Intrapopulation Variability in Wolf Diet Revealed Using a Combined Stable Isotope and Fatty Acid Approach

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Ecology

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ABSTRACT

Naturally occurring stable isotope ratios and fatty acids are two types of chemical biomarkers frequently used to quantitatively estimate consumer diets. Stable isotope values in animal tissues and diets have been evaluated using Bayesian mixing models to provide dietary estimates of consumers in both terrestrial and marine ecosystems. Fatty acids have primarily been used to examine diets of marine species. Using muscle and adipose tissue, we combined the two biomarkers in a Bayesian mixing model to generate quantitative diet estimates for gray wolves (*Canis lupus*, n=78) in the southern Northwest Territories, Canada. Simulation experiments showed that the combined dataset led to more accurate and precise diet estimates than stable isotopes alone. Overall, wood bison (*Bison bison athabascae*) dominated the winter diet (63-96%) of wolves. In one region where bison was not readily available, wolf diet was more variable, with substantial contributions from boreal caribou (*Rangifer tarandus caribou*), moose (*Alces alces*), snowshoe hare (*Lepus americanus*), and beaver (*Castor canadensis*). Surprisingly, fish also comprised 5 – 26 % of wolf diet in the region. Wolves likely scavenged on scraps left behind by commercial ice fishing operations on Great Slave Lake. Our investigation underlines the power of combining these two major analytical tools to investigate diet in an elusive and opportunistic predator.

PREFACE

This thesis is an original work by Sean O'Donovan. Data used in all analyses were generated via stable isotope and fatty acid analyses conducted on wildlife tissue samples. All tissue samples were submitted by wildlife harvesters to the Government of Northwest Territories between 2011 and 2016. Stable isotope samples were prepared and analyzed using mass spectrometry at the Great Lakes Institute for Environmental Research at the University of Windsor. Fatty acid samples were analyzed using gas chromatography at the Marine Lipids Lab at Dalhousie University.

To date, the manuscript has not been submitted for publication in a peer-reviewed journal. S. O'Donovan analyzed the data and wrote most of the manuscript. Co-authors include Suzanne M. Budge, Keith A. Hobson, Allicia P. Kelly, and Andrew E. Derocher. All co-authors, especially S. Budge, provided valuable feedback and edits throughout the analysis and writing processes.

DEDICATION

I dedicate this thesis to my folks, John and Margaret, for their support throughout my academic endeavours.

ACKNOWLEDGEMENT

I'd first like to thank Andrew Derocher for being an awesome supervisor. I feel lucky to have been part of your lab and I really appreciate your supervisory style, particularly the freedom to work a few contracts on the side. Secondly, I thank the co-authors of this manuscript, particularly Sue Budge, for sharing helpful insights and providing critical feedback along the way. Most of all, thank you to Emily Blythe for your support throughout grad school.

For supporting this study I'd like to thank Deninu K'ue First Nation, Smith Landing First Nation, NWT Metis Nation, Forth Smith Metis Council, Hay River Metis Council, Fort Resolution Metis Council, Ka'a'gee Tu First Nation, Deh Gah Got'ie First Nation, Fort Providence Metis. I also thank numerous individual hunters and trappers and the K'atlodeeche First Nation for providing wildlife tissue samples directly to this study. Generous funding for this project was provided by the Natural Sciences and Engineering Research Council of Canada Discovery and Northern Research Supplement grants, and by Environment and Natural Resources, Government of Northwest Territories.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Understanding and monitoring the trophic ecology of predators is an essential component of wildlife management. Apex predators can exert top-down forces on lower trophic levels by regulating or limiting prey populations (Messier 1995, Ripple and Beschta 2012) that may in turn, lead to trophic cascades that affect the structure of communities or ecosystems (Estes et al. 2011, Sergio et al. 2014, Ripple et al. 2015). Although predator-prey relationships are frequently assessed at the population level by studying predator diets, an increasing number of studies have shown that trophic niche width represents an aggregation of often variable individual or group-level diets (Urton and Hobson 2005, Edwards et al. 2011, Matich et al. 2011, Milakovic and Parker 2013). Within a given predator population, variation in diet can be influenced by factors such as prey availability, ease of prey acquisition, individual behavior, and social dynamics (Huggard 1993b, Matich et al. 2011, Metz et al. 2011, Pintor and Byers 2015).

Quantitative diet estimates can be generated using a variety of methods, each characterized by inherent strengths and weaknesses. Traditional methods such as scat and stomach content analysis may be inexpensive, but are limited in spatial and temporal resolution (Bowen and Iverson 2012). Chemical biomarkers such as stable isotopes (SI) and fatty acids (FA) are increasingly being used as dietary tracers, because predators incorporate unique prey biomarker profiles into their tissues after consumption (DeNiro and Epstein 1978, 1981, Iverson et al. 2004, Budge et al. 2006, Ben-David and Flaherty 2012). Combining methods to reconstruct diet can help to increase confidence in estimates. For example, agreement between estimates through qualitative comparison (e.g. Watt and Ferguson 2015, Connan et al. 2017) or positive correlation (e.g. Tucker et al. 2008, Milakovic and Parker 2011) has been used to validate results in past diet studies. Additionally, combining methods can better inform statistical modelling and reduce

uncertainty in diet estimates by incorporating multiple variables, and in the context of Bayesian approaches, by considering prior information (Galloway et al. 2015, Brett et al. 2016).

An advantage of diet biomarkers is that a single tissue sample can provide insights into what an animal was eating over longer time periods than scat or stomach contents (Tiezen et al. 1983, Darimont and Reimchen 2002, Iverson et al. 2004). For example, SI composition of muscle tissue reflects animal diet over the previous 1-2 months depending on body size, and metabolically inactive tissues such as hair incorporate the isotopic ratios of foods consumed while they were growing (Roth and Hobson 2000). FA profiles reflect foods eaten over weeks to months, depending on metabolic rate and activity level (Budge et al. 2006). While SI have been used extensively across taxa and ecosystem types, FA have primarily been used to assess the diets of marine species and their use is rare in terrestrial ecosystems.

