the proportional diet of wolves using stable isotope analysis, which measures the long-term diet of a consumer by comparing carbon and nitrogen stable isotope ratios in tissues of consumers and their prey (Ben-David and Flaherty 1997), providing a holistic view of consumer diet. We used this information to estimate the relative importance of different prey species in

wolf diet (Locke et al. 2013) using a multivariate hierarchical stable isotope Bayesian mixing model (MixSIAR V2.1, Stock and Semmens 2013), after testing several assumptions of the model.

Prey accessibility varies with changes in the environment, so we would expect wolves to consume ungulates in winter, when they are slowed by deep snow, rather than beaver (*Castor canadensis*), which commonly remain protected by ice and stay within their lodges. In summer, we would expect wolf diet to contain a greater proportion of beaver than in winter, since beaver are more accessible as they travel on land to forage. Wolf diet may also depend on prey density, and densities of moose and white-tailed deer (*Odocoileus virginianus*) are concentrated in different regions within our study area. Therefore, we expect wolves from regions with higher densities of moose to have a greater proportion of moose in their diet, and wolves in regions where deer predominate would consume a greater proportion of deer.

Methods

Study site

In this study we focused on Manitoba game hunting area (GHA) 26 (centered at 95°55'5.945"W, 50°55'33.148"N). In 2012, licensed moose hunting was cancelled and First Nations communities voluntarily restricted treaty-based hunting in select areas within GHA 26 in response to declining moose numbers. GHA 26 is located north of Lac du Bonnet, MB (Figure 2.1), and is dominated by boreal shield forest, which consists primarily of white spruce (*Picea glauca*), black spruce (*Picea mariana*), balsam fir (*Abies balsamea*), and trembling aspen (*Populus tremuloides*). Much of the boreal shield landscape is covered by bogs and other wetlands (Bohning et al. 2003). White-tailed deer and moose are the main ungulates found in GHA 26, although caribou (*Rangifer taranadus*) occupy the northeastern region of GHA 26. Moose are primarily concentrated in the north of GHA 26, whereas white-tailed deer are rare in the north and occur at high densities in the south of GHA 26 (Kelly Leavesley pers. comm., Manitoba

Conservation). Smaller prey such as snowshoe hare (*Lepus americanus*) and beaver are also present throughout our study site.

Sample Collection and Preparation

Tissue samples of wolves, moose, and white-tailed deer were collected by Manitoba Conservation from registered trappers, hunters, and road kill in the study area. Caribou hair was collected during concurrent research on caribou in GHA 26. Beaver and snowshoe hare samples were opportunistically collected. Wolf hair and muscle samples as well as prey muscle were collected between 2010-11 and 2013-14 (Table 2.1). All samples were immediately frozen (-20°C) and stored until transport to the University of Manitoba. Tissue samples were processed for analysis 1-6 months post collection date. To prepare muscle tissue, samples were first freeze-dried, then homogenized with a mortar and pestle. We removed lipids using a Soxhlet apparatus with petroleum ether because lipids can significantly influence stable carbon isotope ratio measurement, confounding interpretation of the results (DeNiro and Epstein 1977; Bond and Jones 2009). Guard hair and underfur were separated because they grow at different time periods, then washed using soap and water and rinsed thoroughly with water under high pressure. We homogenized hair samples into a fine powder using scissors or a ball mill (30 Hz; Retsch MM 301 Mixer Mill, Retsch Inc, Newtown, Pennsylvania, USA). We weighed subsamples of muscle (0.4-0.6 µg) or hair (0.6-0.8 µg) into small tin capsules that were sent to the University of Windsor Chemical Tracers Lab, where the stable isotope ratios were measured using a continuous-flow isotope ratio mass spectrometer.

We reconstructed wolf diet using muscle and hair stable isotope ratios. Muscle has a continuous turnover rate, representing the average diet of the consumer during the two months prior to mortality (Tieszen et al. 1983), and so muscle samples collected from wolves trapped in the winter trapping season (mostly Dec-Feb) reflect winter diet. Inert tissues such as hair do not turn over, and thus reflect the diet of the individual during the period of hair growth (Roth and Hobson 2000). Wolves shed their

old coat in spring and grow guard hairs throughout summer, and then in fall wolves begin growing their underfur (Darimont et al. 2009), and so guard hair represents the summer diet and underfur represents the fall diet of wolves. We used prey muscle when available to reconstruct wolf diet because we assumed wolves only assimilate the nutrients from meat that was consumed. However, caribou muscle samples were unavailable, so we used caribou hair isotopic signatures and corrected for the differences between hair and muscle by applying a correction value for nitrogen (+0.3) based on two controlled feeding studies of ungulates (Sponheimer et al. 2003a, b). No differences between hair and muscle were detected in ungulate carbon signatures (Sponheimer et al. 2003a, b); therefore, we did not apply a correction value to carbon.

Diet reconstruction

We tested whether each prey species was isotopically distinct and if temporal differences in prey isotopic signatures existed. To assess whether prey signatures changed among years, we ran a MANOVA for all prey including $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as dependent variables and year as an independent variable, while controlling for species. A Bonferroni correction was applied to account for error associated with multiple comparisons (Bland and Altman 1995).

We then conducted a sensitivity analysis for trophic discrimination, which is the difference between the stable isotope ratios of a consumer and its prey (Martinez del Rio and Anderson-Sprecher 2008). Inaccurate discrimination correction factors can lead to erroneous diet estimates, so we ran several versions of MixSIAR, each time including different discrimination values based on other research to estimate the potential impact of various trophic discrimination factors on our diet estimates. To assess the influence of each set of discrimination values on the geometry of the data in iso-space without interference from other model inputs we ran each model starting with priors that assumed all prey were consumed equally. We used three sets of trophic discrimination values derived from tissues of three canid species based on controlled feeding studies: arctic fox (*Vulpes lagopus*) muscle

($\Delta C=0.37\pm 0.8$, $\Delta N=1.79\pm 0.4$; Lecomte et al. 2011), red fox (*Vulpes vulpes*) muscle ($\Delta C=1.1\pm 0.1$, $\Delta N=3.3\pm 0.1$; Roth and Hobson 2000) and wolf hair ($\Delta C=1.97\pm 0.70$, $\Delta N=3.04\pm 0.31$; Derbridge et al. 2015). The discrimination values for wolf hair were corrected for the difference in muscle and hair by calculating the difference in red fox muscle and hair discrimination values (Roth and Hobson 2000). We applied the correction ($\Delta C=-1.5$, $\Delta N=0.1$) to wolf hair discrimination values to estimate the discrimination values of wolf muscle ($\Delta C=0.47\pm 0.7$, $\Delta N=2.94\pm 0.31$). We corrected for the difference in body size between foxes and wolves because metabolic rate influences the rate of nitrogen assimilation and animals with larger body sizes have a slower metabolism; thus larger animals should have lower $\Delta^{15}N$ than smaller animals (MacAvoy et al. 2005). We altered the ΔN values of foxes by -0.3 to account for this body size difference based on a study of rats and mice (MacAvoy et al. 2006).

For every MixSIAR model in our analysis, we initially ran three chains that were 50,000 iterations long, with a burn-in of 25,000, and thinned every 25th iteration. We determined model convergence for each model using the Gelman-Rubin and Geweke diagnostic tests, as well as trace plots produced by MixSIAR (Stock and Semmens 2013). We also used a pairs plot to examine trade-offs between any strongly correlated prey species before interpreting diet results (Stock and Semmens 2013). If convergence was unsuccessful we increased the number of iterations to 100,000 per chain with a burn-in of 50,000 and thinned every 50th iteration.

We examined the importance of using informative priors in our models. Prior information can improve the accuracy of a model and may strongly influence the resulting diet estimates, depending on the sample size of the consumers and the precision of the priors (Kruschke 2013). Informative priors are prior distributions that were generated from previous knowledge (i.e., professional opinion, previous research, or observations). The default assumption for priors in MixSIAR is that all prey sources are equally important. To acquire our priors we ran the Stable Isotope Analysis in R (SIAR) program (Parnell et al. 2010), correcting for trophic discrimination and grouping wolf carbon and nitrogen signatures by

year, and then used the “siarelicit()” function in SIAR to calculate the priors. The priors produced were then substituted as alpha priors in the MixSIAR “R” code in the “run_model()” function.

To examine the sensitivity of diet estimates to prior information, we ran two different models using the same diet parameters but with different priors. In one model we used uniform priors that initially assumed equal importance of all prey and had a standard deviation of 0.05. For the other model, we produced prior distributions by weighting each prey species based on wolf scat analysis and professional observations (Daniel Dupont, Manitoba Conservation, unpubl. data) with a standard deviation of 0.05. We ran each model using wolf carbon and nitrogen signatures from winter, with year as a fixed effect, and prey signatures were corrected for trophic discrimination ($\Delta C=1.1\pm 0.1$, $\Delta N=3.0\pm 0.1$).

After testing the assumptions of stable isotope analysis and MixSIAR, we then ran MixSIAR to estimate wolf diet each season for each year. Our winter diet analysis included wolf muscle signatures, prey signatures, informative priors, and trophic discrimination factors ($\Delta C=1.1\pm 0.1$, $\Delta N=3.0\pm 0.1$). We also analyzed wolf summer diet using wolf guard hair signatures, prey signatures, informative priors, and trophic discrimination factors ($\Delta C=2.6\pm 0.1$, $\Delta N=2.9\pm 0.1$).

To examine spatial differences in wolf diet, we divided our study site into northern and southern sections based on moose and deer distributions determined from aerial surveys by Manitoba Conservation. We then categorized the wolves based on their geographic locations when killed as northern, where moose are predominant and deer are rare or absent, or southern, where deer are common but moose are rare and caribou are absent (Figure 2.2). We used the same diet parameters as the previous seasonal diet models, but excluded wolves killed during 2010-11 and 2013-14 because several of our samples from those years lacked specific geographic data.

Results

Methods validation

We found no annual differences in the isotopic signatures of prey species (Pillai's Trace, $F_{6, 212} = 1.22$, $p = 0.30$) and no interaction between year and species ($F_{6, 212} = 1.30$, $p = 0.24$), so samples from different years were combined for subsequent analyses. Prey species differed (Pillai's Trace, $F_{8, 212} = 25.6$, $p \leq 0.001$) in both $\delta^{13}\text{C}$ ($F_{4, 106} = 69.6$, $p \leq 0.0001$) and $\delta^{15}\text{N}$ ($F_{4, 106} = 9.51$, $p \leq 0.0001$). Snowshoe hares and white-tailed deer did not differ in $\delta^{13}\text{C}$ (Tukey's HSD, $p = 0.60$) but did differ in $\delta^{15}\text{N}$ ($p = 0.009$). All other prey species differed in $\delta^{13}\text{C}$ ($p \leq 0.02$). $\delta^{15}\text{N}$ values did not differ between beaver and snowshoe hare ($p = 0.90$), caribou and white-tailed deer ($p = 0.99$), or hare and moose ($p = 0.08$). All other pairwise comparisons of prey species were significant for $\delta^{15}\text{N}$ ($p \leq 0.02$). These results suggest that all prey species are isotopically distinct and can be included as separate sources in our mixing models.

Based on the distribution of wolf $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, we removed one outlier wolf from both the winter and summer diet analyses that was likely an immigrant that had consumed prey from a different area and would not represent the diet of the local wolf population (Figure 2.3).

The trophic discrimination sensitivity analysis showed that smaller ΔC and ΔN values removed the consumers from the prey hypervolume within iso-space (Figure 2.4), thus inaccurately estimating wolf diet. The discrimination factors from Roth and Hobson (2000) placed wolves within the prey hypervolume in iso-space, suggesting that these trophic discrimination factors may accurately represent wolf diet-tissue discrimination. The wolf discrimination factors somewhat remove the wolves from the prey hypervolume (Figure 2.4c) compared to the red fox values. We decided to use the red fox values that were adjusted for wolf body size because the location of wolves in iso-space seemed more plausible than the estimated wolf values.

The sensitivity analysis for prior information revealed a large effect of informative priors on diet estimates. The model including informative priors provided a clearer delineation between most likely prey in wolf diet compared to the model using uniform priors (Figure 2.5), in which the diet proportions of each prey species overlap greatly. With informative priors (Figure 2.5b), the diet proportions of moose and deer were separate from all other prey distributions, allowing us to determine the most important prey in wolf diet.

Proportional wolf diet estimates

Overall, deer were the most prominent prey in wolf diet during winter (Figure 2.6). Moose were only important prey for wolves in 2010-11 (median = 59%, 95% Credible Interval [48, 71%]) and 2012-13 (37%, [12, 71%]) during winter. Deer (32% [7, 63%]) were nearly as important as moose in 2012-13, and the most important in 2011-12 (52%, CI=10-82%) as well as in 2013-14 (56%, CI=8-91%). In summer, moose and beaver were the most important prey to wolves overall (Figure 2.7). Moose were the most important prey to wolves in 2010-11 (64%, CI=48-80%), and in 2012-13 beaver (33%, CI=5-71%) were the most dominant prey during summer. In 2012-13 and 2013-14, wolves most likely consumed moose (median = 24% and 48%, respectively) and beaver (35% and 40%, respectively) during summer. Deer were only important to wolves during summer in 2012-13 (30%, [8-58%]).

Wolves in the north predominantly consumed deer (median=50%) and moose (30%) in winter (Figure 2.8), despite the absence of deer in the north. In the south, wolves in winter were eating mainly deer (75%) and hardly any moose (15%). In summer, wolves in the north primarily consumed moose (39%) and some caribou (25%) (Figure 2.9). In the south, wolves ate mainly deer (44%) and some moose (25%) in summer. In 2012, caribou consisted of approximately 25% of wolf summer diet in the south as well, suggesting wolves trapped in the south during winter, where caribou are absent, had been foraging in the north the previous summer. Snowshoe hares were excluded from the summer spatial diet analysis

because the model would not converge when hares were included and snowshoe hares were a minor component of wolf diet in previous analyses.

Discussion

Our overall diet results indicated deer were the predominant prey in wolf winter diet, and that moose and beaver were the most likely prey to wolves in summer. A shift in wolf diet would be expected as environmental conditions change, both seasonally and annually, thus affecting prey availability. Deer populations are limited by deep snow and cold temperatures (-30°C) in Manitoba (Chranowski 1983). Winter weather, specifically deep snow, can inhibit white-tailed deer movements because deer have shorter legs and smaller hooves, unlike other northern ungulates such as moose and caribou (Nelson and Mech 1986). Historically, white-tailed deer did not exist in Manitoba (Barto and Vogel 1978), but have expanded north as humans settled and agriculture spread across the landscape, creating food subsidies for deer (Latham et al. 2011). More recently, climate change has been credited with the expansion of deer farther north (Latham et al. 2013), resulting in an available prey item to wolves that historically did not exist. Wolves are opportunistic hunters, making deer an easy target for these predators in winter.

Beaver are generally unavailable to wolves in winter because they reside in their lodges and stay beneath the ice until spring (Müller-Schwarze 2011), which may explain the prominence of beaver in our summer diet estimates. Moose proportions were much higher in summer compared to winter when deer were the most important prey. As previously mentioned, the mechanism for this switch in diet across seasons may be due to the increase in yearling moose that have become independent from their mothers in spring (Franzmann and Schwartz 1998). These yearling moose are inexperienced and may be preferred over adult moose prey because they would presumably be easier to kill. Joly and Messier (2004) found that wolves mainly prey upon weak, old, or young moose and so it is likely that wolves in GHA 26 are consuming young moose. Moose calves born in early spring are also vulnerable to predation

(Van Ballenberghe and Ballard 1994), but we do not know if wolves were mainly consuming moose calves in summer. Many neonate animals while still nursing are enriched in ^{15}N compared to adults (Jenkins et al. 2001), but we do not have neonate moose samples to corroborate. Carbon and nitrogen signatures of young moose would be equivalent to the signatures of adults because young moose will have been weaned and consume the same foods as adults. If wolves are consuming young moose or calves, then it is possible that they are limiting moose recruitment. Black bears (*Ursus americanus*) coexist with wolves in Manitoba, and when this occurs bears can inflate the effect of wolf predation of moose calves because black bears also prey on moose calves (Gasaway et al. 1992).

Prey density can also have major effects on the proportional diet of a predator. In summer, wolves in the north mainly consumed moose, and wolves in the south ate predominantly deer. These results support our hypothesis that wolves will prey on the most abundant prey species available in an area. Wolves in the north may feed on young moose that are more easily killed than adult moose, thus increasing the proportion of moose in wolf summer diet. However, wolves captured in the north, where deer are scarce, had high proportions of deer in their winter diet, suggesting that these wolves travelled south to feed despite the dense population of moose in the north. Thus, the opportunity to consume prey with a limited handling time and small likelihood of causing injury during the attack apparently overrides the importance of prey density in winter. Wolf telemetry data from a monitoring program conducted by Manitoba Conservation suggests that wolves within GHA 26 move north to south frequently (Manitoba Conservation, unpublished data). Travel can be very energetically costly, so there must be a benefit for wolves that travel to hunt deer when a supply of moose was available within their territory that outweighs that travel cost. Moose can be deadly when defending themselves during a wolf attack, making them a difficult resource to obtain, unless wolves are hunting in a large pack (Mech 1970), so the risk of hunting moose may be greater than the cost of traveling to hunt deer.