Quantitative diet estimation using SI has embraced Bayesian mixing models, which have undergone substantial development in recent years (Moore and Semmens 2008, Parnell et al. 2010, Phillips 2012, Parnell et al. 2013). The newest models address some of the complexities in ecological systems by allowing for explicit integration of uncertainty in prey isotopic variability, and diet-tissue isotopic discrimination factors (Ward et al. 2010, Parnell et al. 2013, Stock and Semmens 2016). Despite these advances, a common problem associated with SI analysis is poor source (i.e., prey) resolution because typically only the SI ratios of carbon and nitrogen are used to inform statistical modeling. For example, Milakovic and Parker (2011), were unable to distinguish moose (*Alces alces*) and beaver (*Castor canadensis*) in northern British Columbia using these two isotopes. In addition to poor source resolution, the accuracy and precision of diet estimates can suffer when systems are mathematically underdetermined (i.e., when the number

of sources (n) relative to tracers is greater than $n + 1$) as is the case in most ecosystems (Phillips and Gregg 2003, Fry 2013, Brett 2014, Galloway et al. 2015).

A potential solution to poor source resolution and underdetermined constraints is to incorporate additional dietary tracers into analyses, thereby increasing dimensionality and better informing Bayesian statistical modelling. FA profiles for an individual animal often consist of many different individual FA. Accordingly, marine animal studies have shown that FA alone, and in combination with SI, hold great promise in overcoming these problems (Dethier et al. 2013, Galloway et al. 2014, Galloway et al. 2015, Neubauer and Jensen 2015, Brett et al. 2016). However, the integration of SI and FA in Bayesian mixing models remains untested on terrestrial animals.

We used stomach content surveys, SI (δ^{13}C and δ^{15}N), and FA analyses to gain insights into the diet of an apex terrestrial predator, gray wolves (*Canis lupus*) in the southern Northwest Territories, Canada. Although wolves exploit a diversity of species, ungulates tend to be primary prey throughout their North American range (Peterson and Ciucci 2003). Our study area had three regions, each with a unique species assemblage of the commonly occurring ungulates in the southern Northwest Territories: moose, wood bison (*Bison bison athabascae*), and boreal caribou (*Rangifer tarandus caribou*). Studies in other regions have quantified wolf diet, documenting intrapopulation variability using SI only (Urton and Hobson 2005, Milakovic and Parker 2011, Derbridge et al. 2012). However, our study represents the first use of FA to assess wolf diet.

Our objectives were to 1) assess the efficacy of combining SI and FA in Bayesian mixing models to generate quantitative diet estimates for a terrestrial predator, 2) reconstruct the winter diet of wolves from three regions of our study area characterized by spatially heterogeneous distributions of different ungulate species. We hypothesized that combining SI and FA would

result in better prey species resolution in multivariate space and more precise diet estimates than SI alone. Secondly, we hypothesized that wolf diets would be variable between the three regions, and specifically that they would reflect differential availability of ungulate prey species.

STUDY AREA

The study area is located south and west of Great Slave Lake in the southern Northwest Territories, Canada (Figure 1), within the Taiga Plains Mid-Boreal Ecoregion (Ecological Land Classification Group 2007). There is little topographic relief in the area. Peatlands and water comprise approximately 40% and 18% of total land cover, respectively (Ecological Land Classification Group 2007). Fens are characterized by black spruce (*Picea mariana*), larch (*Larix laricina*), dwarf birch (*Betula glandulosa*), sedges (*Carex* spp.), and mosses. Peat plateaus are dominated by open black spruce forests. Well-drained soils closer to the Slave and Mackenzie rivers support large mixed-wood, deciduous, and coniferous forests where white spruce (*P. glauca*), aspen (*Populus tremuloides*), and jack pine (*Pinus banksiana*) are common. The most common human disturbances are exploratory seismic lines, roads, human settlements, and timber harvest. The study area is comprised of three regions (Figure 1), delineated *a priori* based on known distributions of ungulate prey. The Slave River Lowlands (SRL) are just outside boreal caribou range, but are inhabited by moose and wood bison. Boreal caribou and moose inhabit the Pine Point/Buffalo Lake region (PPBL), but wood bison do not. The PPBL overlaps a zone known as the Bison Control Area, which is kept free of wood bison to prevent disease transmission between herds (Shury et al. 2015). All three ungulate species occur in the Mackenzie Region (MACK).

METHODS

Tissue sample collection

All wildlife tissue samples used in this study were submitted by local wildlife harvesters. In winter 2012-2016, muscle and adipose tissue samples were collected from 78 wolf carcasses, and muscle samples were collected from potential wolf prey species, including: boreal caribou, moose, bison, beaver, and snowshoe hare (*Lepus americanus*). Additionally, lake whitefish (*Coregonus clupeaformis*), lake trout (*Salvelinus namaycush*), and white sucker (*Catostomus commersonii*) muscle samples were collected, as fish are often used as trapline bait. Samples were stored at approximately -20°C in a conventional freezer.

FA sample preparation and analysis

Lipid was extracted from wolf adipose tissue and prey muscle tissue using the Folch et al. (1957) technique, modified to prevent oxidation and maximize lipid yield as described by Budge et al. (2006). Accordingly, samples were immersed and agitated in a 2:1 chloroform:methanol ($CHCl_3$:MeOH) solution with 0.01% butylated hydroxytoluene (BHT). Water and proteins were removed using 0.7% NaCl solution, and the isolated lipids dissolved in hexane. Secondly, the lipids were converted to FA methyl esters (FAME) via a base-catalyzed transmethylation reaction using sodium methoxide as the catalyst. Lastly, FAME dissolved in hexane were analyzed with by gas chromatography with flame ionization detection at the Marine Lipids Lab, Dalhousie University. An RTX-2330 column (90% biscyanopropyl/10% phenylcyanopropyl polysiloxane; 105 m, 0.25 mm ID, 0.2 um d_f) was used with the following temperature program: 150 °C was held for 2 min, then ramped up at 2 °C/min to 245 °C which was held for 13 mins. Helium was used as carrier gas and the detector was held at 270 °C. The injector was isothermal at 250 °C and a 1/100 split ratio was used. Gas chromatography (GC) separates and selectively

retains individual FAME according to carbon chain length and the number of double bonds present in each molecule. FA were identified by comparison of retention times with standards and by evaluation of spectra from GC-mass spectrometry.

SI sample preparation and analysis

Wolf and prey muscle samples were prepared and analyzed using mass spectrometry at the Chemical Tracers Laboratory, Great Lakes Institute for Environmental Research, University of Windsor. Samples were freeze-dried and ground into fine powder using a mortar and pestle. Lipids can alter $\delta^{13}C$ measurements (DeNiro and Epstein 1978, Rau et al. 1992), so lipids were removed using a 2:1 chloroform:methanol solution. Prepared samples were weighed into tin capsules. A Thermo Finnigan Delta Plus mass spectrometer (Thermo Finnigan, San Jose, CA, USA) coupled with an elemental analyzer (Costech, Valencia, CA, USA) was used to measure $\delta^{13}C$ and $\delta^{15}N$ natural abundances. Values of $\delta^{13}C$ and $\delta^{15}N$ are reported relative to Viena PeeDee Belemnite (VPDB) and Air standards, respectively. Based on replicate measurements (n=32) of internal laboratory standards (tilapia, NIST1577c, USGS 40, and urea) we estimate measurement error to be ±0.1 ‰ and ±0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$ measurements, respectively.

Source selection

Results from stomach content surveys conducted on a subset of 64 wolves were used to choose appropriate prey species to include during SI and FA modeling. To assess whether our proposed model fit the dataset, we employed the method of Smith et al. (2013), which uses a Monte Carlo simulation to iterate mixing polygons based on consumer and prey SI data. The simulation estimates a 95% mixing region that all consumers should fall within if the proposed model fits the data. The approach accounts for uncertainty in SI profiles and diet-tissue discrimination factors.

Variable selection

A requirement of Bayesian mixing models is that sources are isotopically different (Phillips et al. 2014). Accordingly, we visualized prey species separation using three profile categories: SI-only, FA-only, and combined SI-FA. Biplots of δ^{13}C and δ^{15}N prey profiles were created for the SI-only dataset, and non-metric dimensional scaling (NMDS) ordination plots generated in the R package Vegan (Oksanen et al. 2017) were used to visualize multivariate datasets that included FA. We measured 68 individual FA, but excluded those for which diet-tissue calibration coefficients have not been calculated, resulting in a FA-only dataset of 39 FA. Next, the two biomarkers were merged, as the two tissue types they were derived from (muscle and adipose) reflect diet over similar temporal scales (weeks to months). This combined SI-FA dataset included δ^{13}C and δ^{15}N values and a subset of three FA that were found to maximize prey species separation in multivariate space. Permutational ANOVAs were run on each of the 39 FA using proportion as the dependent variable and species as a factor. FA were then ranked according to their *f*-statistic, which in this case is a ratio of between-species variance / within species variance. The three FA with the highest *f*-statistics were used in the combined SI-FA dataset. This approach reduced dimensionality, while selectively retaining FA that contributed to among source variation. We tested for significant differences between prey species using one-way multivariate analyses of variance (MANOVA) on the SI-only dataset and permutational analyses of variance (PERMANOVA; Anderson 2001) within the *adonis* function in Vegan for the FA-only and SI-FA datasets. SI data are continuous and reported as the ratio of heavy to light isotopes in relation to an internationally recognized standard. Alternatively, FA data are compositional, measured as proportions that sum to 1. Importantly, the two biomarkers cannot be merged and used in the Bayesian mixing model without a transformation to put them on the same

scale of measurement. Accordingly the SI-FA dataset was transformed by subtracting the mean and dividing by the standard deviation (Dethier et al. 2013).

Simulated wolf diet

Simulated wolf diets were generated from the actual prey data to demonstrate the utility of reconstructing diet with the SI-FA dataset. Additionally, model performance was compared between the SI-only and SI-FA datasets. Four simulated diet categories (Diets A-D) were created with 10 wolves in each. For the 10 wolves in each category, the proportion of each prey species in the diet was generated randomly within a set range. Diets A-C simulated situations where bison, moose, and caribou were primary prey species, respectively, while Diet D simulated a generalist diet. Specifically, simulated wolf diet was comprised of 70-80% bison (Diet A), 75-85% moose (Diet B), and 85-95% caribou (Diet C). The remaining 4 prey species in Diets A-C comprised random percentages between 0-10%. In Diet D all prey species contributed between 20-30%. The proportional contributions of all 5 prey species to each simulated wolf diet were then normalized to sum to 1. Next, to generate the biomarker profiles for each simulated wolf the randomly generated prey diet proportions were multiplied by the corresponding mean prey isotopic or fatty acid values. Lastly, we fit Bayesian mixing models in the R package MixSIAR (Stock and Semmens 2015) to see if we could properly categorize simulated diets.

Harvested wolves

For the harvested wolves, we used the same suite of two SI and three FA for all analyses. We tested for differences between wolf age classes, sex, and harvest region using PERMANOVA. Diets were reconstructed at the population level and by harvest region; any wolves with unknown harvest location were excluded. We applied $\delta^{13}C$ and $\delta^{15}N$ diet-tissue discrimination factors estimated by Derbridge et al. (2015). Because species-specific diet-tissue calibration

coefficients have not been published for wolves, we applied calibration coefficients to our wolf FA profiles calculated by Thiemann et al. (2008) for mink (*Mustela vision*) fed a poultry diet. We generated informative priors using results from stomach content surveys conducted on 64 of the harvested wolves. As outlined by Stock and Semmens (2015), the informative priors were rescaled to have the same weight as the uninformative prior. Models were run twice, once with the informative prior and once with the uninformative prior. Lastly, to serve as a check on our diet estimates we qualitatively compared prey species and wolves from different regions using two trans fatty acids (TFA; 11t-18:1 and 16t-18:1) that are known to be prevalent in domestic ungulates (Kramer et al. 2002, Kramer et al. 2008).