Typically, wolves living in packs take larger prey, such as moose (Larter et al. 1994), because they provide more meat for the effort shared by each pack member (Zimmermann et al. 2015). Top predators, such as wolves, are self-regulating species whose populations are often limited by social interactions rather than food (Wallach et al. 2015). Wolf harvest by trappers may cause social fragmentation resulting in the demise of pack groups (Brainerd et al. 2014). Similarly, the trapper incentive program that was designed to increase wolf harvest in 2011-2012 may have caused social fragmentation in the wolf population, resulting in a greater number of individuals or pairs of hunting wolves. Wolves hunting individually or in pairs may be more likely to take smaller prey, such as white-tailed deer. If pack size of wolves in our study area has decreased, deer could have become the preferred prey species. A change in the social structure could alter the functional impact of wolves on their prey populations, which may explain the patterns that we have observed in wolf winter diet.

As opportunistic generalists, wolves will eat a wide variety of food items, thus allowing them to switch their diet as needed. Diet switching allows wolf populations to sustain themselves, even if a certain prey's abundance becomes critically low, and reduces the predation pressure on any one prey species (Metz et al. 2012). Wolves in both the northern and southern areas increased consumption of caribou in some summers relative to other prey, but in different years. It is unlikely that caribou are important to wolves in GHA 26 because they occur at low densities, and are confined to the northeastern region (Daniel Dupont, pers. comm., Manitoba Conservation), and the median proportions of caribou in wolf diet never exceeded 25%.

Several wolves had higher nitrogen signatures than the prey included in the model, suggesting we may have omitted an important prey species. Other foods in the area that likely have high nitrogen signatures include domestic livestock, fish, and human refuse. Domestic livestock consume agricultural foods that are enriched in ^{15}N due to the use of fertilizer compared to native plant species (Bol and Pflieger 2002). However, a wolf would have to acquire a large proportion of its diet from livestock for

them to be reflected in wolf tissue. Farmers receive reparations for wolf-killed livestock (Manitoba Conservation, Predator Management) and livestock depredation is not a common occurrence in our study area. Marine and aquatic species also have elevated $\delta^{15}\text{N}$ compared to terrestrial species (DeNiro and Epstein 1981). Fisheries by-catch from Lake Winnipeg, which is adjacent to GHA 26, may also provide a food subsidy for wolves. Local residents and fisherman have observed wolves waiting nearby to scavenge by-catch as fishing boats offload their catch, although the availability of this resource is ephemeral. Human refuse is also a possible food resource for wolves. Humans eat domestic livestock and agricultural crops, so human refuse is typically enriched in ^{15}N (Bol and Pflieger 2002). Two wolves tracked by the provincial wolf monitoring program visited dump sites and scavenged on human refuse (Daniel Dupont, Manitoba Conservation, pers. comm.). Many fisherman discard fish viscera as trash, which may increase wolf $\delta^{15}\text{N}$ if wolves are scavenging at dumps. Many trappers also use unwanted fish to bait wolf traps (Manitoba Conservation, unpublished data), which may have contributed to greater consumption of fish by wolves depending on baiting duration prior to trapping.

Manitoba Conservation has taken a proactive approach to mitigate some of the potential impacts that may have influenced local moose populations. In 2011, licensed moose harvest was suspended, and with the cooperation of First Nations communities, treaty-based hunting was also restricted in 2012. Manitoba Conservation barricaded unused logging roads, blocking access into deep-forested areas. Additionally, the province launched a study on the prevalence of *Parelaphostrongylus tenuis* in white-tailed deer within Manitoba; this nematode parasite causes mortality in moose but is benign in deer who act as a carrier (Duffy et al. 2002). Lastly, beginning in 2011, the province implemented a five-year trapper incentive program that offered registered trappers a monetary incentive for each wolf carcass brought to Manitoba Conservation. The trapper incentive program was initiated to stimulate moose recovery by temporarily easing wolf predation pressure on moose populations.

Our wolf diet analyses show that despite the prominence of white-tailed deer in wolf diet, moose are still an important prey species to wolves, especially in summer. Our diet estimates may have been affected by the management actions taken against wolves in response to the decline in moose populations, if the additional removals affected wolf behavior or social structure. For example, wolf predation may have been affected by dividing a pack after the removal of a pack member, thus forcing the remaining wolves to disperse or form a new pack (Brainerd et al. 2008, Borg et al. 2015), changing the predator landscape and possibly space use by wolves. Also, the removal of a predator opens territory for competing individuals or groups, and so wolf removal may instigate recolonization or territory expansion by neighboring packs (Brainerd et al. 2008), which may change wolf foraging strategy. Moose decline in Manitoba is a complex issue that requires a multi-faceted solution. Wolf diet analysis revealed some information regarding the impact of wolves on declining moose populations and Manitoba Conservation has taken preliminary steps toward a moose management plan. Additional research on habitat suitability and prey abundance could help explain some of the trends that we have observed in wolf diet. Wolf predation pressure on moose is greatest during summer, but the presence of beaver may mitigate predation impacts on moose populations. Due to insufficient data we are unable to estimate if wolf predation on moose is additive or compensatory in these areas. However, maintaining alternate prey populations could mitigate wolf predation impacts on moose populations.

Acknowledgements

We thank Hank Hristienko, Kelly Leavesley, and Daniel Dupont at Manitoba Conservation and Water Stewardship for providing samples and logistical support for this study. We would also like to thank Manitoba Conservation and Water Stewardship, the Natural Sciences and Engineering Research Council of Canada (NSERC), and the Canada Foundation for Innovation (CFI) for providing funding. Additionally, we thank the Trophic Ecology lab at the University of Manitoba for preparation of stable isotope samples.

Literature Cited

- Barto, W.P., and C.G. Vogel. 1978. Agro-Manitoba information package. Technical Report No. 78-79. Manitoba Department of Mines, Natural Resources, and Environment, Lands and Surveys Division, Winnipeg, Manitoba, CA.
- Ben-David, M., and E. Flaherty. 1997. Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. *Oecologia* 111:280-291.
- Bland, J.M., and D.G. Altman. 1995. Multiple significance tests: the Bonferroni method. *The BMJ* 310:170.
- Bol, R., and C. Pflieger. 2002. Stable isotope analysis of the hair of modern humans and their domestic animals. *Rapid Communications in Mass Spectrometry* 16:2195-2200.
- Bohning, R.A., Bell, J.L., Bulloch, D., and A. Ascher. 2003. Manitoba's forests. Natural Resources Canada, Canadian Forest Service, Northern Forestry Centre, Edmonton, AB.
- Bond, A., and I. Jones. 2009. A practical introduction to stable-isotope analysis for seabird biologists: approaches, cautions and caveats. *Marine Ornithology* 37:183–188.
- Borg, B.L., Brainerd, S.M., Meier, T.J., and L.R. Prugh. 2015. Impacts of breeder loss on social structure, reproduction and population growth in a social canid. *Journal of Animal Ecology* 84:177-187.
- Brainerd, S.M., Adrén, H., Bangs, E.E., Bradley, E.H., Fontaine, J.A., Hall, W., Iliopoulos, Y., Jimenez, M.D., Jozwiak, E.A., Liberg, O., Mack, C.M., Meier, T.J., Niemeyer, C.C., Pedersen, H.C., Sand, H., Schultz, R.N., Smith, D.W., Wabakken, P., and A.P. Wydeven. 2008. The effects of breeder loss on wolves. *Journal of Wildlife Management* 72:89-98.
- Charnov, E.L. 1976. Optimal foraging, the marginal value theorem. *Theoretical Population Biology* 9:129-136.
- Chranowski, D.J. 1983. Winter weather and white-tailed deer in the southwestern region. Technical Report No. 83-118. Manitoba Department of Natural Resources, Brandon, Manitoba, CA.
- Darimont, C.T., Pacquet, P.C., and T.E. Reimchen. 2009. Landscape heterogeneity and marine subsidy generate extensive intrapopulation niche diversity in a large terrestrial vertebrate. *Journal of Animal Ecology* 78:126-133.
- DeNiro, M., and S. Epstein. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* 197:261–263.
- DeNiro, M.J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45:341-351.
- Derbridge, J.J., Merkle, J.A., Bucci, M.E., Callahan, P., Koprowski, J.L., Polfus, J.L., and P.R. Krausman. 2015. Experimentally derived $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ discrimination factors for gray wolves and the impact of prior information in Bayesian mixing models. *PLoS ONE* 10: e0119940.

- Duffy, M.S., Greaves, T.A., Keppie, N.J., and M.D.B. Burt. 2002. Meningeal worm is a long-lived parasitic nematode in white-tailed deer. *Journal of Wildlife Diseases* 38:448-452.
- Franzmann, A.W., and C.C. Schwartz. 1998. Ecology and management of the North American moose. Smithsonian Institution Press, Washington D.C., USA.
- Gasaway, W.C., Boertje, R.D., Grangaard, D.V., Kelleyhouse, D.G., Stephenson, R.O., and D.G. Larsen. 1992. The role of predation in limiting moose at low densities in Alaska and Yukon: implications for conservation. *Wildlife Monographs* 120:3-59.
- Jenkins, S.G., Partridge, S.T., Stephenson, T.R., Farley, S.D., and C.T. Robbins. Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. *Oecologia* 29:336-341.
- Joly, D.O., and F. Messier. 2004. The distribution of *Echinococcus granulosus* in moose: evidence for parasite-induced vulnerability to predation by wolves? *Oecologia* 140:586-590.
- Keech, M.A., Lindberg, M.S., Boertje, R.D., Valkenburg, P., Taras, B.D., Boudreau, T.A., and K.B. Beckmen. 2011. Effects of predator treatments, individual traits, and environment on moose survival in Alaska. *The Journal of Wildlife Management* 75:1361-1380.
- Kruschke, J.K. 2013. Bayesian estimation supersedes the t-test. *Journal of Experimental Psychology* 142:573-603.
- Larter, N.C., Sinclair, A.R.E., and C.C. Gates. 1994. The response of predators to an erupting bison (*Bison bison athabasca*) population. *The Canadian Field-Naturalist* 108:318-327.
- Latham, A.D.M., Latham, M.C., McCutchen, N.A., and S. Boutin. 2011. Invading white-tailed deer change wolf-caribou dynamics in northeastern Alberta. *Journal of Wildlife Management* 75:204-212.
- Latham, A.D.M., Latham, M.C., Knopff, K.H., Hebblewhite, M., and S. Boutin. 2013. Wolves, white-tailed deer, and beaver: implications of seasonal prey switching for woodland caribou declines. *Ecography* 36:1276-1290.
- Leavesley, K. 2010. Big game aerial survey report moose survey GHA 26: winter 2009-2010. Manitoba Conservation, Winnipeg, Manitoba, CA.
- Lecomte, N., Ahlstrøm, Ø., Ehrich, D., Fuglei, E., Ims, R.A., and N.G. Yoccoz. 2011. Intrapopulation variability shaping isotope discrimination and turnover: experimental evidence in arctic foxes. *PLoS ONE* 6: e21357.
- Lindell, L.E., and A. Forsman. 1996. Density effects and snake predation: prey limitation and reduced growth rate of adders at high density of conspecifics. *Canadian Journal of Zoology* 74:1000-1007.
- Locke, S.A., Bulté, G., Forbes, M.R., and D.J. Marcogliese. 2013. Estimating diet in individual pumpkinseed sunfish using stomach contents, stable isotopes and parasites. *Journal of Fish Biology* 82:522-537.

- MacAvoy, S.E., Macko, S.A., and L.S. Arneson. 2005. Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis. *Canadian Journal of Zoology* 83:631-641.
- MacAvoy, S.E., and L.S. Arneson. 2006. Correlation of metabolism with tissue carbon and nitrogen turnover rate in small mammals. *Oecologia* 150:190-201.
- Martinez del Rio, C., and R. Anderson-Sprecher. 2008. Beyond the reaction progress variable: the meaning and significance of isotopic incorporation data. *Oecologia* 156:765-772.
- Mech, L.D. 1970. The wolf: ecology and behavior of an endangered species. Natural History Press (Doubleday Publishing Co., N.Y.) 389 pp. (Reprinted in paperback by University of Minnesota Press, May 1981).
- Messier, F. and M. Crête. 1985. Moose-wolf dynamics and the natural regulation of moose populations. *Oecologia*, 65:503-512.
- Metz, M.C., Smith, D.W., Vucetich, J.A., Stahler, D.R., and R.O. Peterson. 2012. Seasonal patterns of predation for gray wolves in the multi-prey system of Yellowstone National Park. *Journal of Animal Ecology* 81:553-563.
- Müller-Schwarze, D. 2011. The beaver: its life and impact. Second Edition. Cornell University Press, Ithaca, New York, USA.
- Naughton, D. 2012. The natural history of Canadian mammals. Canadian Museum of Nature and University of Toronto Press, Toronto, CA.
- Nelson, M.E. and L.D. Mech. 1986. Relationship between snow depth and gray wolf predation on white-tailed deer. *Journal of Wildlife Management* 50:471-474.
- Parnell, A.C., Inger, R., Bearhop, S., and A.L. Jackson. 2010. Source partitioning using stable isotopes: coping with too much variation. *PloS ONE* 5:e9672.
- Roth, J.D., and K.A. Hobson. 2000. Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction. *Canadian Journal of Zoology* 78:848-852.
- Sponheimer, M., Robinson, T., Ayliffe, L., Roeder, B., Hammer, J., Passey, B., West, A., Cerling, T., Dearing, D. and J. Ehleringer. 2003a. Nitrogen isotopes in mammalian herbivores: hair $\delta^{15}\text{N}$ values from a controlled feeding study. *International Journal of Osteoarchaeology* 13:80-87.
- Sponheimer, M., Robinson, T., Ayliffe L., Passey, B., Roeder, B., Shipley, L., Lopez, E., Cerling, T., Dearing, D., and J. Ehleringer. 2003b. An experimental study of carbon-isotope fractionation between diet, hair, and feces of mammalian herbivores. *Canadian Journal of Zoology* 81:871-876.
- Stock, B.C., and B.X. Semmens. 2013. MixSIAR GUI User Manual, version 1.0. <http://conserver.iugocafe.org/user/brice.semmens/MixSIAR>.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., and N.A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues : implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* 57:32–37.

Van Ballenberghe, V., and W.B. Ballard. 1994. Limitation and regulation of moose populations: the role of predation. *Canadian Journal of Zoology* 72:2071-2077.

Wallach, A.D., Izhaki, I., Toms, J.D., Ripple, W.J., and U. Shanas. 2015. What is an apex predator? *Oikos* 000:001-009.

Zimmermann, B., Sand, H., Wabakken, P., Liberg, O., and H.P. Andreassen. 2015. Predator-dependent functional response in wolves: from food limitation to surplus killing. *Journal of Animal Ecology* 84:102-112.

Table 2.1. Sample sizes for wolves and prey species in eastern Manitoba.

Year	Wolf	Beaver	Caribou	Moose	Snowshoe hare	White-tailed deer
2010-11	16	2	0	4	0	0
2011-12	26	10	13	8	4	18
2012-13	31	19	0	6	6	0
2013-14	34	0	0	0	0	27
Total	107	31	13	18	10	45



Figure 2.1 Map of Game Hunting Area (GHA) 26, Manitoba where moose have declined and moose hunting has been canceled.