RESULTS

Source selection

The simulated mixing region (Appendix Figure 1) suggested that the proposed suite of prey species (bison, caribou, moose, hare, beaver, and fish) were appropriate sources to explain the $\delta^{13}C$ and $\delta^{15}N$ profiles of all 78 wolves.

Variable selection

Using the SI-only dataset (Figure 2) beaver and moose profiles were not significantly different from each other (MANOVA; Pillai's Trace = 0.16, $F_{2,16} = 1.49$, $P = 0.26$). Consequently, beavers were excluded from simulation experiments, where the goal was to explicitly compare diet estimates from the SI-only and SI-FA datasets. With the FA-only dataset (Figure 3a) bison, moose, and caribou profiles were not significantly different from each other (PERMANOVA; bison-moose, Pseudo-$f = 0.47$, $p = 0.55$; bison-caribou, Pseudo-$f = 1.32$, $p = 0.28$; caribou-moose, Pseudo-$f = 2.15$, $p = 0.12$). The three FA with the highest corresponding f-statistics were

*iso*17:0, 20:2*n*-6, and 20:5*n*-3 (Appendix Table 1). When merged with the SI-dataset (Figure 3b) all prey species profiles were significantly different from each other.

Simulated wolf diet

For all simulated diets, estimates using the combined SI-FA dataset was both more accurate and precise than those from the SI-only dataset, indicating better overall model performance (Table 1). For the combined dataset, mean posterior density estimates were the same or closer to the true mean value for all source contribution estimates (Table 1). Additionally, tighter 95% CI estimates reveal that uncertainty was reduced for every diet estimate when compared to the SI-only dataset.

Harvested wolves

Combined SI-FA profiles of the harvested wolves suggested no difference between age classes or sex (Appendix Table 2) but significant differences between regions (PERMANOVA; Pseudo-$F = 5.37$, $p = 0.001$). Bison dominated wolf diet at the population level (Mean and [95% CI] for estimates using informative priors: 84% [63-96%]; Table 1), in the SRL (94% [85-100%]) and in MACK (98 % [93-100%]). Bison was also the primary prey in PPBL (45% [24-67%]), although proportionately lower than elsewhere in the study area. In PPBL, dietary contributions from caribou (12% [1-27%]) and moose (7% [0-30%]) were higher than in SRL (3% [0-12%], 3% [0-10%], respectively) or MACK (0% [0%], 0% [0%]). Similarly, more beaver (8% [0-28%]) and hare (13% [0-29%]) were consumed by wolves in PPBL than in SRL (0% [0%], 3% [0-12%]) or MACK (1% [0-4%], 1% [0-4%]). Fish contributed minimally to diet, except in PPBL (15% [6-25%]).

Results from stomach content surveys showed that bison contributed more to wolf diet than other prey species at the population level (43%), in PPBL (33%), SRL (70%), and MACK

(75%; Table 2). Caribou made up a higher proportion of diet in PPBL (17%) than in SRL (10%) or MACK (0%). Fish contributed most to wolf diet in PPBL (25%), with proportionately less consumed in MACK (8%), and none found in stomachs of wolves from SRL.

Qualitative comparison of prey using TFAs showed that in general, ungulates had higher proportions of 11t-18:1 than other species, while beavers generally had the highest levels of 16t-18:1 (Figure 4a). Overall, wolves from PPBL had the lowest proportion of both TFAs (Figure 4b). Additionally, the proportions of both 11t-18:1 and 16t-18:1 were more variable in PPBL wolves (s^2 = 0.336 and 0.007, respectively) than wolves from SRL (s^2 = 0.279 and 0.006) or MACK (s^2 = 0.029 and 0.003).

DISCUSSION

We demonstrate the benefit of combining FA and SI data to reconstruct the diet of a terrestrial predator. Most notably, our simulation experiments showed that the integration of SI and FA data in Bayesian mixing models substantially reduced uncertainty and improved the accuracy of estimated source contributions to predator diet (Table 1). We also showed that combining SI and FA profiles lead to greater prey species resolution in multivariate space (Figures 2 - 3). Our methodology allowed us to 1) select enough predictor variables (i.e. FA) to provide significant discrimination between relevant sources, and to 2) avoid working on a mathematically underdetermined system, while 3) keeping the relative influence of the SI predictors as high as possible due to a wider body of knowledge related to SI and our study organism.

Similar to our results, simulation studies focused on diet reconstruction of marine organisms that combined SI and FA biomarkers also reported more precise and accurate diet estimates (e.g. Dethier et al. 2013, Neubauer and Jensen 2015). However, using a dataset consisting only of FA, Brett et al. (2016) showed that the precision and accuracy of Bayesian mixing models could be

greatly improved by increasing the number of predictor FA from 2 to 7. Intuitively, increasing the number of predictor variables should better inform statistical modelling and lead to better diet estimates. Better model performance for our combined SI-FA dataset may therefore simply reflect a higher number of predictor variables rather than the explicit integration of SI and FA data.

Increasing the number of tracers in marine consumers improves discrimination between sources (Crawley et al. 2009, Dethier et al. 2013). However, we found that the effect of more tracers was not always beneficial. When we ordinated the full FA-only dataset in NMDS plots there was very little difference among ungulate species (Figure 3a), a possible reflection of the effects of rumination on FA profiles (Berkley et al. 2014). It was therefore necessary to select and retain those FA that contributed most to between-species separation. A number of methods have been described for FA selection, including constrained ordination (Neubauer and Jensen 2015), ranking by standard deviations (Brett et al. 2016), running similarity percentage analyses, or by keeping only the most abundant (Dethier et al. 2013). While none of these methods proved successful for separating ungulate species in our study, ranking by *f*-statistic did. We posit that this may be a simple yet effective means of selecting appropriate predictor variables in diet studies for terrestrial organisms.

The suite of prey species included in our analysis would not have been possible using the SI-only dataset, due to isotopic overlap between beaver and moose (Figure 2). Given the millions of possible combinations, there was likely some subset of FA that would have resulted in significant separation of all prey species as a standalone dataset. However, incorrectly accounting for trophic modification of biomarkers can lead to inaccurate diet estimates (Budge et al. 2012, Milakovic and Parker 2013, McLaren et al. 2015, Brett et al. 2016, Bromaghin et al.

2016). Because species-specific calibration coefficients have not been estimated for wolves, we felt it was essential to use SI as the foundation of the analysis and add only enough FA to avoid working in a mathematically underdetermined system and provide significant discrimination between sources.

General agreement between diet estimates using uninformative priors (Table 1) and estimates derived from stomach content analyses (Table 2) helped to validate our results and justify the use of stomach contents as informative priors. The most substantial difference between biomarker and stomach content estimates is the relative contributions of bison and fish. When compared to stomach contents, biomarker estimates suggest a higher proportion of bison and a lower proportion of fish. Because biomarkers reveal the proportion of prey species assimilated into the predator's tissue, it is possible that the discrepancy between the two methods can be explained by the much higher amount of consumable biomass on a bison vs. a fish. When informative priors were incorporated into the mixing models, uncertainty was reduced for most prey species contributions to wolf diet (Table 1).

Overall, our results suggest that bison is by far the primary prey species of wolves during winter across the study area (Table 1). Diets of wolves from SRL and MACK were similar, with the vast majority being made up of bison, while moose and caribou were less important. In the PPBL, the only region where bison was not readily available, wolf diet was more variable, with substantial dietary input from other species. A contributing factor may be that our sample size was larger in PPBL compared to SRL or MACK. Sampling more wolves in PPBL may have captured more wolf diet variability than elsewhere. Despite this, bison still contributed the most to wolf diet in PPBL, suggesting that highly mobile wolves accessed bison in other areas before being harvested in the PPBL. Although contrary to our hypothesis, it is perhaps unsurprising that

wolf diet did not entirely reflect regional ungulate distribution, as wolves commonly display preferential selection of certain prey species over others (Potvin and Jolicoeur 1988, Huggard 1993b, Smith et al. 2004, Merkle et al. 2017, Stanek et al. 2017).

Qualitative analysis of wolf and prey TFA profiles served as an additional layer of evidence for our diet estimates using data that were not included during modeling. Apart from 16t-18:1 in beaver, both TFAs were most abundant in ungulates. Overall, wolves from MACK and SRL had higher levels of both TFAs than those from PPBL. When viewed in relation to regional diet estimates from both biomarker and stomach content analyses, it is logical that proportions would be higher in MACK and SRL wolves given the dominance of bison in the diet. It follows that elevated levels of 16t-18:1 most likely came from bison, as beavers contributed minimally to wolf diet. Furthermore, higher variances for both TFAs in PPBL wolves parallel the diet estimates, which were much more variable than in MACK or SRL.

Our results are consistent with Carbyn et al. (1993) who found that during winter, bison accounted for 82% of the biomass consumed by wolves in Wood Buffalo National Park. Larter et al. (1994) also estimated that bison comprised more of the biomass consumed by wolves during winter than other prey species in their study area west of Great Slave Lake. However, they concluded that moose was the preferred wolf prey species based on the amount of consumable biomass that each species represented on the landscape. Although we did not estimate available biomass for our prey species, this finding was not supported by our results in MACK, as the contribution of moose to wolf diet was negligible.

Where they co-occur, wolves tend to prey upon bison more commonly during winter than at other times of year (Carbyn et al. 1993, Smith et al. 2000, Jaffe 2001). Generally, wolves target prey that are most vulnerable (Bergman et al. 2006), such as calves or individuals in poor

body condition. Snow depth is also positively related to wolf hunting success, as wolves take advantage of prey whose movement is hindered by snow (Huggard 1993a). Bison, particularly calves, are hindered by shallower snow than moose (Larter et al. 1994) and likely more than caribou (Larter et al. 2017), a phenomenon that may contribute to the high proportion of bison in the winter diet of wolves.

Bison may also benefit wolves energetically, as the amount of consumable biomass on an adult bison is greater than any other prey species in the region. Bison are also the most gregarious ungulate species in the area and it is possible that the relative ease and reliability of locating bison herds compared to more solitary prey may play a role in their dominance in wolf diet. Additionally, during the summer of 2012 an outbreak of anthrax (*Bacillus anthracis*) killed hundreds of bison in the Mackenzie population (New et al. 2017). At least 52 of the wolves in our dataset were harvested the following winter, so it is possible that wolves scavenged on bison carcasses into the winter months in MACK.

Anthropogenic foods likely made up a substantial proportion of wolf diet, but in most cases the variety of different possible food types prevented us from including them as sources during modeling. Numerous wolves were known to be scavenging in dumps and plastic or Styrofoam garbage was found in wolf stomachs 16 times (Appendix Table 3). Especially apparent was the dietary contribution from fish in the PPBL (Table 1), which was possible to include as a distinct source in the mixing models. Fish is a surprising wolf food source, especially in non-coastal areas and particularly during winter. Recent telemetry data show that wolves scavenge on discarded fish scraps from commercial ice fishing operations on Great Slave Lake near Hay River. Because most of the wolves in the dataset were harvested near areas of

human activity (communities and traplines) our diet estimates may be biased toward anthropogenic foods rather than being representative of the wider wolf population.

Our results suggest that diet reconstruction using SI benefitted from incorporating FA as additional predictor variables. This approach allowed us to include more prey species than an SI-only analysis by increasing source resolution, making the model more representative of complex real-world food webs. Furthermore, it resulted in more accurate and precise simulated diet estimates. Ultimately the combination increased the effectiveness and utility of diet estimation in Bayesian mixing models for wolves in our study area, and may be widely applicable to other regions and species.

Table 1. Summary of four simulated wolf diet categories (Diets A-D). For the 10 wolves in each category, the proportion of each prey species in the diet was generated randomly within a set range. Specifically, simulated wolf diet was comprised of 70-80% bison (Diet A), 75-85% moose (Diet B), and 85-95% caribou (Diet C). The remaining 4 prey species in Diets A-C comprised random percentages between 0-10%. In Diet D all prey species contributed between 20-30%. The proportional contributions of all 5 prey species to each simulated wolf diet were then normalized to sum to 1. To generate the biomarker profiles for each simulated wolf the randomly generated prey diet proportions were multiplied by the corresponding mean prey isotopic or fatty acid value. Mean diet proportions (%) for 10 simulated wolves in each diet group are shown here. Mean and posterior density estimates (95% credible intervals) from Bayesian mixing models are compared for the SI-only and combined SI-FA datasets.