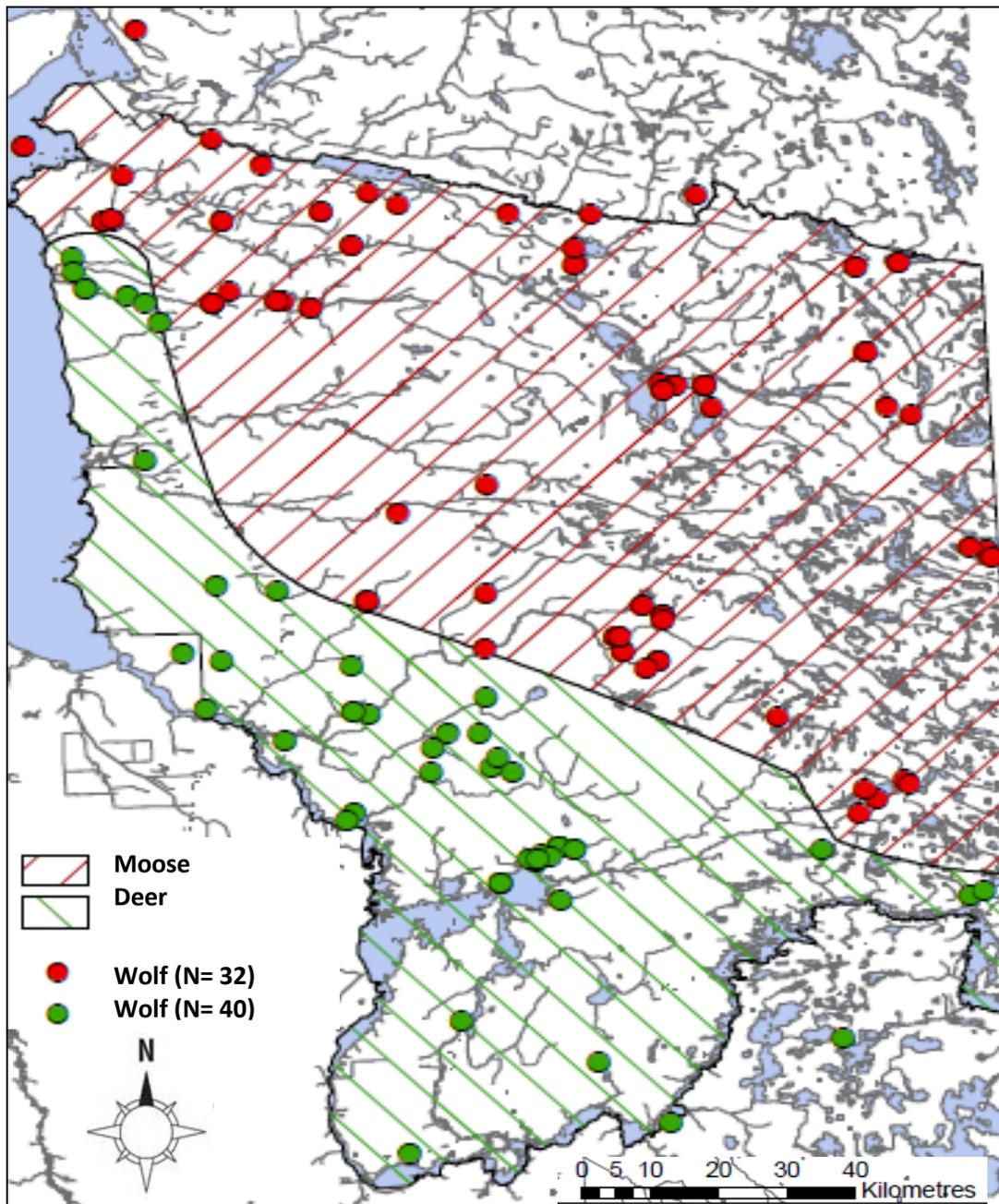


Figure 2.2 Map of GHA 26 wolves divided into northern (red) and southern (green) regions based on the predominance of either moose or white-tailed deer. Map courtesy of Daniel Dupont (Manitoba Conservation).

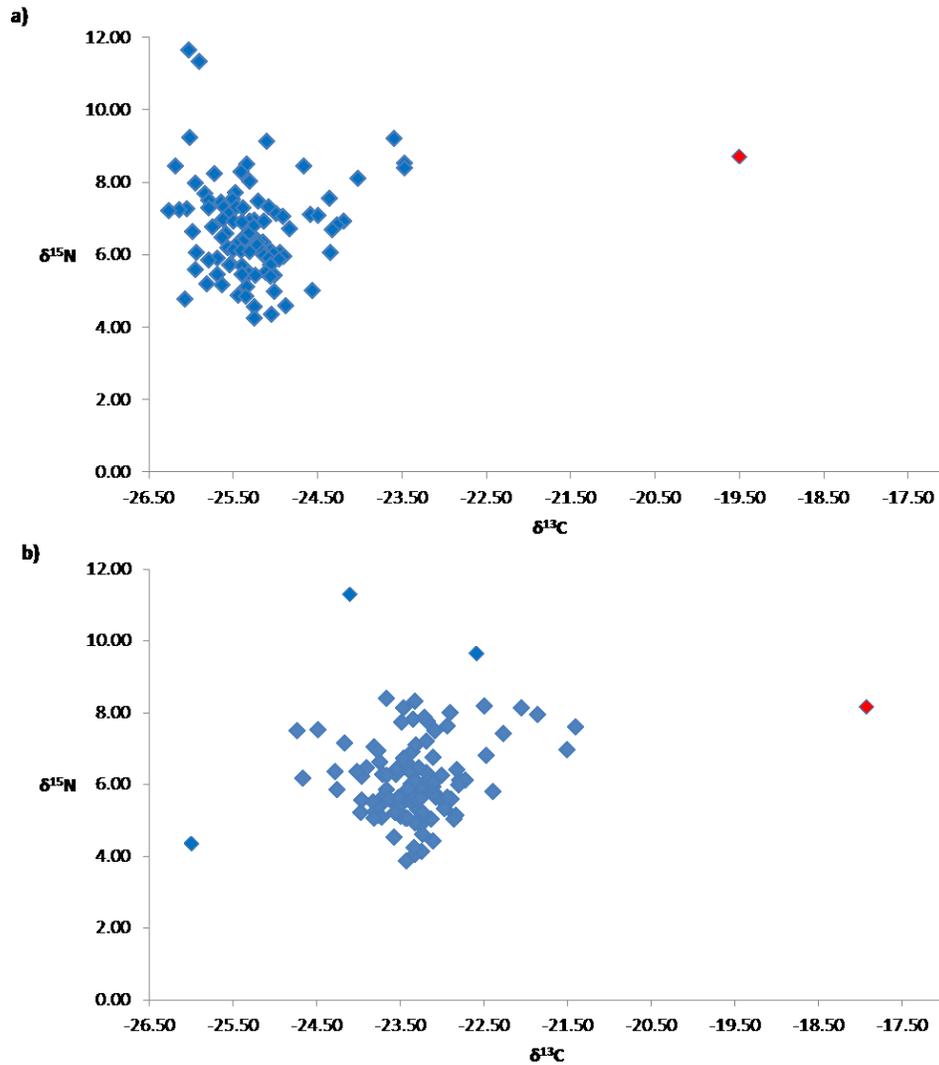


Figure 2.3 Distribution of carbon and nitrogen signatures of wolves reflecting a) winter diet (from muscle), and b) summer diet (guard hair) in GHA 26. Red markers indicate outliers that were removed because they do not reflect the diet of the local wolf population (i.e., likely immigrant wolves that had consumed different prey).

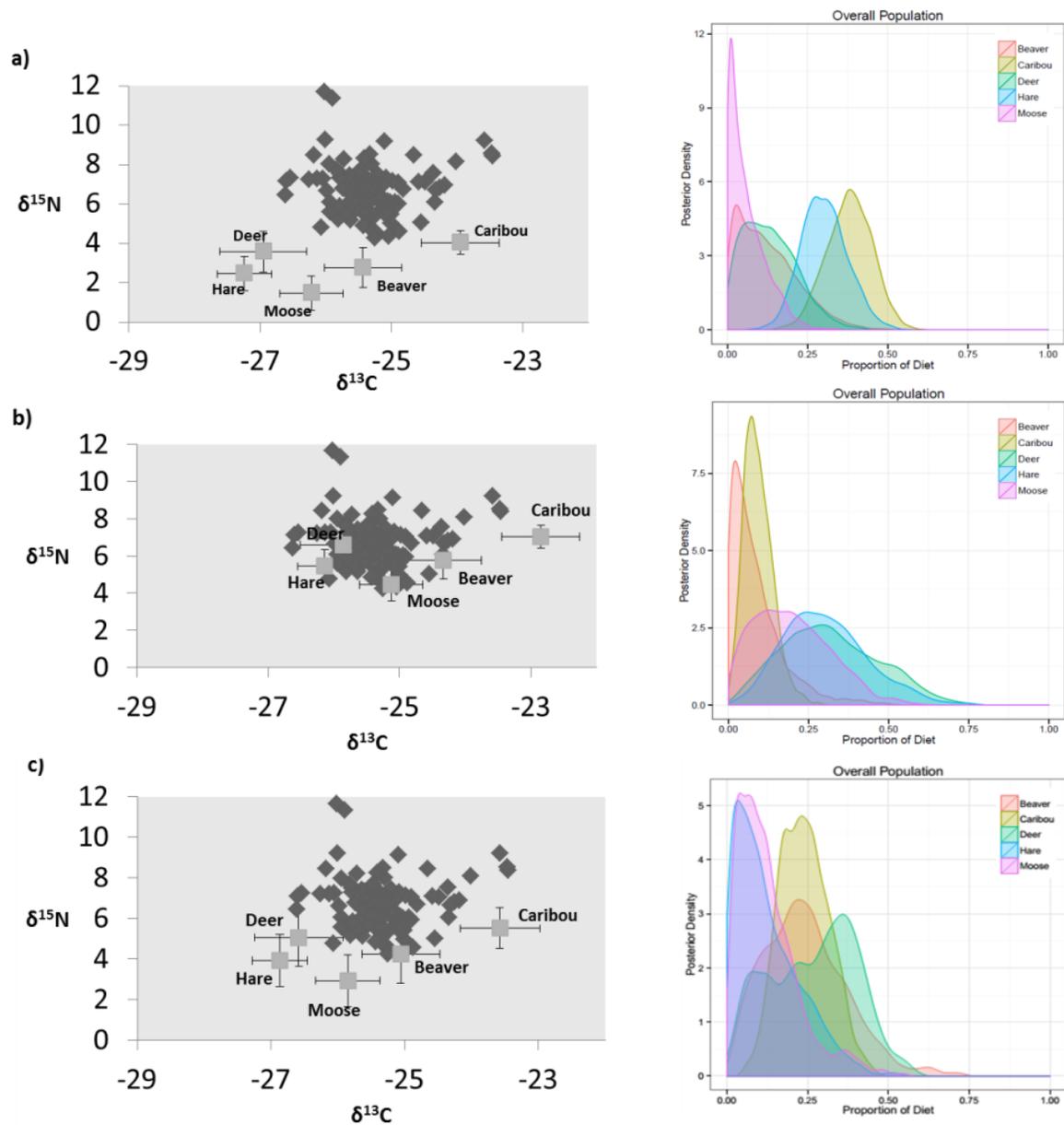
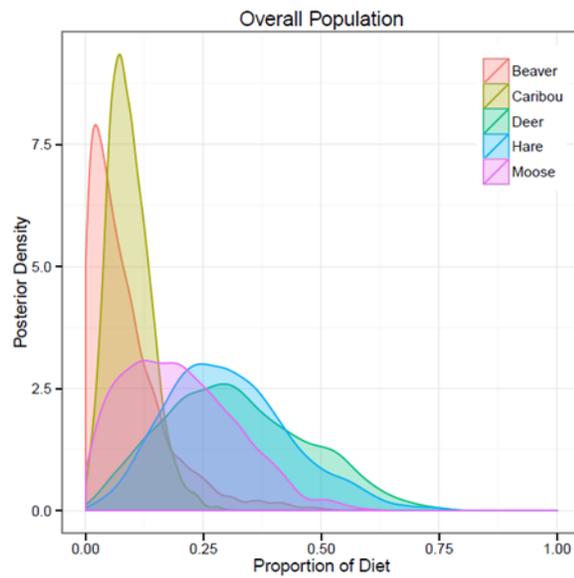


Figure 2.4 Trophic discrimination sensitivity analysis results. We ran three separate models using trophic discrimination factors (mean \pm SD) based on previously measured values for a) arctic fox ($\Delta C=0.37\pm 0.8$, $\Delta N=1.49\pm 0.4$), b) red fox ($\Delta C=1.1\pm 0.1$, $\Delta N=3.0\pm 0.1$), and c) wolves ($\Delta C=0.47\pm 0.1$, $\Delta N=2.94\pm 0.1$). The left panel contains the nitrogen and carbon iso-space plot for individual wolves (black) and mean prey signatures (\pm standard deviation) corrected using the assumed trophic discrimination values. The right panel displays the probability density plot for overall wolf winter diet. Each distribution in the right

panel corresponds with a prey species (denoted by color) and reflects the modal diet proportion (peak of distribution), as well as the associated uncertainty in each estimate (width of distribution). All models were run using uniform priors. Both models using fox values were corrected for body size differences between foxes and wolves by subtracting 0.3 from nitrogen discrimination values. Wolf muscle trophic discrimination was estimated from measured wolf hair values.

a)

Prey	Prior information
Beaver	20.0%
Caribou	20.0%
Deer	20.0%
Hare	20.0%
Moose	20.0%



b)

Prey	Prior information
Beaver	6.0%
Caribou	5.0%
Deer	21.0%
Hare	3.0%
Moose	65.0%

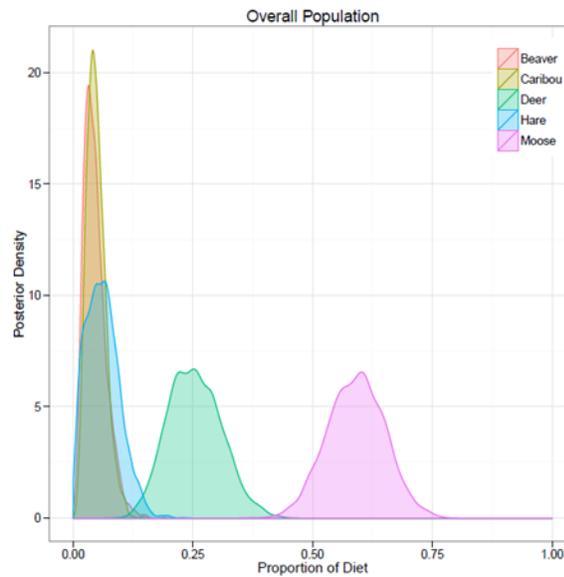


Figure 2.5 Results of the prior information sensitivity analysis using eastern wolf winter data. Winter wolf diet estimates were produced using a) uniform priors and b) informative priors. The diet estimate including informative priors shows a clearer delineation between the most likely prey species in wolf diet.

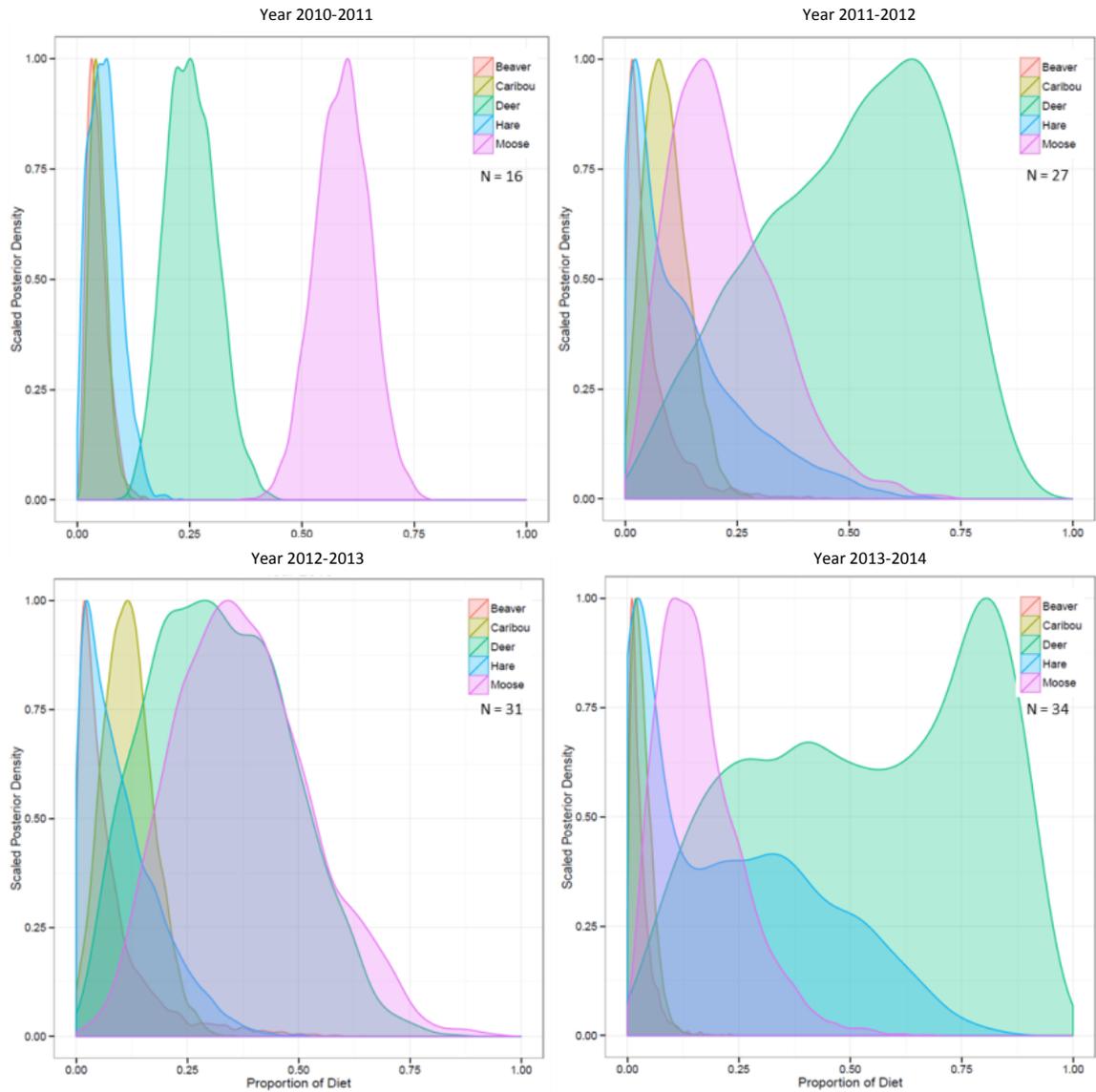


Figure 2.6 Wolf winter diet estimates from 2010-11 to 2013-14, including the posterior distributions of each prey species in wolf diet. Mid-point of the distribution represents the modal diet estimate and the total width of the distribution represents the 95% credible interval. All diet models were corrected for trophic discrimination and evaluated for model convergence. Weights assigned to each prey species to calculate priors include: beaver (6.0%), caribou (5.0%), deer (21.0%), snowshoe hare (3.0%), and moose (65.0%).