Prey	**Diet A**			**Diet B**			**Diet C**			**Diet D**		
Species	Mean	SI	SI & FA	Mean	SI	SI & FA	Mean	SI	SI & FA	Mean	SI	SI & FA
Bison	78	56 (16-85)	60 (38-81)	5	13 (1-31)	14 (2-28)	4	9 (0-26)	8 (1-19)	21	38 (2-73)	25 (7-47)
Caribou	7	15 (1-33)	14 (2-25)	6	10 (1-22)	6 (1-13)	83	78 (65-87)	80 (72-87)	20	14 (1-34)	18 (6-28)
Fish	4	10 (1-24)	6 (1-12)	5	4 (0-12)	4 (1-8)	4	4 (0-13)	3 (0-7)	20	14 (2-29)	18 (13-23)
Hare	5	9 (1-24)	5 (0-11)	5	29 (3-61)	9 (2-17)	4	4 (0-11)	4 (0-10)	20	17 (1-35)	20 (10-30)
Moose	6	11 (0-29)	15 (2-29)	79	44 (5-79)	67 (51-82)	5	5 (0-14)	6 (0-15)	19	18 (1-43)	20 (4-37)

Table 2. MixSIAR results summary (using the SI-FA dataset) for all wolves in the dataset (n=78) and those harvested in Pine Point/Buffalo Lake (n=24), Mackenzie (n=16), and Slave River Lowlands (n=18). Results represent the mean and 95% credible interval (CI) for the proportion of each prey species in wolf diet.

	All Wolves		**Pine Point/Buffalo Lake**		**Mackenzie**		**Slave River Lowlands**	
Prey Species	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Uninformative Prior								
Beaver	3	0-10	10	0-27	2	0-6	4	0-14
Bison	76	50-92	39	10-61	89	71-97	83	65-94
Caribou	10	0-27	13	1-29	5	0-22	5	0-19
Fish	3	0-9	15	6-26	1	0-4	1	0-4
Hare	4	0-12	13	1-28	2	0-6	4	0-13
Moose	5	0-15	10	0-34	2	0-7	3	0-11
Informative Prior								
Beaver	2	0-8	8	0-28	1	0-4	0	0
Bison	84	63-96	45	24-67	98	93-100	94	85-100
Caribou	7	0-22	12	1-27	0	0	0	0
Fish	3	0-8	15	6-25	1	0-3	0	0
Hare	2	0-10	13	0-29	1	0-4	3	0-12
Moose	3	0-12	7	0-30	0	0	3	0-10

Table 3. Percent occurrence (%) of prey species in the stomach contents of winter harvested wolves in the southern Northwest Territories. Results shown here exclude items that were deemed non-primary prey including plastic garbage, vegetation, small mammals, birds, lynx (*Lynx canadensis*), domestic chicken (*Gallus gallus domesticus),* and domestic dog (*Canis familiaris*). See Appendix Table 3 for a full summary of stomach content surveys.

Prey Species	Study Area (*n*=64)	Pine Point/Buffalo Lake (*n*=15)	Mackenzie (*n*=10)	Slave River Lowlands (*n*=13)
Beaver	7	8	8	0
Bison	43	33	75	70
Caribou	17	17	0	10
Fish	17	25	8	0
Hare	9	8	8	10
Moose	7	8	0	10

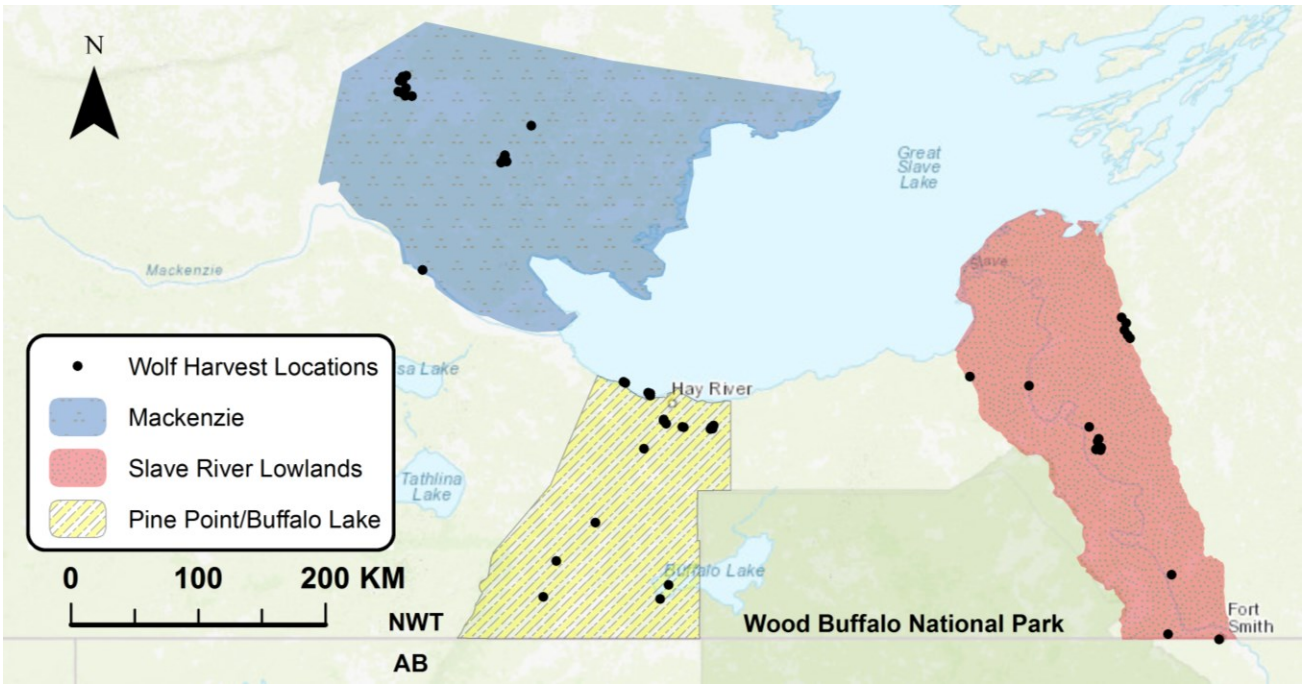

Figure 1. Map of the study area in the southern Northwest Territories, Canada. The three regions were delineated based on spatially heterogeneous distributions of ungulate species. Boreal caribou and moose occur in the Pine Point/Buffalo Lake region, while bison and moose inhabit the Slave River Lowlands. All three ungulate species are present in the Mackenzie region.

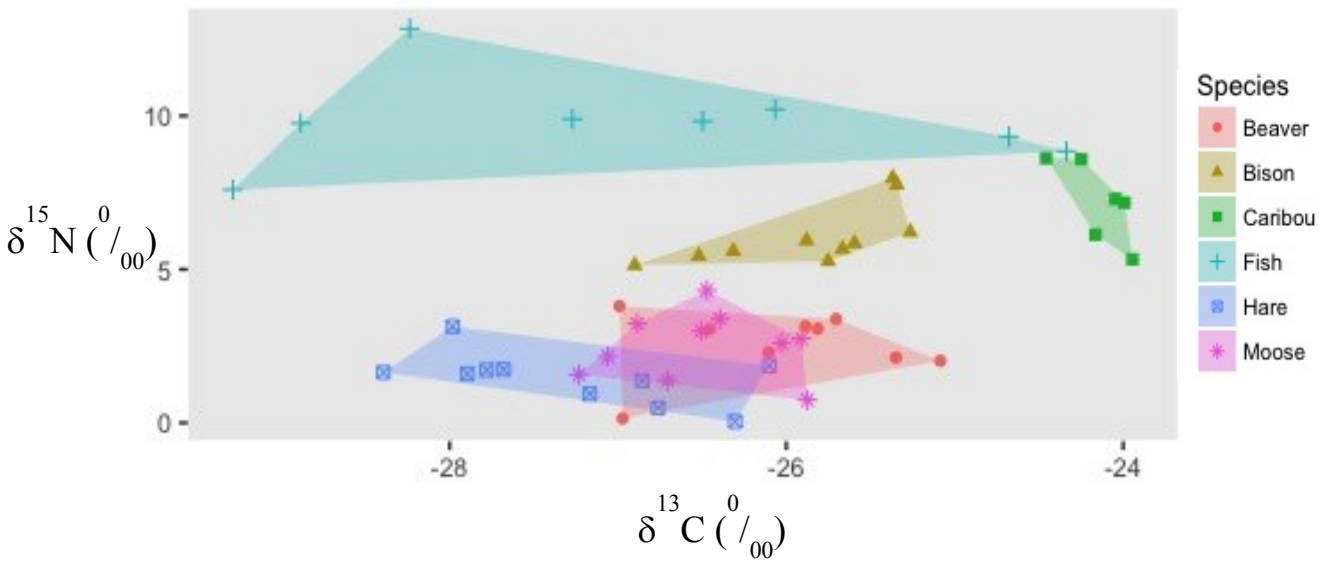

Figure 2. Carbon and nitrogen stable isotope profiles of prey species used for estimating the diets of simulated wolves. The high degree of overlap between moose and beaver means that the two species cannot be distinguished from each other and were not significantly different (MANOVA; Pillai's Trace = 0.16, $F_{2,16} = 1.49$, $P = 0.26$), violating a major assumption of Bayesian mixing models.