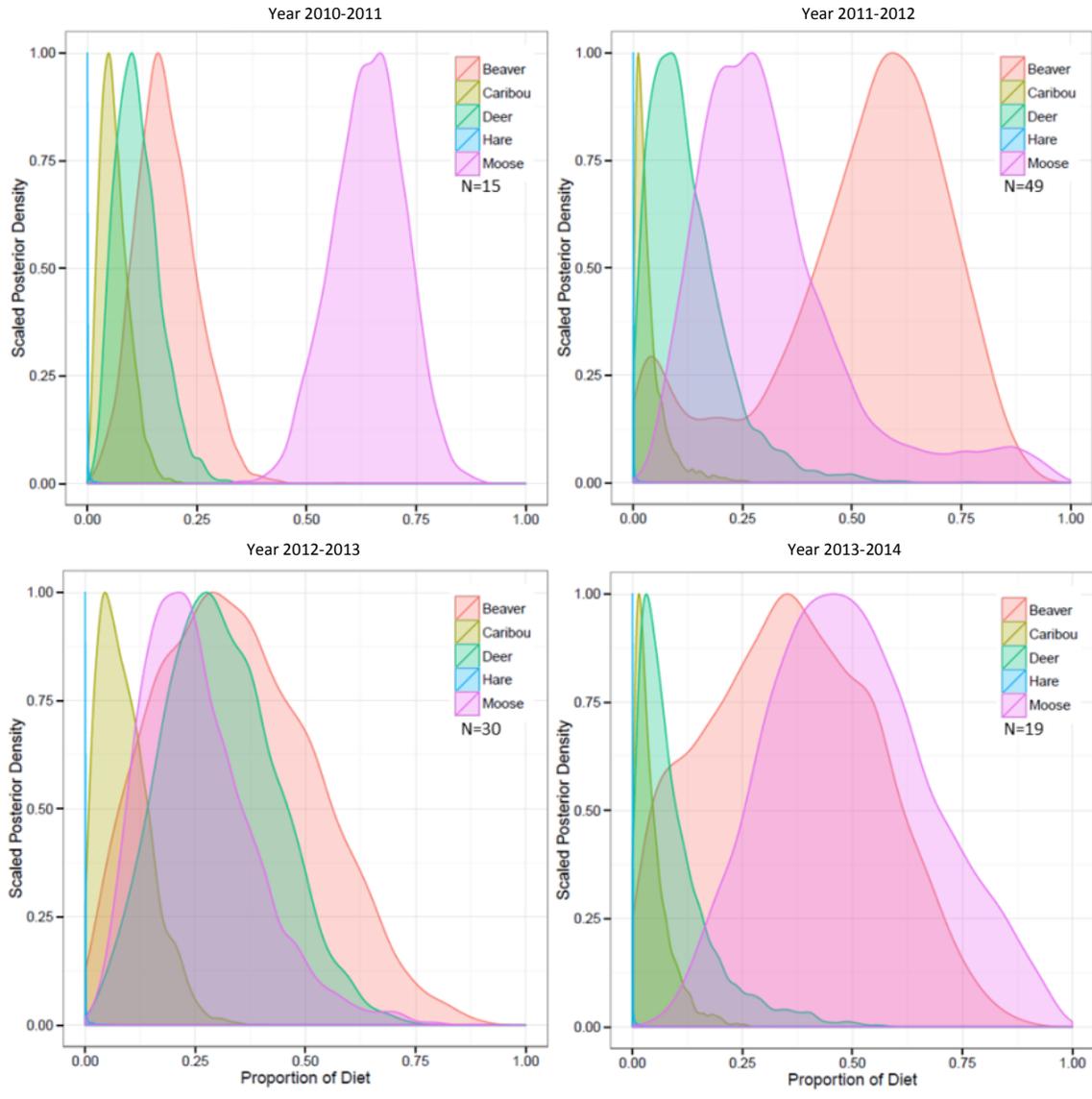


Figure 2.7 Wolf summer diet estimates from 2010 to 2013, including the posterior distributions of each prey species in wolf diet. Mid-point of distribution represents the modal diet estimate and the total width of the distribution represents the 95% credible interval. All diet models were corrected for trophic discrimination and evaluated for model convergence. Weights assigned to each prey species to calculate priors include: beaver (11.8%), caribou (7.9%), deer (7.6%), snowshoe hare (0.1%), and moose (72.6%).

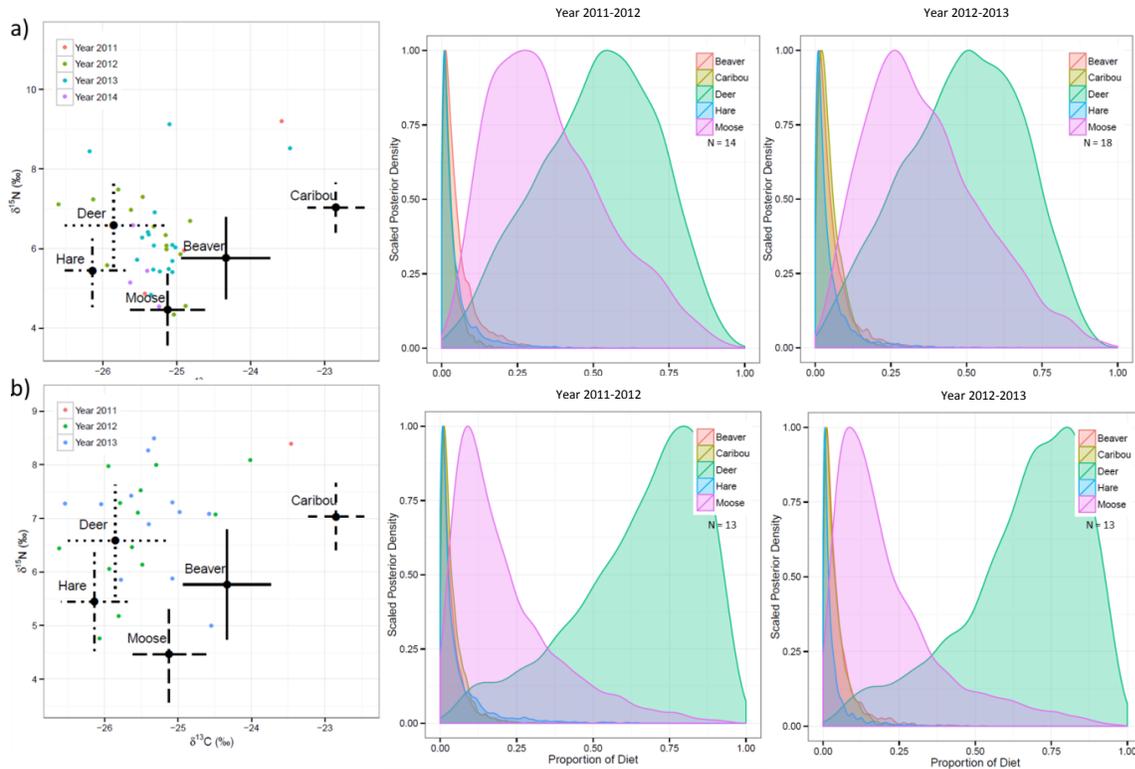


Figure 2.8 Estimates of wolf winter diet located in a) the northern section of GHA 26 and b) the southern section of GHA 26, are reflected in the posterior distributions of each prey species in wolf diet in 2011-12 and 2012-13. Mid-point of the distribution represents the modal diet estimate and the total width of the distribution represents the 95% credible interval. All diet models were corrected for trophic discrimination and evaluated for model convergence. Weights assigned to each prey species to calculate priors include: beaver (6.0%), caribou (5.0%), deer (21.0%), snowshoe hare (3.0%), and moose (65.0%).

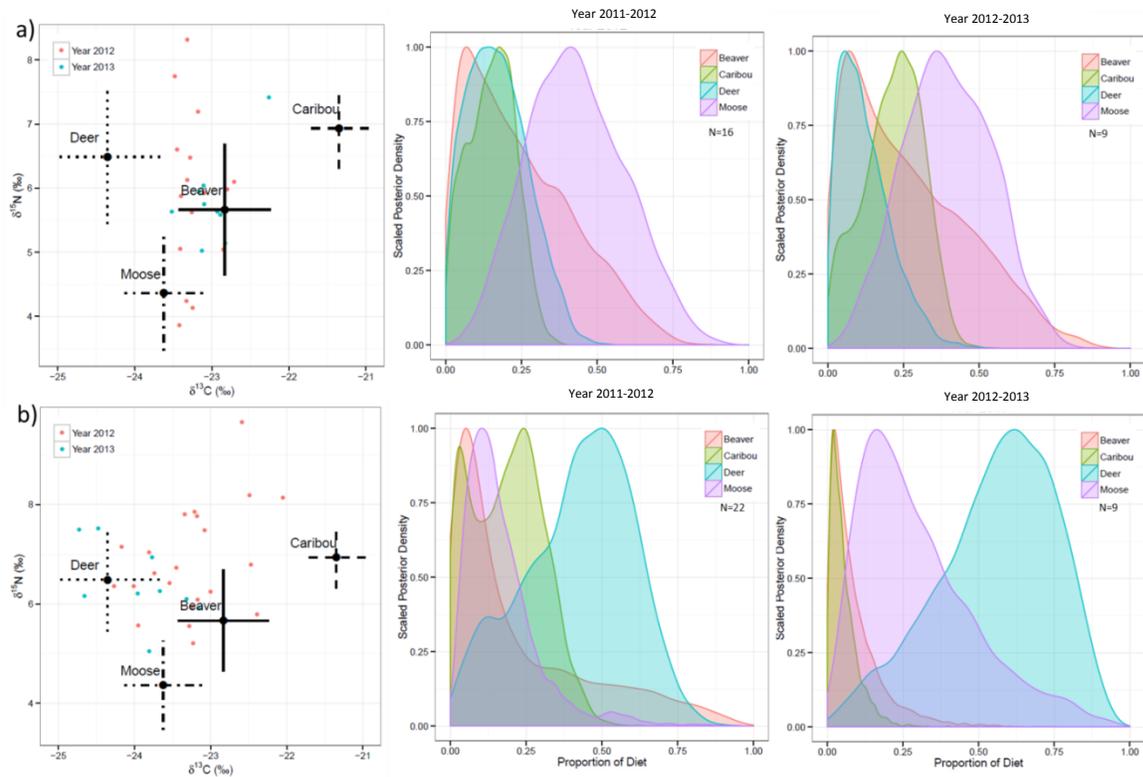


Figure 2.9 Wolf summer spatial diet analysis from 2011 and 2012 for wolves sampled in the a) northern section and b) southern section of GHA 26. Colors represent posterior distributions of each prey species in wolf diet. Mid-point of the distribution represents the modal diet estimate and the total width of the distribution represents the 95% credible interval. All diet models were corrected for trophic discrimination and evaluated for model convergence. Weights assigned to each prey species to calculate priors include: beaver (11.8%), caribou (8.0%), deer (7.6%), and moose (72.6%). Snowshoe hare were excluded from this analysis because the model would not converge.

Chapter 3: Effects of prey distribution and availability on wolf diet in a complex community

Abstract

Ungulate species can affect each other through interactions with a shared predator, such as the wolf (*Canis lupus*). These effects can be negative if the predator is a generalist and responds numerically to changes in prey availability, or positive if the predator demonstrates a preference for one prey and is limited by factors other than prey availability. Wolves in some areas select moose (*Alces americanus*) for their large body mass, but research on wolf diets in eastern Manitoba has suggested wolves preferentially select deer in winter, despite the energetic benefits of consuming larger-bodied prey, and even when moose were much more abundant locally. This preference suggests moose consumption would be even lower in areas where deer and moose are more evenly distributed, with greater spatial overlap.

We estimated wolf diet in an area of western Manitoba where moose, deer, and elk (*Cervus canadensis*) coexist and compared diet estimates to previous wolf diet reconstruction in eastern Manitoba, where moose and deer segregate spatially and elk are absent (but caribou occur at low densities). Wolf diet in western Manitoba mainly consisted of deer during winter and moose in summer, consistent with our eastern Manitoba wolf diet estimates, where beaver (*Castor canadensis*) were also important summer prey. However, in the west beaver were not important to summer diet, most likely because beaver populations have declined in this area. These results suggest wolves switch their diet based on the presence of the most easily attained prey, and the presence of beaver may reduce wolf predation on moose during summer, the season when wolves have the greatest impact on moose. Manipulating alternative prey populations may mitigate predation impacts on moose.

Introduction

Animal populations and communities can be strongly influenced by predator selection of prey, trait-mediated effects of predators, behavioral modifications of predators and prey, and the effects of apparent competition (Bonsall and Hassell 1997, Hebblewhite and Pletscher 2002, Keech et al. 2011, Montgomery et al. 2014, Stephenson et al. 2015). A generalist predator that feeds on multiple prey species or the invasion of an alternate prey species may induce apparent competition (Holt 1977, Latham et al. 2011). Apparent competition occurs when the presence of one species alters the population status of another species through changes in predation pressure from a shared predator, regardless of whether the prey species are competing (Holt 1977). Apparent competition can enhance predation on a focal prey species, a phenomenon that has been observed in many systems, and can have dire effects on threatened prey populations (Holt and Lawson 1994, Wittmer et al. 2005, Latham et al. 2011). For example, in Alberta, Canada, incidental wolf (*Canis lupus*) predation of caribou (*Rangifer tarandus*) increased with increasing moose (*Alces americanus*) abundance (Latham et al. 2011). Similarly, the encroachment of white-tailed deer (*Odocoileus virginianus*) on moose range with the advancement of agriculture and as humans moved northward (Barto and Vogel 1978) has increased prey availability for wolves, which indirectly may have increased predation pressure on other ungulates through apparent competition. However, if predators are not food-limited then a preference for one prey species will reduce predation on non-preferred prey.

Moose populations in accessible areas of southern Manitoba have declined in recent years, but the primary cause of this decline is unknown. Several strategies to mitigate additional damage to moose populations have been implemented, including a pecuniary incentive program enacted in 2011 to encourage registered trappers to harvest more wolves within the designated moose conservation zones, thereby decreasing predation pressure on moose. Other measures to conserve moose in areas of

concern include suspending licensed moose harvest, barricading unused logging roads to inhibit access into deep-forested areas, and an investigation of deer-induced ungulate disease (Chapter 2).

Recent research on wolf diets in eastern Manitoba suggested wolves preferentially consumed white-tailed deer in winter, despite the energetic benefits of consuming larger-bodied prey, even in areas where moose were much more abundant locally (Chapter 2). Compared to moose, deer may provide an alternate, more catchable prey source for wolves. This preference for deer suggests moose consumption would be even lower in areas where deer and moose are more evenly distributed because wolves that prefer deer do not have to travel long distances to procure their preferred prey. Further, wolves in nearby Riding Mountain National Park, Manitoba predominantly preyed on elk and white-tailed deer, and secondarily on moose (Paquet 1992). Under this hypothesis, we would predict that wolves in western Manitoba, where moose, deer, and elk (*Cervus canadensis*) coexist, will have a greater proportion of elk and deer in their diet and consume less moose. Alternatively, wolves searching for their preferred prey in areas where deer and moose coexist may be more likely to encounter moose, and this greater encounter rate may lead to greater wolf predation pressure on moose.

The objective of this study was to estimate wolf diet to gain insight into the effects of prey distribution and prey availability on predation. We used stable isotope analysis and Bayesian mixing models to estimate wolf diet in a region of western Manitoba where moose populations have declined. We compared western Manitoba wolf diet estimates to previous wolf diet reconstruction in eastern Manitoba, where moose and deer are spatially segregated and elk are absent. Winter survival of ungulates has been linked to winter severity in northern ecosystems (Mech et al. 2001, DelGiudice et al. 2002), so we also examined the relationship between deer winter severity indices and wolf diet. We would expect wolves to consume more deer in severe winters compared to milder winters because deer are less well-adapted to deep snow and cold temperatures relative to moose.

Methods

Study Site and Sample Collection

Samples of wolves, moose, white-tailed deer, elk, and other prey (snowshoe hare, *Lepus americanus* and beaver, *Castor canadensis*) were collected by Manitoba Conservation from trappers, hunters, and road kill in an area of moose population decline in western Manitoba. This region (centered at 100°59'0.373"W, 51°38'58.902"N) includes the Porcupine Mountains (Game Hunting Areas (GHA) 13-13A), the Duck Mountains (GHA 18-18C), and the Swan-River area (GHA 14-14A) (Figure 3.1). Hair and muscle samples from several years were collected for both wolves and prey species (Table 3.1), although small sample sizes of wolves in 2007-08, 2008-09, and 2009-10 precluded a rigorous diet analysis for those years. Wolf trapping occurs from October 14 to March 31. We categorized our samples based on the calendar at the end of the trapping season (e.g., any wolves harvested between October 2010 and March 2011 were considered year 2011).

The study area is dominated by boreal plains forest and is surrounded by agricultural development. The dominant tree species in these boreal plains forests are: white spruce (*Picea glauca*), black spruce (*Picea mariana*), balsam fir (*Abies balsamea*), trembling aspen (*Populus tremuloides*), and various willow (*Salix* spp.) species. Elk, white-tailed deer, and moose are the three main ungulate species, and all three coexist.

Diet reconstruction

Stable isotope analysis is useful for reconstructing animal diets because it measures the long-term diet of a consumer by comparing stable isotope ratios in tissues of consumers and their prey (Ben-David and Flaherty 1997). Various tissues turn over at different rates, and so using multiple tissues we can estimate the annual diet of a consumer over different timeframes. We measured carbon and nitrogen stable isotope ratios of wolf muscle and hair and used them to estimate winter (approx. Nov-Mar) and summer (approx. May-Aug) diet of wolves in west-central Manitoba, Canada with a Bayesian

stable isotope mixing model known as MixSIAR. This multivariate hierarchical Bayesian mixing model uses mass balance, Bayesian inference, and Markov Chain Monte Carlo (MCMC) methods to estimate proportional consumer diet from consumer and prey stable isotope ratios (Stock and Semmens 2013). We tested for differences in prey stable isotope ratios with a MANOVA, ANOVA, and Tukey's HSD post hoc analysis, controlling for year and region. We also ran two sensitivity analyses to examine the effect of trophic discrimination and prior information on our diet models (Appendix A).

All prey signatures were corrected for trophic discrimination ($\Delta C=1.1\pm 0.1$, $\Delta N=3.0\pm 0.1$, in winter and $\Delta C=2.6\pm 0.1$, $\Delta N=2.9\pm 0.1$, in summer; Chapter 2). Prior information used in MixSIAR for western wolf diet was derived from a preliminary wolf scat analysis conducted in Duck Mountain Provincial Park by Manitoba Conservation and Parks Canada that was corrected for prey biomass using the conversion equation $Y = 0.439 + 0.008X$ (Weaver 1993), where X is the mean body mass of the prey species and Y is the prey mass consumed per scat. For each model we ran three chains consisting of 50,000 iterations, with a burn-in of 25,000 and thinned every 25th iteration. We determined model convergence using the Gelman-Rubin and Geweke diagnostic tests, as well as trace plots produced by MixSIAR (Stock and Semmes 2013).

We compared the proportional diets of wolves in western Manitoba to previous estimates of wolf diets from eastern Manitoba (Chapter 2). We also compared our diet estimates with the winter severity index (WSI) for white-tailed deer used by Manitoba Conservation. This index is derived by taking daily temperature and snow depth measurements starting in November, and applying one point per day that the ambient temperature was $\leq -7^{\circ}\text{C}$, and another point for each day that snow depth $\geq 35\text{cm}$ (Baccante and Woods 2010). These thresholds used to gather data are specifically for white-tailed deer, and are summed at the end of the season (April) resulting in the winter severity index for deer. The index ranges from 1 to ≥ 180 , with 1-49 as the most mild and >180 as most severe.

Results

Methods validation

We found no annual differences among western prey species (Pillai's Trace, $F_{12, 272} = 0.846$, $p = 0.60$) and no interaction between species and year ($F_{8, 272} = 1.26$, $p = 0.27$), so prey samples from different years were combined for subsequent analyses. Moose and elk did not differ in $\delta^{13}\text{C}$ (Tukey's HSD, $p = 0.99$), but their $\delta^{15}\text{N}$ values differed ($p = 0.0001$). Moose and beaver differed in $\delta^{13}\text{C}$ ($p = 0.02$), but not in $\delta^{15}\text{N}$ ($p = 0.14$). Beaver, deer, and snowshoe hare differed in $\delta^{13}\text{C}$ ($p \leq 0.0004$). $\delta^{15}\text{N}$ of beaver differed from deer and elk ($p \leq 0.0001$), but not snowshoe hare ($p = 0.66$). Because all western Manitoba wolf prey species differed isotopically in at least one element we concluded that western prey are isotopically distinct.

We also compared our prey signatures to previous isotopic data for the same species measured from eastern Manitoba (excluding elk and caribou, which do not exist in both regions). Prey muscle signatures differed between eastern and western Manitoba (ANOVA, $F_{2, 190} = 39.4$, $p \leq 0.0001$), and there was an interaction between species and region (Pillai's Trace, $F_{6, 382} = 6.85$, $p \leq 0.0001$). $\delta^{13}\text{C}$ did not differ between regions for beaver ($t_{49} = 1.67$, $p = 0.94$), but $\delta^{15}\text{N}$ did differ ($t_{43} = -2.63$, $p = 0.006$). Snowshoe hare differed in $\delta^{13}\text{C}$ ($t_{11} = -2.88$, $p = 0.015$) but not $\delta^{15}\text{N}$ ($t_{11} = 1.02$, $p = 0.84$). Moose from eastern and western Manitoba did not differ in either $\delta^{13}\text{C}$ ($t_{28} = 2.20$, $p = 0.99$) or $\delta^{15}\text{N}$ ($t_{28} = 3.50$, $p = 0.99$). Similarly, neither $\delta^{13}\text{C}$ ($t_{95} = 1.80$, $p = 0.96$) nor $\delta^{15}\text{N}$ ($t_{95} = 4.50$, $p = 1.0$) differed spatially in white-tailed deer. The raw stable isotope values for all prey (only muscle) and wolves (muscle and guard hair) are reported in Table 3.2.

Proportional diet results

Overall, in western Manitoba where moose and deer coexist, wolves ate mainly deer (median diet proportion, 25-60%) in winter (Figure 3.2) and moose (30-40%) during summer (Figure 3.3). However, there was some annual variation in diet in both winter and summer. In winter 2011 wolves

predominantly ate elk (66%, 95% credible interval [21-90%]), while deer remained the most important prey in the other three years. Yet winter severity did not affect winter diet, as deer were prominent in wolf diet during years of both severe and mild winter weather (Figure 3.4). Snowshoe hare were also important prey from 2011-12 to 2013-14 during winter. Although moose were the most important summer prey overall, in 2012-13 deer were consumed in similar amounts to moose during summer, and deer became the most important prey in summer 2013-14.

Winter moose consumption by wolves in this area was lower overall compared to wolves in eastern Manitoba, where moose and deer are spatially segregated, with less inter-annual variability (Table 3.3). Moose consumption in summer was similar between the two regions overall, but with greater variability between years in eastern Manitoba (Table 3.3). Beaver consumption in summer was much lower in western Manitoba.

Discussion

Wolves were less likely to prey on moose during winter in western Manitoba, where moose coexist with deer and elk, than in eastern Manitoba, where moose are spatially segregated from deer. Instead, western Manitoba wolves focused on elk and deer during winter, despite the presence of moose. These results are consistent with a study in nearby Riding Mountain National Park (RMNP), where wolves preferentially preyed on elk and deer, and secondarily ate moose during winter (Paquet 1992). Hunting is not allowed in Riding Mountain National Park, and so interactions between wolves and their prey are not interrupted by regulated hunting. Our findings and the data collected by Paquet 1992 suggest that wolves prefer smaller ungulates, even when moose, which are considered energetically optimal prey for wolves (Wittmer et al. 2005), are available. These results are also consistent with Potvin and Jolicouer (1988), who found that in areas where moose and deer were present, wolves primarily ate deer, regardless of their density during winter. Kunkel and Pletscher (2001) also found that wolves in Montana killed more deer in areas with lower deer densities compared to areas with higher deer

densities. The recent decline in deer harvest in Manitoba suggests deer population numbers may be low following several severe winters (Hank Hristienko, Manitoba Conservation, pers. comm.), but our results suggest the apparent low deer densities do not deter wolves from consuming deer in winter.

These results support our prediction that wolf predation on moose would be greater if moose were spatially segregated from the wolves' preferred prey (as in eastern Manitoba) due to the costs of travel. Higher encounter rates with moose that were expected in the area with a more even distribution of moose and deer did not appear to increase predation on moose in winter, potentially due to different microhabitat preferences by deer and moose within the study area. Latham et al. (2013), on the other hand, found that seasonal differences in wolf diet were linked with incidental wolf predation of caribou, whose population suffered from apparent competition with moose.

The annual and seasonal changes in wolf diet we observed may be a result of changes in prey abundance and accessibility over time. In particular, the impact of winter on deer behavior and movement may have played a role in high deer proportions in wolf winter diet. In the colder months white-tailed deer group together, and in severe winter weather conditions deer may become vulnerable to predation through malnutrition, impeded movement in deep snow, and the effects of consecutive harsh winters (Kunkel and Pletscher 2001, Delgiudice et al. 2002, Kunkel et al. 2004, Taillon et al. 2006). Prey capture efficiency can increase during severe winters for wolves, because deer are not well-adapted to deep snow and prolonged cold temperatures (Fuller 1991; Huggard et al. 1993). However, deer were prominent in wolf diet during years of both severe and mild winter weather (Figure 3.4), indicating that winter severity for deer did not affect wolf prey selection. For example, wolves primarily ate elk and moose in the winter of 2010-11 (the highest WSI in 13 years of data), and in the following year wolves ate mainly deer, despite it being a mild winter (low WSI). Our results disagree with Fuller (1991), who found that wolves consumed a greater number of deer when snow levels were high compared to low snow levels. The absence of an effect of winter severity for deer on wolf diet may

indicate that deer densities were high enough to remain stable post winter (Simard et al. 2010), or that prey selection by wolves is unaffected by winter weather conditions, which parallels a study by Potvin and Jolicoeur (1988), who established that wolves primarily ate deer during winter regardless of deer density.

Wolves in eastern Manitoba, where moose and deer are spatially segregated, consumed high proportions of moose and beaver during summer (Chapter 2). The presence of beaver may decrease predation pressure on moose if wolves eat mainly beaver during summer. Beaver are a common ephemeral prey resource because they are less accessible in winter until the ice melts in spring and they can forage on land where wolves may easily hunt them (Müller-Schwarze 2011).

However, beaver were not important for wolves in western Manitoba, where moose and deer were the most important prey. Tremblay et al. (2001) found that wolf diet can change with fluctuations in beaver density, so the difference in beaver consumption between the two wolf populations may be due to differences in relative beaver abundance. Trapper harvest data suggest beaver populations are declining in Manitoba (Manitoba Conservation, Figure 3.5). More specifically, aerial surveys by Parks Canada show that beaver population numbers have declined in Riding Mountain National Park, MB, which is directly south of our western study site (Sallows 2013). It is possible that the encounter rate between wolves and moose increased as wolves foraged for beaver because beaver and moose coexist in similar areas while feeding on aquatic plants during summer. Similarly, Latham et al. (2013) found that wolves that prey on beaver in summer were more likely to encounter caribou, resulting in an increase in wolf predation of caribou.

Moose and deer were similarly important to western wolf diet during summer. Zimmermann (2013) found that wolves in Scandinavia increased their consumption rate of moose in summer over winter, but the percent biomass of moose in wolf diet remained the same, indicating that wolves likely consumed moose calves in summer. Following birth in spring, moose calves remain with the cow until

the next birthing, and are extremely vulnerable to bear and wolf predation in summer (Franzmann and Schwartz 1998, Boutin 1992). Zimmermann (2013) found that wolf consumption rates of moose during summer decreased as summer progressed, perhaps due to fewer moose present on the landscape or an increase in calf biomass. Wolf predation of moose calves can have limiting effects on moose populations (Bergerud et al. 1983).

Our data suggest summer is the period of highest predation risk for moose in Manitoba. Demographic data on moose populations would be useful to understand the impact of wolf predation on moose calves. Maintaining high densities of beaver could lessen wolf predation pressure on moose during summer in areas where moose and beaver coexist. Additional experimental studies that manipulate the availability of deer to wolves throughout winter and summer would help explain the role deer play in this complex wolf-moose-deer system. Our examination of wolf-moose-deer interactions provides insight into the possible mechanisms behind wolf predation of moose in a human-influenced community.

Acknowledgements

We thank Hank Hristienko, Rich Davis, Brian Kiss, Brent Fuchs, and Gerald Shelemy at Manitoba Conservation and Water Stewardship for providing samples, logistical support, and funding for this study. We would also like to thank the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canada Foundation for Innovation (CFI) for providing funding. Additionally, we thank the Trophic Ecology lab at the University of Manitoba for preparation of stable isotope samples.

Literature Cited

- Baccante, D., and R. Woods. 2010. Relationship between winter severity and survival of mule deer fawns in the Peace Region of British Columbia. *BC Journal of Ecosystems and Management* 10:145-153.
- Barto, W.P., and C.G. Vogel. 1978. Agro-Manitoba information package. Technical Report No. 78-79. Manitoba Department of Mines, Natural Resources, and Environment, Lands and Surveys Division, Winnipeg, Manitoba, CA.
- Bearhop, S., Waldron, S., Votier, S.C., and R.W. Furness. 2013. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiological and Biochemical Zoology* 75:451–458.
- Ben-David, M., and E. Flaherty. 1997. Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. *Oecologia* 111:280-291.
- Ben-David, M., and E. Flaherty. 2012. Stable isotopes in mammalian research: a beginner's guide. *Journal of Mammalogy* 93:312–328.
- Bergerud, A.T., Wyett, W., and B. Snider. 1983. The role of wolf predation in limiting a moose population. *Journal of Wildlife Management* 47:977-988.
- Bonsall, M.B., and M.P. Hassell. 1997. Apparent competition structures ecological assemblages. *Nature* 388:371-373.
- Boutin, S. 1992. Predation and moose population dynamics: a critique. *Journal of Wildlife Management* 56:116-127.
- DelGiudice, G.D., Riggs, M.R., Joly, P., and W. Pan. 2002. Winter severity, survival, and cause-specific mortality of female white-tailed deer in north-central Minnesota. *Journal of Wildlife Management* 66:698-717.
- Franzmann, A.W., and C.C. Schwartz. 1998. Ecology and management of the North American moose. Smithsonian Institution Press, Washington D.C., USA.
- Fuller, T.K. 1991. Effect of snow depth on wolf activity and prey selection in north central Minnesota. *Canadian Journal of Zoology* 69:283-287.
- Hebblewhite, M., and D.H. Pletscher. 2002. Effects of elk group size on predation by wolves. *Canadian Journal of Zoology* 80:800-809.
- Holt, R.D. 1977. Predation, apparent competition, and the structure of prey communities. *Theoretical Population Biology* 12:197-229.
- Holt, R.D., and J.H. Lawton. 1994. The ecological consequences of shared natural enemies. *Annual Review of Ecology and Systematics* 25:495-520.
- Huggard, D.J. 1993. Effect of snow depth on predation and scavenging by gray wolves. *Journal of Wildlife Management* 7:382-388.

- Keech, M.A., Lindberg, M.S., Boertje, R.D., Valkenburg, P., Taras, B.D., Boudreau, T.A., and K.B. Beckmen. 2011. Effects of predator treatments, individual traits, and environment on moose survival in Alaska. *Journal of Wildlife Management* 75:1361-1380.
- Kunkel, K.E., and D.H. Pletscher. 2001. Winter hunting patterns of wolves in and near Glacier National Park, Montana. *Journal of Wildlife Management* 65:520-530.
- Kunkel, K.E., Pletscher, D.H., Boyd, D.K., Ream, R.R., and M.W. Fairchild. 2004. Factors correlated with foraging behavior of wolves in and near Glacier National Park, Montana. *Journal of Wildlife Management* 68:167-178.
- Lankester, M.W. 2010. Understanding the impact of meningeal worm, *Parelaphostrongylus tenuis*, on moose populations. *Alces* 46:53-70.
- Latham, A.D.M., Latham, M.C., McCutchen, N.A., and S. Boutin. 2011. Invading white-tailed deer change wolf-caribou dynamics in northeastern Alberta. *Journal of Wildlife Management* 75:204-212.
- Latham, A.D.M., Latham, M.C., Knopff, K.H., Hebblewhite, M. and S. Boutin. 2013. Wolves, white-tailed deer, and beaver: implications of seasonal prey switching for woodland caribou declines. *Ecography* 36:1276-1290.
- Mech, L.D., Smith, D.W., Murphy, K.M., and D.R. MacNulty. 2001. Winter severity and wolf predation on a formerly wolf-free elk herd. *Journal of Wildlife Management* 65:998-1003.
- Montgomery, R.A., Vucetich, J.A., Roloff, G.J., Bump, J.K., and R.O. Peterson. 2014. Where wolves kill moose: the influence of prey life history dynamics on the landscape ecology of predation. *PLoS ONE* 9: e91414.
- Müller-Schwarze, D. 2011. Mortality and predators. Pages 123-127 in Müller-Schwarze, editor. *The beaver its life and impact*, Second Edition. Cornell University Press, Ithaca, New York, USA.
- Paquet, P. 1992. Prey use strategies of sympatric wolves and coyotes in Riding Mountain National Park, Manitoba. *Journal of Mammalogy* 73:337-343.
- Potvin, F., and H. Jolicoeur. 1988. Wolf diet and prey selectivity during two periods for deer in Quebec: decline versus expansion. *Canadian Journal of Zoology* 66:1274-1279.
- Sallows, T. 2013. Riding Mountain National Park of Canada - Aerial Beaver Survey 2013. Unpublished Report. Resource Conservation, Manitoba, CA.
- Serrouya, R., Wittmann, M.J., McLellan, B.N., Wittmer, H.U., and S. Boutin. 2015. Using predator-prey theory to predict outcomes of broadscale experiments to reduce apparent competition. *American Naturalist* 185:000.
- Simard, M.A., Coulson, T., Gigras, A., and S.D. Côté. 2010. Influence of density and climate on population dynamics of a large herbivore under harsh environmental conditions. *Journal of Wildlife Management* 74:1671-1685.

- Stephenson, J.F., Van Oosterhout, C., Mohammed, R.S., and J. Cable. 2015. Parasites of Trinidadian guppies: evidence for sex- and age-specific trait-mediated indirect effects of predators. *Ecology* 96:489-498.
- Stock, B.C., and B.X. Semmens. 2013. MixSIAR GUI User Manual, version 1.0. <http://conserver.iugocafe.org/user/brice.semmens/MixSIAR>.
- Taillon, J., Sauvé, D.G., and S.D. Côté. 2006. The effects of decreasing winter diet quality on foraging behavior and life-history traits of white-tailed deer fawns. *Journal of Wildlife Management* 70:1445-1454.
- Thompson, A.K., Samuel, M.D., and T.R. Van Deelen. 2006. Alternative feeding strategies and potential disease transmission in Wisconsin white-tailed deer. *Journal of Wildlife Management* 72:416-421.
- Tremblay, J.P., Jolicoeur, H., and R. Lemieux. 2001. Summer food habits of gray wolves in the boreal forest of the Lac Jacques-Cartier Highlands, Québec. *Alces* 37:1-12.
- Weaver, J.L. 1993. Refining the equation for interpreting prey occurrence in gray wolf scats. *Journal of Wildlife Management* 57:534-538.
- Wittmer, H.U., Sinclair, R.E., and B.N. McLellan. 2005. The role of predation in the decline and extirpation of woodland caribou. *Oecologia* 144:257-267.
- Zimmermann, B. 2013. Predatory behavior of wolves in Scandinavia. PhD Thesis, Hedmark University College, Hedmark, Norway.

Table 3.1 Sample sizes for wolves and prey species in western Manitoba.

Year	Wolf	Beaver	Elk	Moose	Snowshoe hare	White-tailed deer
2007-08	1	0	0	1	0	0
2008-09	10	0	0	2	0	0
2009-10	4	0	1	2	0	0
2010-11	23	4	0	7	1	1
2011-12	88	16	44	0	2	28
2012-13	74	0	0	0	0	19
2013-14	28	0	19	0	0	4
Total	228	20	64	12	3	52

Table 3.2 Raw $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures for all prey (only muscle) and wolves (muscle and hair) from samples collected in 2010-11 to 2013-14.

Year	Prey	Western Manitoba		Eastern Manitoba	
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
2011	Beaver	-25.05±0.38	3.24±0.81	-25.52±0.55	2.22±0.52
	Caribou				
	Deer				
	Elk	-25.68	8.19		
	Moose	-25.47±0.91	5.91±2.4	-26.05±0.55	1.34±0.95
	Hare	-28.44	1.63		
	Wolf (muscle)	-25.57±0.78	6.40±2.2	-25.10±0.81	6.60±1.4
	Wolf (hair)	-24.23±0.41	5.72±0.77	-23.32±0.56	6.01±1.1
2012	Beaver	-25.24±0.39	1.70±0.68	-25.03±0.46	2.47±0.51
	Caribou			-23.95±0.36	3.73±0.59
	Deer	-25.52±1.0	5.97±2.1	-26.88±0.70	3.61±1.1
	Elk	-25.55±0.89	5.57±2.3		
	Moose			-26.10±0.48	1.40±0.89
	Hare	-27.97±0.52	3.87±0.32	-27.46±0.48	2.85±0.40
	Wolf (muscle)	-25.32±0.98	7.17±2.1	-25.14±1.0	6.70±1.4
	Wolf (hair)	-23.48±1.4	6.52±1.7	-23.31±0.8	6.18±1.3
2013	Beaver			-25.64±0.52	2.96±1.2
	Caribou				
	Deer	-25.62±1.0	5.89±2.2	-27.01±0.64	3.57±0.99
	Elk	-25.85±0.43	4.97±1.2		
	Moose			-26.47±0.38	1.59±0.82
	Hare			-25.51±0.41	2.79±0.87
	Wolf (muscle)	-25.63±0.64	7.62±1.5	-25.38±0.60	7.32±1.2
	Wolf (hair)	-23.58±0.49	6.87±1.6	-23.49±0.80	6.28±0.98
2014	Beaver				
	Caribou				
	Deer	-26.99±0.62	3.71±1.1		
	Elk	-25.85±0.38	4.97±1.1		
	Moose				
	Hare				
	Wolf (muscle)	-25.61±0.66	7.30±1.5	-25.02±0.82	6.59±1.5
	Wolf (hair)	-25.53±0.60	6.74±1.6	-23.34±0.55	6.11±1.4

Table 3.3 Comparison of wolf consumption (median and 95% Credible Interval) of common prey (moose, deer, and beaver) between western and eastern Manitoba in a) winter and b) summer from 2010-2011 to 2013-2014.

a) Summer		West		East	
Year	Prey	Median Diet Proportion (%)	95% Credible Interval	Median Diet Proportion (%)	95% Credible Interval
2010-11	Moose	46	15-77%	64	48-80%
	Deer	18	4-47%	11	4-23%
	Beaver	4	0.6-19%	17	6-32%
2011-12	Moose	47	19-73%	28	9-85%
	Deer	20	4-43%	11	1-36%
	Beaver	7	1-21%	56	3-82%
2012-13	Moose	47	21-69%	24	7-57%
	Deer	41	22-62%	30	8-58%
	Beaver	3	0.4-14%	33	5-71%
2013-14	Moose	26	4-61%	49	17-87%
	Deer	48	19-75%	7	0.7-37%
	Beaver	12	1-36%	36	3-73%
b) Winter		Western Manitoba		Eastern Manitoba	
Year	Prey	Median diet	95% CI	Median diet	95% CI
2010-11	Moose	9	1-36%	59	48-71%
	Deer	18	3-57%	25	15-37%
	Beaver	1	0.1-6%	4	1-10%
2011-12	Moose	13	4-29%	20	5-50%
	Deer	3	14-55%	52	10-82%
	Beaver	3	0.3-8%	4	0.3-24%
2012-13	Moose	6	2-16%	37	12-71%
	Deer	69	50-86%	32	7-63%
	Beaver	1	0.1-5%	5	0.5-34%
2013-14	Moose	23	4-57%	15	3-39%
	Deer	45	11-77%	56	8-91%
	Beaver	3	0.2-17%	2	0.2-10%

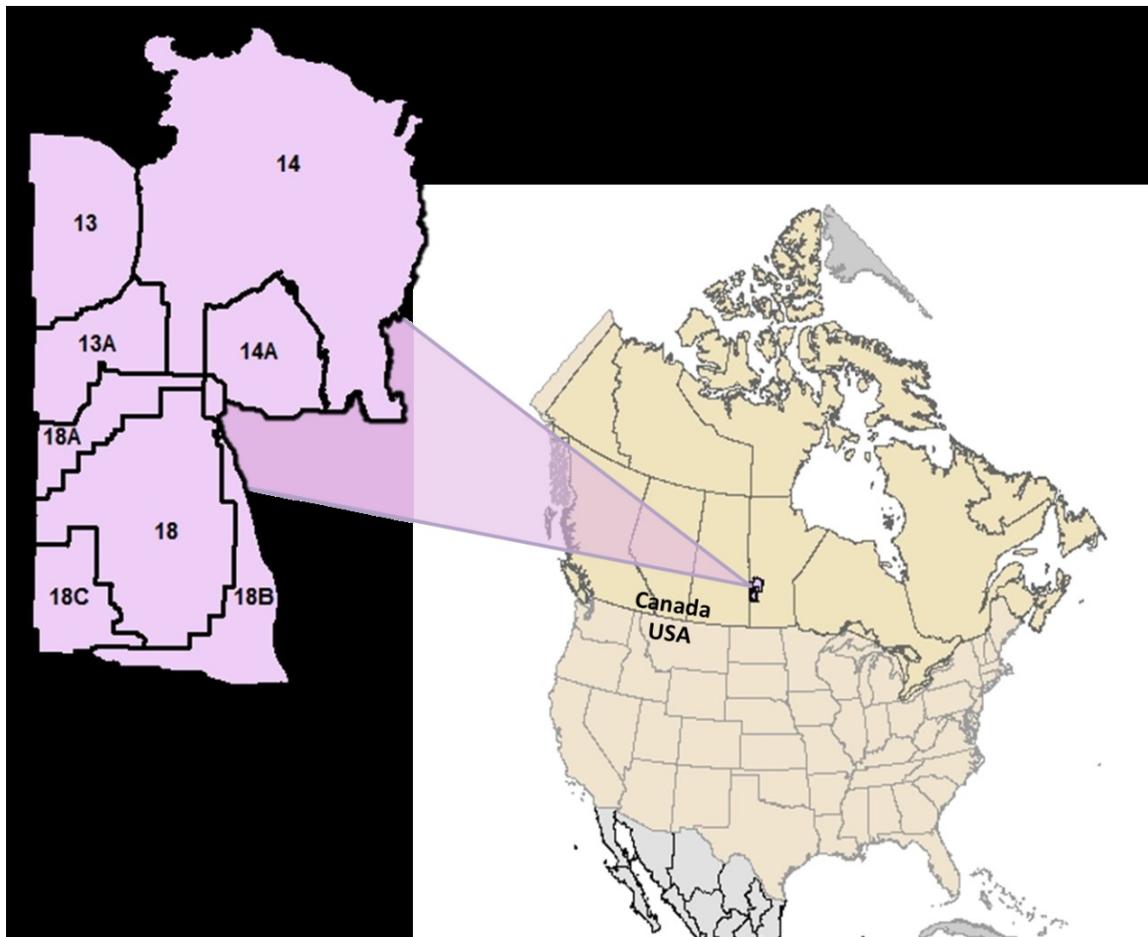


Figure 3.1. Map of Manitoba and the western Game Hunting Areas where moose have declined and moose hunting has been canceled.

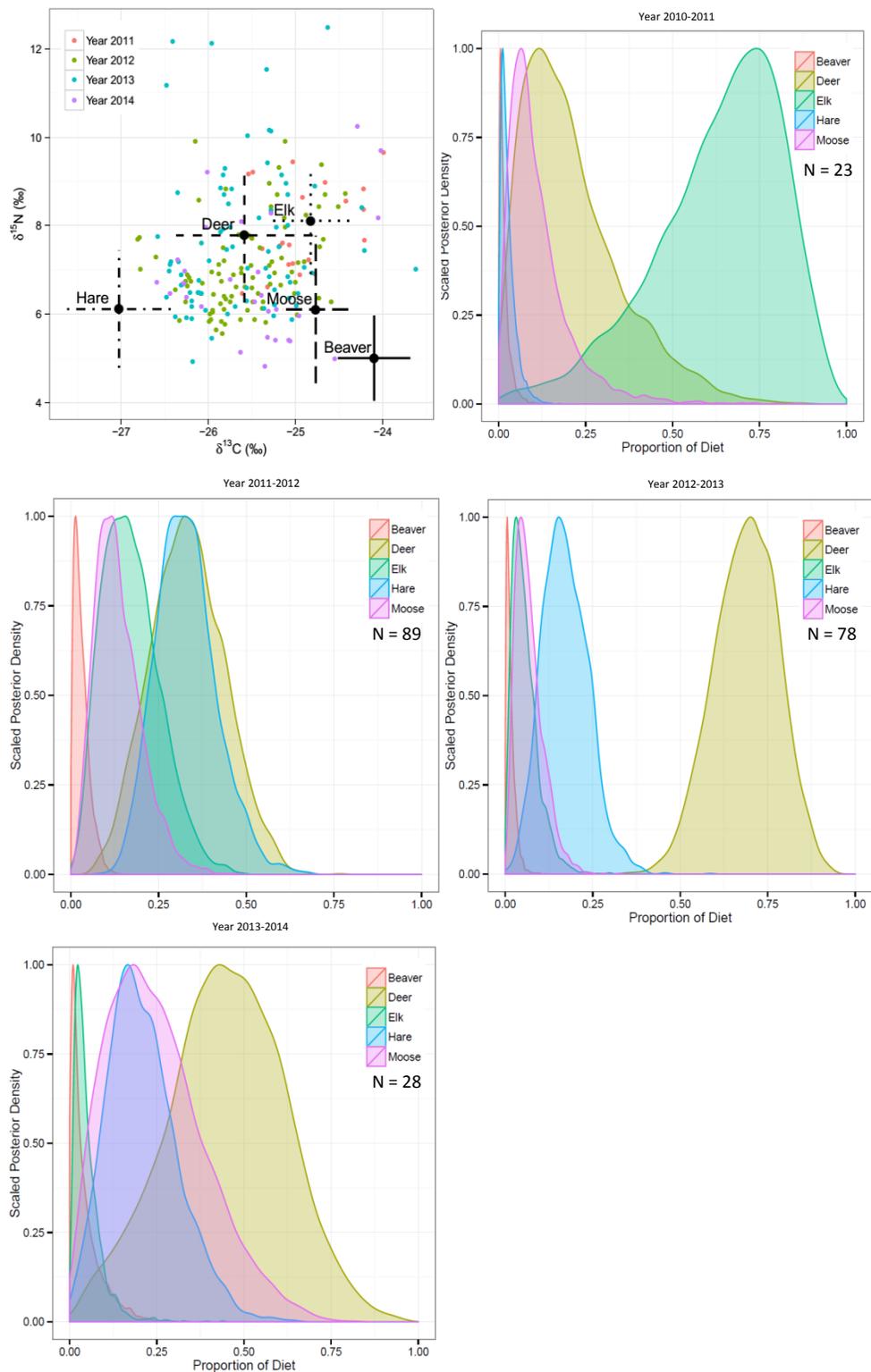


Figure 3.2 Wolf winter diet results from 2010-11 to 2013-14. All mean prey signatures were corrected for trophic discrimination and priors were used to weight each prey. The first panel shows the position of wolf signatures in iso-space relative to mean prey signatures. The other panels show the diet estimates by year. Each distribution represents a proportion of wolf diet by a prey species. The mid-

point of the distribution represents the modal diet estimate and the width of the distribution reflects the uncertainty in the diet estimate. The wider the distribution, the more uncertainty is present in the model.

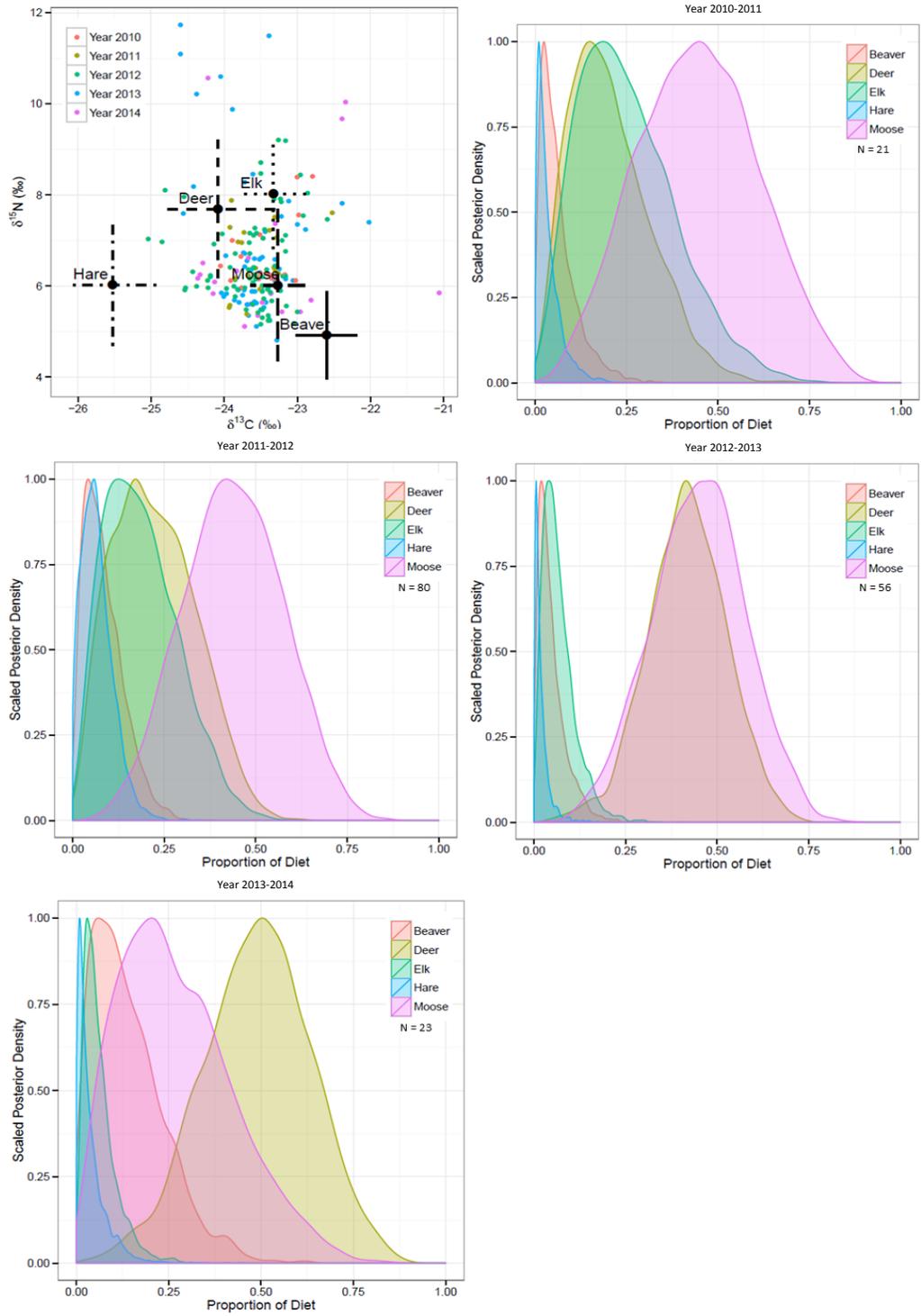


Figure 3.3 Wolf summer diet results from 2010-11 to 2012-13. All mean prey signatures were corrected for trophic discrimination. The first panel shows the position of wolf signatures in iso-space relative to mean prey signatures. The other panels show the diet estimates by year. Each distribution represents a

proportion of wolf diet by a prey species. The mid-point of the distribution represents the modal diet estimate and the width of the distribution reflects the uncertainty in the diet estimate.

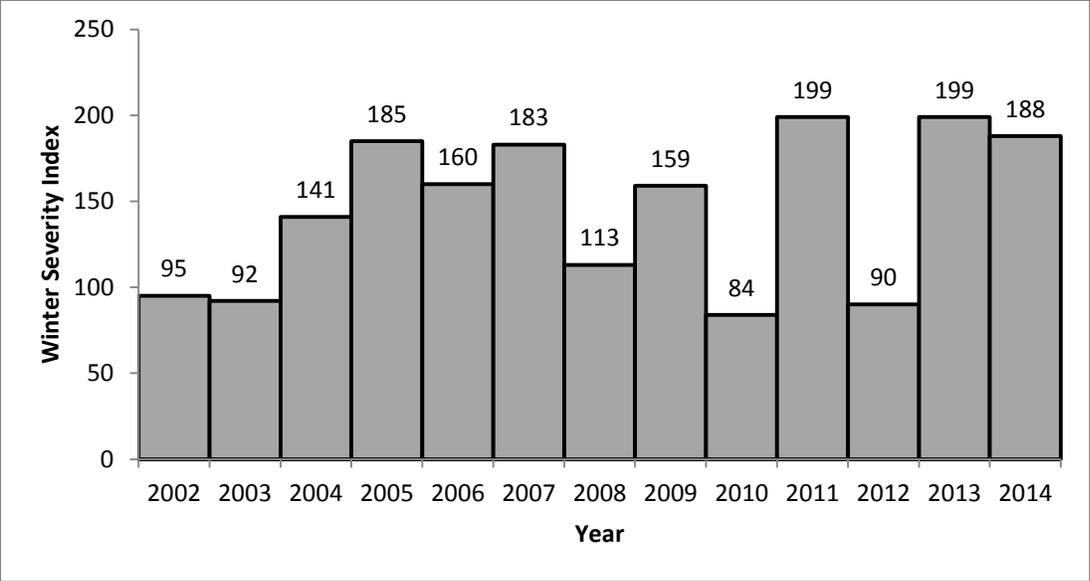


Figure 3.4 Winter severity index based on criteria for white-tailed deer. Wolves consumed mainly deer during mild (2011-12) and severe winters (2010-11, 2012-13, 2013-14). Data provided by Manitoba Conservation.

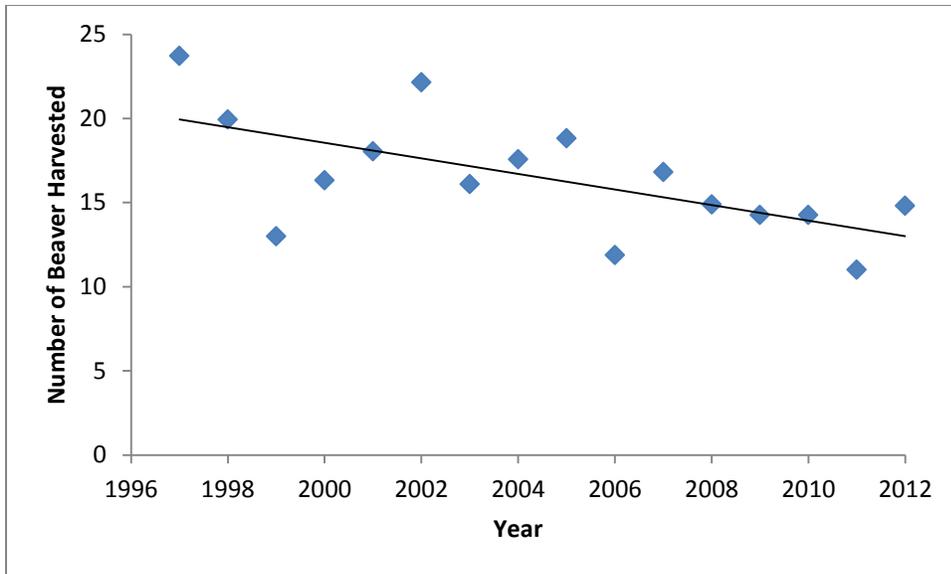


Figure 3.5 Beaver harvest record data from 1996 to 2012 shows a decline in western Manitoba beaver populations ($y=-0.082x + 1656.5$, $R^2=0.5586$, $p=0.0004$). Harvest information corrected for trapper effort and provided by Manitoba Conservation.

Chapter 4: Thesis Conclusion

Wolves (*Canis lupus*) in Manitoba mainly consume deer (*Odocoileus virginianus*) during winter. In summer, wolf diet predominantly consisted of moose (*Alces americanus*), except in southeastern Manitoba where wolves ate both moose and beaver (*Castor canadensis*). Our results show that seasonal shifts in wolf diet occur, and that summer may be a critical time period for moose survival regarding wolf predation.

Changes in prey availability may occur due to seasonal differences in weather conditions. Beaver availability is one example of how seasonal changes may affect wolf diet. During winter beaver are confined to their lodges and rarely emerge because they have cached food beneath the ice to survive the winter (Müller-Schwarze 2011). Wolves occasionally visit beaver lodges during winter and sometimes will attempt to dig into the lodge (Daniel Dupont, Manitoba Conservation, pers. comm.), but those attempts could be driven by food shortages for those wolves. Wolves in GHA 26 that have been collared by Manitoba Conservation were tracked to beaver ponds throughout winter. One wolf resting site that we visited in GHA 26 was on the shore of a beaver pond, and a beaver lodge was located approximately 50 meters from the rest site. Wolf tracks led from the rest site to the lodge and then circled the perimeter of the pond. There has been controversy amongst managers questioning the availability of beaver to wolves in winter. We included beaver in our winter diet models and found that beaver are not a significant component of wolf winter diet. However, beaver are highly important in wolf summer diet in southeastern Manitoba where moose and deer were segregated spatially. Beaver are easily accessible to wolves in summer because they often move between their lodges and land to forage, and are not very agile on land (Müller-Schwarze 2011). Although beaver were not important to wolf summer diet in western Manitoba, beaver densities may be low in western Manitoba, resulting in a low encounter rate between beaver and wolves. Another possibility may be that wolves are consuming deer instead of beaver in this portion of western Manitoba where deer and moose coexist. White-tailed

deer are preferred prey for lone wolves or small packs because they are easier and less dangerous to catch than moose, yet provide more meat than beaver, so it is feasible that western wolves replaced beaver with deer.

Optimal foraging theory suggests that the most catchable and abundant prey in an area will dominate predator diet (Charnov 1976). However, our spatial analysis results show that catchability of a prey species overrides the importance of prey density. Wolves in the northern region of GHA 26, where deer are rare, had high proportions of deer in their diet, suggesting that these wolves travelled south to feed, where deer density is high. These results also show that wolves sought easier prey regardless of the dense population of moose in the north. Our results do not support our hypothesis that wolves select prey based solely on relative prey densities within their territory. Wolf telemetry data from a monitoring program conducted by Manitoba Conservation suggest that wolves within GHA 26 move north to south frequently (Daniel Dupont, Manitoba Conservation, unpublished data). Travel is energetically costly and seemingly unnecessary if a supply of moose prey is available within the wolves' territory. Moose can be deadly when defending themselves during a wolf attack, making them a difficult prey species to obtain (Mech 1970), unlike deer, which are less dangerous and easier to catch. Wolves living in packs preferentially select moose because they have a greater body mass, providing more meat for the effort shared by each pack member (Zimmermann et al. 2015). Wolves in Manitoba may not be hunting in large packs, and so deer may have become the ideal prey species for wolves.

Wolves may be hunting individually because the trapper incentive program could have caused social fragmentation within packs by removing individuals from the group. Wolves hunting individually or in pairs may be more likely to take smaller prey, such as white-tailed deer. Our results show that prior to the trapper incentive program wolves were mainly consuming larger prey like elk (in the western area) and moose, but in the years after the trapper incentive program began, wolves ate smaller prey such as snowshoe hare (west) and deer during winter. Previous research suggests that top predators,

such as wolves, are self-regulating species, whose populations are limited by social interactions (Wallach et al. 2015). Predator control can increase stress in wolves, affecting their reproduction and social dynamics (Brainerd et al. 2008, Bryan et al. 2014). Any deterioration of their social structure could change the functional impact of wolves on their prey populations, which may explain the trends that we have observed in wolf winter diet.

Research shows that wolf predation alone may not be a major limiting factor on some moose populations, but when black bears (*Ursus americanus*) are also present on the landscape, wolf predation then appears to be a major source of predation (Boutin 1992). In spring and early summer, black bears prey upon moose calves, typically at a greater percent than wolves (Boutin 1992). Black bears and wolves coexist in Manitoba and so it is possible that black bear predation on moose calves is confounding the effect of wolf predation on moose populations. Our results for both study areas in Manitoba during summer show that wolves were eating a greater proportion of moose compared to winter diet estimates. It is likely that wolves are eating moose calves in the summer when calves are most vulnerable (Ballard and Larsen 1987; Van Ballenberghe 1987). Wolves hunting in a pack are able to separate the cow moose from her calf to procure moose calf prey. Therefore, if wolf packs have disintegrated due to predator control, then it is unlikely that wolves are killing calves with cows. During early summer naïve, yearling moose have also dispersed from the cow prior to parturition (Schwartz et al. 1987). Depending on the physical condition or predator avoidance skills of individual yearling moose, small groups of wolves or perhaps an individual could kill a sickly yearling moose, thereby increasing the proportional representation of moose in wolf summer diet.

Additionally, the relatively high presence of moose in wolf summer diet may be because wolves are consuming more carrion, rather than expending energy and resources to chase and kill prey (Peterson and Ciucci 2003). Harsh winter conditions can be fatal to bull moose who lack fat resources that were expended during the fall rut, and were meant to carry them through to spring when forage is

more plentiful and nutritious (Schwartz et al. 1987). Therefore, if moose are dying for other reasons such as starvation or disease, and wolves are feeding mainly on carcasses, then our models may be inflating the effect wolf predation is having on moose populations. For this reason, it would be prudent for managers to consider all possible mechanisms behind consumption before enacting a predator control program.

Parasitism may also play a key role in moose population declines in Manitoba. The distribution of some parasites, such as *Parelaphostrongylus tenuis* and liver fluke (*Fascioloides magna*) (Lankester and Foreyt 2011, Hoberg 2008), has increased with the expansion of white-tailed deer range. Furthermore, the increasing frequency of mild winters and the spread of human development have altered northern, otherwise inhospitable habitats, allowing the expansion of deer populations (Lankester and Samuel 1998). The expansion of white-tailed deer range into the boreal forests of Canada may be increasing spatial overlap between moose and deer, thus increasing moose susceptibility to parasitism and possibly predation (Wasel et al. 2003). *Parelaphostrongylus tenuis* is a nematode parasite that is benign in white-tailed deer, but symptomatic in moose causing an increase in moose mortality (Lankester 2010). The parasite was initially thought to exist in only the southeastern region of Manitoba but recent studies show that the parasite is present in southwestern Manitoba as well (Brian Kiss, Manitoba Conservation, unpubl. data).

Trophically transmitted parasites use trophic interactions such as predation as a means of transmission to their next host (Lafferty et al. 2008; Amundsen et al. 2009). *Echinococcus canadensis* (Cestoda: Taeniidae) is a trophically transmitted parasite that uses wolf predation on moose to complete its life cycle (Messier et al. 1989). The adult *E. canadensis* tapeworm resides in the intestine of wolves and sheds its eggs into the environment through wolf feces, which are then accidentally ingested by cervids (moose, deer, elk, etc.) while foraging (Joly and Messier 2004). The eggs flow through the blood stream of the cervid to the lungs, and occasionally to other organs, where the larvae encyst within

the tissue (Romig 2003). In moose, severe infestations of *E. canadensis* can reduce the endurance of animals when being pursued by a predator (Messier et al. 1989; Jenkins et al. 2005; Thompson 2008). Many researchers have debated whether or not *E. canadensis* directly influences the predatory relationship between moose and wolves by purposely altering the condition of moose to enhance predation (Rausch 1959; Mech 1970; Petersen 1977; Addison et al. 1978; Rau and Caron 1979; Messier et al. 1989; Joly and Messier 2004); however, no study to date has provided empirical evidence for this phenomenon in *E. canadensis* and its host species (Lagrué and Poulin 2010).

Evolutionarily, it is reasonable for a parasite to adopt some method of host control when its mode of transmission is dependent on a predation event. Wolves selectively prey on weaker animals and often consume the internal organs of their prey before any other part of the animal (Joly and Messier 2004). Therefore, natural selection may have favored the location of *E. canadensis* cysts in the lungs of moose (Sand et al. 2006; Lagrué and Poulin 2010). If moose in Manitoba are heavily infected with *E. canadensis*, and possibly more susceptible to predation, then this predator-prey-parasite system may also be enhancing the effect of wolf predation on moose populations.

Although population data on wolves and ungulate prey in Manitoba are limited, government officials have implemented several strategies to mitigate additional damage to moose populations. One major factor that was immediately addressed is hunter harvest of moose. Licensed moose hunting in areas of moose decline in Manitoba have been canceled by Manitoba Conservation. Rights-based hunters (First Nation communities, Metis) voluntarily restricted their hunting activities in certain moose conservation zones within our study sites. A study in Manitoba revealed that blocking and destroying unused logging roads is an effective way to reduce human impacts on moose (Crichton et al. 2004). To mitigate hunting efforts Manitoba Conservation barricaded and destroyed old logging roads, so licensed and unlicensed hunters could not access areas where moose are located. The government also launched a study on the prevalence of *P. tenuis* in white-tailed deer populations located in and around moose

conservation zones. Lastly, to increase trapping effort for wolves, the province enacted a five year trapper incentive program that awards trappers \$250.00 CAD per trapped wolf in the hope of temporarily decreasing wolf predation pressure on moose populations. Predator control is a controversial management technique that is commonly discussed amongst the general public in popular magazines and internet sources. Some people are uncomfortable with euthanizing one species to conserve another species, and support non-lethal management techniques. Other people see predator control as an economically sound and effective method to conserve ungulate populations. These social differences between stakeholders typically drive the allocation of government funding, and sometimes the biology of the animals is given less consideration during the decision making process.

Stable isotope analysis may be an alternative method to expensive and time consuming techniques such as telemetry tracking of wolves and their ungulate prey, which is typically used when studying these species. Scat analysis is commonly used in large predator studies because it is easier to attain compared to other techniques; however, scat analysis only provides diet information for a feeding event and may misrepresent the actual biomass consumed (Weaver 1993). Stable isotopes and Bayesian mixing models can provide the proportional diet of a consumer and percent biomass of the prey that was assimilated over various time periods in an individual's or consumer population's diet. These techniques may serve as an approximate alternative to population data when funding resources are limited.

Moose decline in Manitoba is a complex issue that requires a multi-faceted solution. Our study of wolf-moose-deer interactions provides insight into the possible mechanisms behind wolf predation of moose in a human-influenced community. Demographic data on moose populations would be useful for understanding these mechanisms, and maintaining high densities of beaver could lessen wolf predation pressure on moose during summer. Additional experimental studies that manipulate the availability of deer to wolves throughout winter and summer would help explain the role deer play in this complex

wolf-moose-deer system. Diet studies using stable isotopes and mixing models can contribute to management by providing long-term information of a predator and its prey.

Literature cited

- Addison, E., Pybus, M., and H. Rietveld. 1978. Helminth and arthropod parasites of black bear in central Ontario. *Canadian Journal of Zoology* 56:2122–2126.
- Amundsen, P.A., Lafferty, K.D., Knudsen, R., Primicerio, R., Klemetsen, A., and A.M. Kuris. 2009. Food web topology and parasites in the pelagic zone of a subarctic lake. *Journal of Animal Ecology* 78:563–572.
- Ballard, W.B., and D.G. Larsen. 1987. Implications of predator-prey relationships to moose management. *Swedish Wildlife Resources* 1:581-602.
- Boutin, S. 1992. Predation and moose population dynamics: a critique. *Journal of Wildlife Management* 56:116-127.
- Brainerd, S.M., Adrén, H., Bangs, E.E., Bradley, E.H., Fontaine, J.A., Hall, W., Iliopoulos, Y., Jimenez, M.D., Jozwiak, E.A., Liberg, O., Mack, C.M., Meier, T.J., Niemeyer, C.C., Pedersen, H.C., Sand, H., Schultz, R.N., Smith, D.W., Wabakken, P., and A.P. Wydeven. 2008. The effects of breeder loss on wolves. *Journal of Wildlife Management* 72:89-98.
- Bryan, H.M., Smits, J.E.G., Koren, L., Paquet, P.C., Wynne-Edwards, K.E., and M. Musiani. 2014. Heavily hunted wolves have higher stress and reproductive steroids than wolves with lower hunting pressure. *Functional Ecology* doi: 10.1111/1365-2435.12354.
- Crichton, V., Barker, T., and D. Schindler. 2004. Response of a wintering moose population to access management and not hunting—a Manitoba experiment *Alces* 40:87-94.
- Hoberg, E.P., Polley, L., Jenkins, E.J., Kutz, S.J., Veitch, A.M., and B.T. Elkin. 2008. Integrated approaches and empirical models for investigation of parasitic diseases in northern wildlife. *Emerging Infectious Diseases* 14:10-17.
- Jenkins, D. J., Romig, T., and R.C.A. Thompson. 2005. Emergence/re-emergence of *Echinococcus* spp.—a global update. *International Journal for Parasitology* 35:1205–1219.
- Joly, D.O., and F. Messier. 2004. The distribution of *Echinococcus granulosus* in moose: evidence for parasite-induced vulnerability to predation by wolves? *Oecologia* 140:586–590.
- Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., DeLeo, G., Dobson, A.P., Dunne, J.A., Johnson, P.T.J., Kuris, A.M., Marcogliese, D.J., Martinez, N.D., Memmott, J., Marquet, P.A., McLaughlin, J.P., Mordecai, E.A., Pascual, M., Poulin, R., and D.W. Thieltges. 2008. Parasites in food webs: the ultimate missing links. *Ecology Letters* 11:533–546.
- Lagrange, C., and R. Poulin. 2010. Manipulative parasites in the world of veterinary science: implications for epidemiology and pathology. *Veterinary Journal* 184:9–13.
- Lankester, M. W., and W. M. Samuel. 1998. Pests, parasites and diseases. Pages 479–517 in A.W. Franzmann, and C.C. Schwartz, editors. *Ecology and management of the North American moose*. Smithsonian Institution, Washington, D.C., USA.

- Lankester, M.W. 2010. Understanding the impact of meningeal worm, *Parelaphostrongylus tenuis*, on moose populations. *Alces* 46:53-70.
- Lankester, M.W., and W.J. Foreyt. 2011. Moose experimentally infected with giant liver fluke. *Alces* 47:9-15.
- Mech, L.D. 1970. The wolf: the ecology and behavior of an endangered species. Natural History Press (Doubleday Publishing Co., N.Y.) 389 pp. (Reprinted in paperback by University of Minnesota Press, May 1981).
- Messier, F. 1985. Social organization, spatial distribution, and population density of wolves in relation to moose density. *Canadian Journal of Zoology* 63:1068-1077.
- Messier, F., Rau, M., and M. McNeill. 1989. *Echinococcus granulosus* (Cestoda: Taeniidae) infections and moose-wolf population dynamics in southwestern Quebec. *Canadian Journal of Zoology* 67:216–219.
- Müller-Schwarze, D. 2011. Mortality and predators. Pages 123-127 in Müller-Schwarze, editor. *The beaver its life and impact*, Second Edition. Cornell University Press, Ithaca, New York, USA.
- Peterson, R.O. 1977. Wolf ecology and prey relationships on Isle Royale. National Park Service Scientific Monograph no. 11. Washington D.C., USA.
- Peterson, R.O., and P. Ciucci. 2003. The wolf as a carnivore. In *Wolves: Behavior, Ecology, and Conservation*. Edited by L.D. Mech and L. Boitani. The University of Chicago Press, Chicago, USA.
- Rau, M., and F. Caron. 1979. Parasite-induced susceptibility of moose to hunting. *Canadian Journal of Zoology* 57:2466–2468.
- Rausch, R. 1959. Notes on the prevalence of hydatid disease in Alaskan moose. *Journal of Wildlife Management* 23:122.
- Romig, T. 2003. Epidemiology of echinococcosis. *Langenbeck's Archives of Surgery. Deutsche Gesellschaft für Chirurgie* 388:209–217.
- Schwartz, C.C., Regelin, W.L., and A.W. Franzmann. 1987. Seasonal weight dynamics of moose. *Swedish Wildlife Resources* 1:301-310.
- Thompson, R.C.A. 2008. The taxonomy, phylogeny and transmission of *Echinococcus*. *Experimental Parasitology* 119:439–446.
- Van Ballenberghe, V. 1987. Effects of predation on moose numbers: a review of recent North American studies. *Swedish Wildlife Resources* 1:431-460.
- Wallach, A.D., Izhaki, I., Toms, J.D., Ripple, W.J., and U. Shanas. 2015. What is an apex predator? *Oikos* 000:001-009.
- Wasel, S.M., Samuel, W.M., and V. Crichton. 2003. Distribution and ecology of meningeal worm, *Parelaphostrongylus tenuis* (Nematoda), in northcentral North America. *Journal of Wildlife Diseases* 39:338–346.

Weaver, J.L. 1993. Refining the equation for interpreting prey occurrence in gray wolf scats. *Journal of Wildlife Management* 57:534-538.

Zimmermann, B., Sand, H., Wabakken, P., Liberg, O., and H.P. Andreassen. 2015. Predator-dependent functional response in wolves: from food limitation to surplus killing. *Journal of Animal Ecology* 84:102-112.

Appendix A: western Manitoba sensitivity analyses

Trophic discrimination sensitivity analysis

Trophic discrimination can strongly influence diet estimates when using Bayesian mixing models. We ran three models using uniform priors and discrimination values derived from muscle of arctic foxes (*Vulpes lagopus*) ($\Delta C=0.37\pm 0.8$, $\Delta N=1.79\pm 0.4$; Lecomte et al. 2011), red foxes (*Vulpes vulpes*) ($\Delta C=1.1\pm 0.1$, $\Delta N=3.3\pm 0.1$; Roth and Hobson 2000) and wolf hair ($\Delta C=1.97\pm 0.70$, $\Delta N=3.04\pm 0.31$; Derbridge et al. 2015). The discrimination values from wolf hair were corrected for the difference stable isotope values in muscle and hair by calculating the difference in red fox muscle and hair discrimination values. We applied the correction ($\Delta C=-1.5$, $\Delta N=0.1$) to wolf hair discrimination values to estimate the discrimination values of wolf muscle ($\Delta C=0.47\pm 0.7$, $\Delta N=2.94\pm 0.31$). To correct for the difference in body size between foxes and wolves we altered the ΔN values of foxes by -0.3 based on a study of rats and mice ($\Delta C=1.1\pm 0.1$, $\Delta N=3.0\pm 0.1$ for muscle and $\Delta C=2.6\pm 0.1$, $\Delta N=2.9\pm 0.1$ for hair) (MacAvoy et al. 2006).

The results of the sensitivity analysis show that wolf and red fox trophic discrimination values are slightly different, but do not have a large effect on wolf diet estimates in western Manitoba, unlike arctic fox values. Red fox discrimination values produce diet estimates with less uncertainty (narrower distribution) compared to wolf discrimination values (Figure A.1).

Prior information sensitivity analysis

We also examined the effect of priors on western wolf diet estimates. Prior information used in MixSIAR for western wolf diet was derived from a preliminary wolf scat analysis conducted in Duck Mountain Provincial Park by Manitoba Conservation and Parks Canada that was corrected for prey biomass using the conversion equation by Weaver (1993). All prey signatures were corrected for trophic discrimination ($\Delta C=1.1\pm 0.1$, $\Delta N=3.0\pm 0.1$, in winter and $\Delta C=2.6\pm 0.1$, $\Delta N=2.9\pm 0.1$, in summer). One model was run using uniform priors and the other model was run using priors from preliminary data (Figure

A.2). The model using informative priors showed a clear delineation between most likely prey species, and so subsequent diet analyses included informative priors.

Literature Cited

- Derbridge, J.J., Merkle, J.A., Bucci, M.E., Callahan, P., Koprowski, J.L., Polfus, J.L., and P.R. Krausman. 2015. Experimentally derived $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ discrimination factors for gray wolves and the impact of prior information in Bayesian mixing models. *PLoS ONE* 10: e0119940.
- Lecomte, N., Ahlstrøm, Ø., Ehrich, D., Fuglei, E., Ims, R.A., and N.G. Yoccoz. 2011. Intrapopulation variability shaping isotope discrimination and turnover: experimental evidence in arctic foxes. *PLoS ONE* 6: e21357.
- MacAvoy, S.E., Macko, S.A., and L.S. Arneson. 2005. Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis. *Canadian Journal of Zoology* 83:631-641.
- MacAvoy, S.E., and L.S. Arneson. 2006. Correlation of metabolism with tissue carbon and nitrogen turnover rate in small mammals. *Oecologia* 150:190-201.
- Roth, J.D., and K.A. Hobson. 2000. Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction. *Canadian Journal of Zoology* 78:848-852.

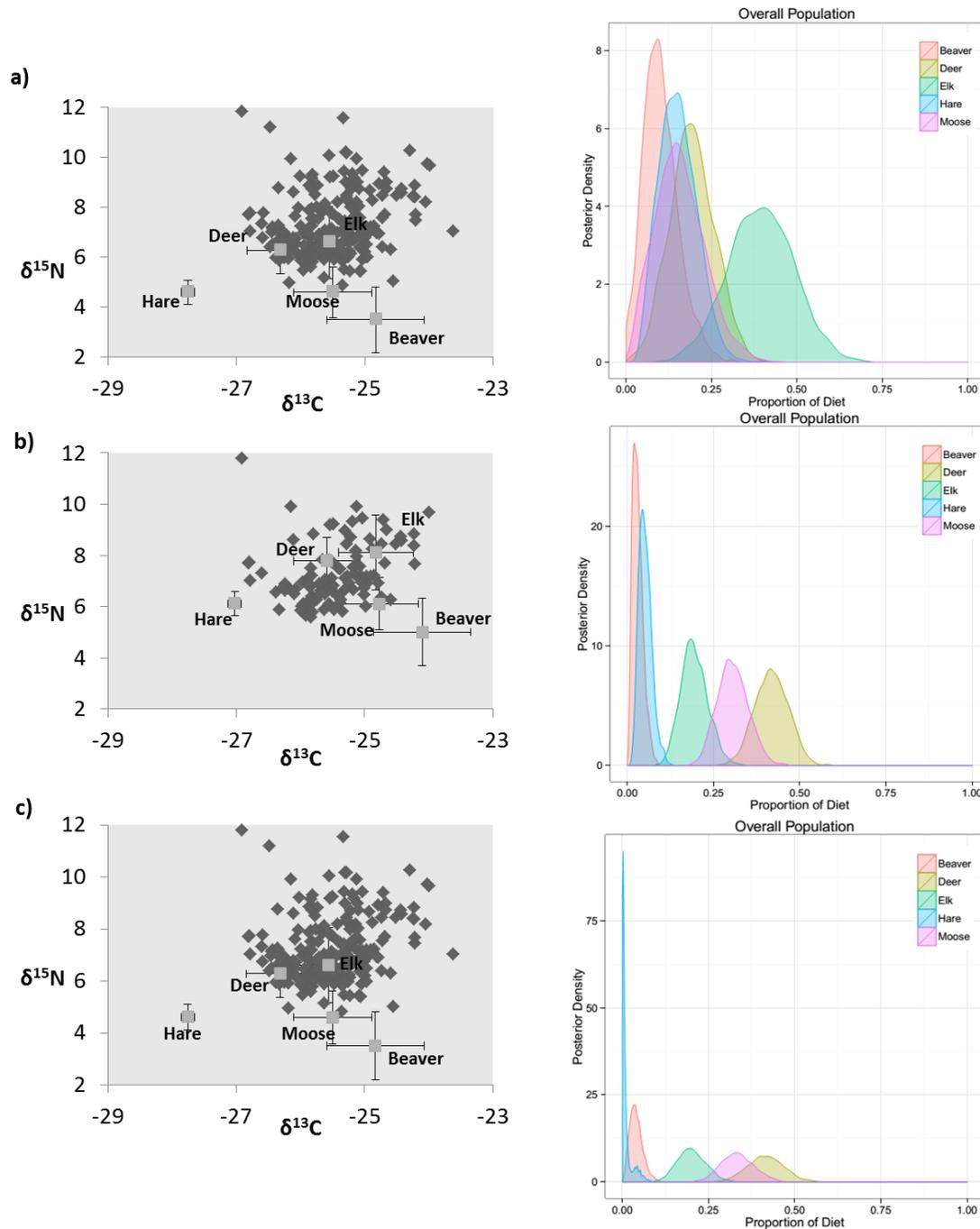
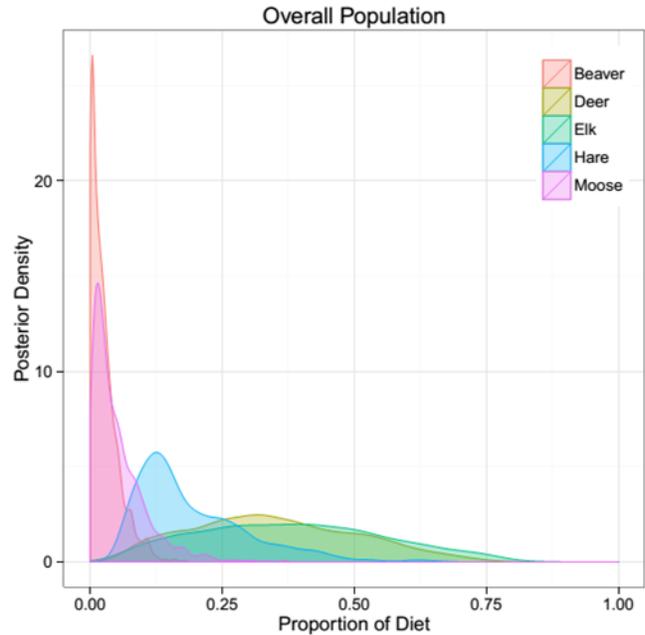


Figure A.1 Trophic discrimination sensitivity analysis results for western Manitoba wolf diet models. We ran three separate models using trophic discrimination factors (mean \pm SD) based on previously measured values for a) arctic fox ($\Delta C=0.37\pm 0.8$, $\Delta N=1.49\pm 0.4$), b) red fox ($\Delta C=1.1\pm 0.1$, $\Delta N=3.0\pm 0.1$), and c) wolf ($\Delta C=0.47\pm 0.1$, $\Delta N=2.94\pm 0.1$) (Chapter 2). The left panel contains the nitrogen and carbon iso-space plot for individual wolves (black) and mean prey signatures (\pm standard deviation). The right panel

displays the probability density plot for overall wolf winter diet. All models were run using uniform priors. Wolf muscle trophic discrimination was estimated from measured wolf hair values.

a)

Prey	Prior information
Beaver	20.0%
Deer	20.0%
Elk	20.0%
Hare	20.0%
Moose	20.0%



b)

Prey	Prior information
Beaver	4.1%
Deer	41.4%
Elk	19.3%
Hare	0.7%
Moose	34.5%

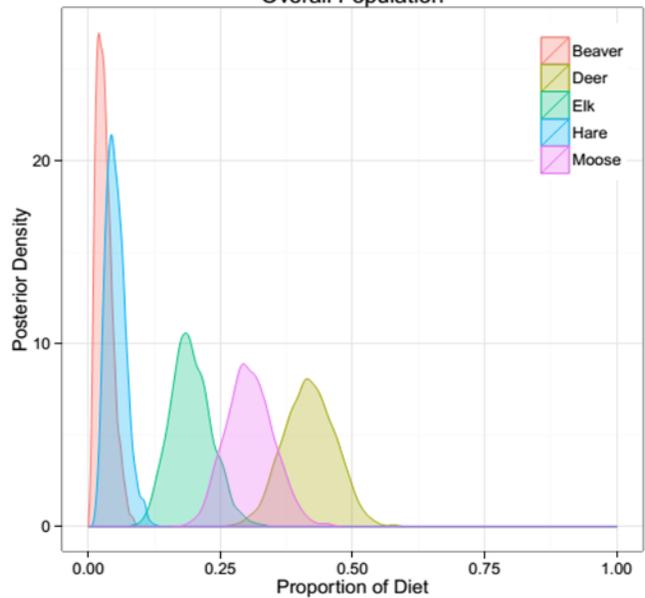


Figure A.2 Results of the prior information sensitivity analysis using western wolf winter data. Winter wolf diet estimates were produced using a) uniform priors and b) informative priors. The diet estimate including informative priors shows a clearer delineation between the most likely prey species in wolf diet.