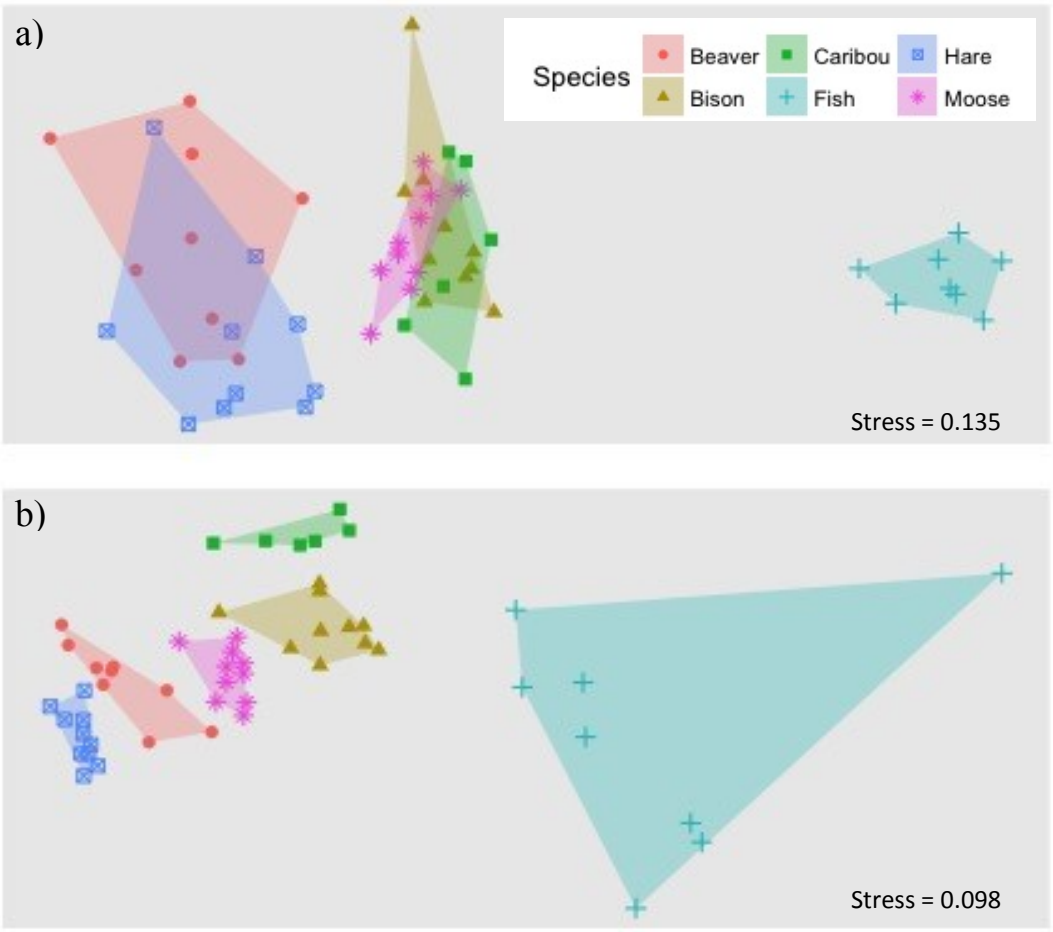

Figure 3. Non-metric dimensional scaling (NMDS) plots of prey FA profiles (a) and combined SI-FA profiles (b). Extensive overlap between species in a) means that the ungulates are indistinguishable from each other and unsuitable to use as distinct sources in Bayesian mixing models. Following variable selection, the combined SI-FA profiles in b) show higher discriminatory power between species and all pairwise comparisons of prey species were significantly different.

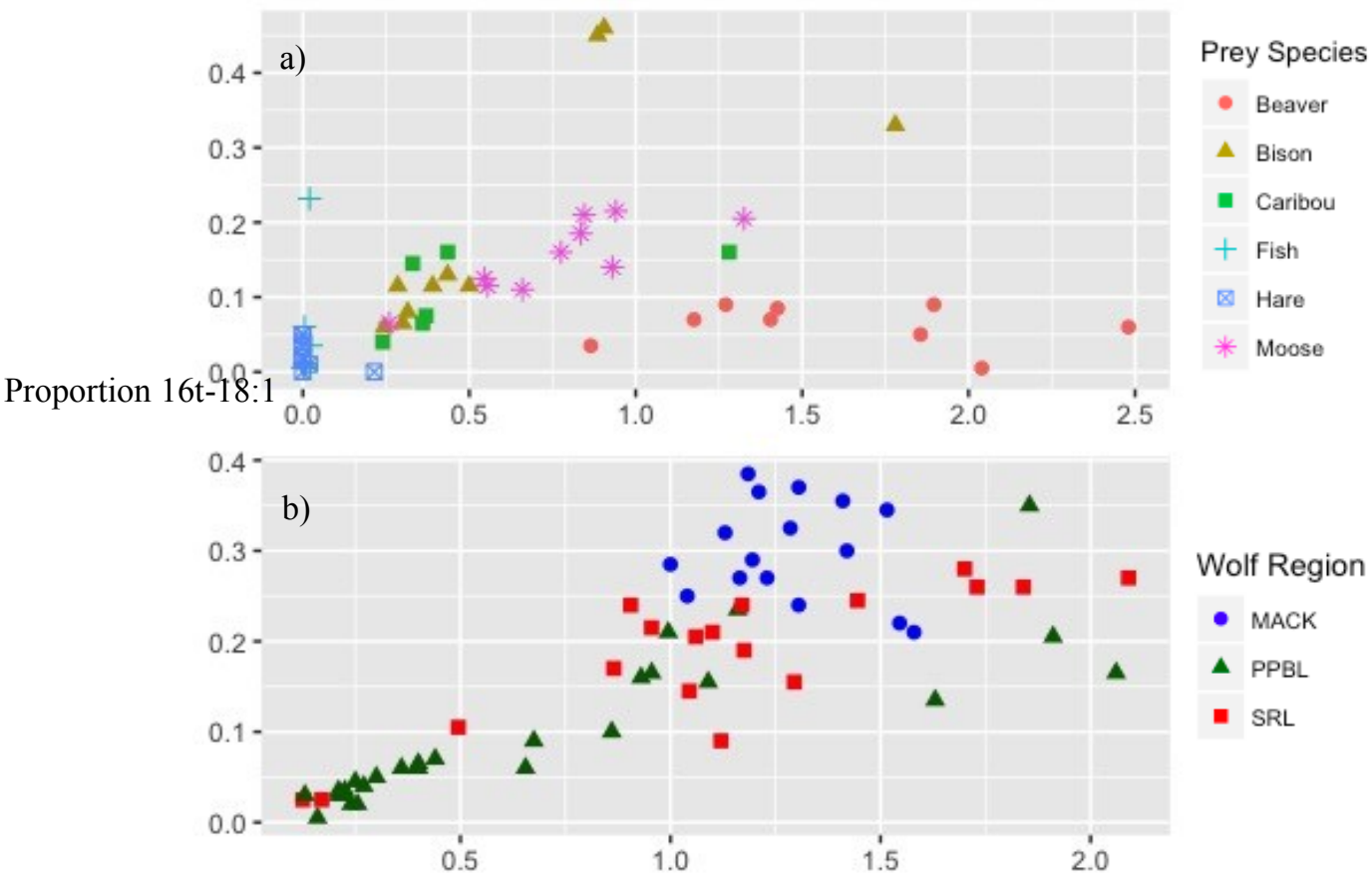

Figure 4. TFA profiles of prey species (a) and wolves by region (b). Higher proportions of 16t-18:1 and 11t-18:1 in wolves from MACK and SRL compared to PPBL wolves suggest greater dietary contribution from ungulates, which is consistent with diet estimates.

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APPENDIX

Appendix Table 1. Fatty acids were evaluated individually using permutational ANOVA and ranked by their corresponding *f*-statistic.

Fatty acid	***f*-statistic**
*iso*17:0	50.577
20:2*n*-6	34.498
20:5*n-3*	27.686
18:2*n-6*	27.156
18:1*n-9*	22.428
18:1*n-5*	19.083
15:0	16.489
*iso*15:0	16.174
*iso*16:0	16.167
18:0	15.768
20:4n-3	14.112
17:1	13.384
*ai*15:0	10.438
20:1*n-11*	8.9914
16:1*n-9*	7.8161
18:1*n-11*	7.5335
22:1*n*-9	6.7112
22:1*n-11*	6.5413
18:3*n*-6	6.3777
18:1*n*-7	6.3394
14:0	5.8303
20:0	5.5035
22:6*n*-3	5.4738
17:0	5.4469

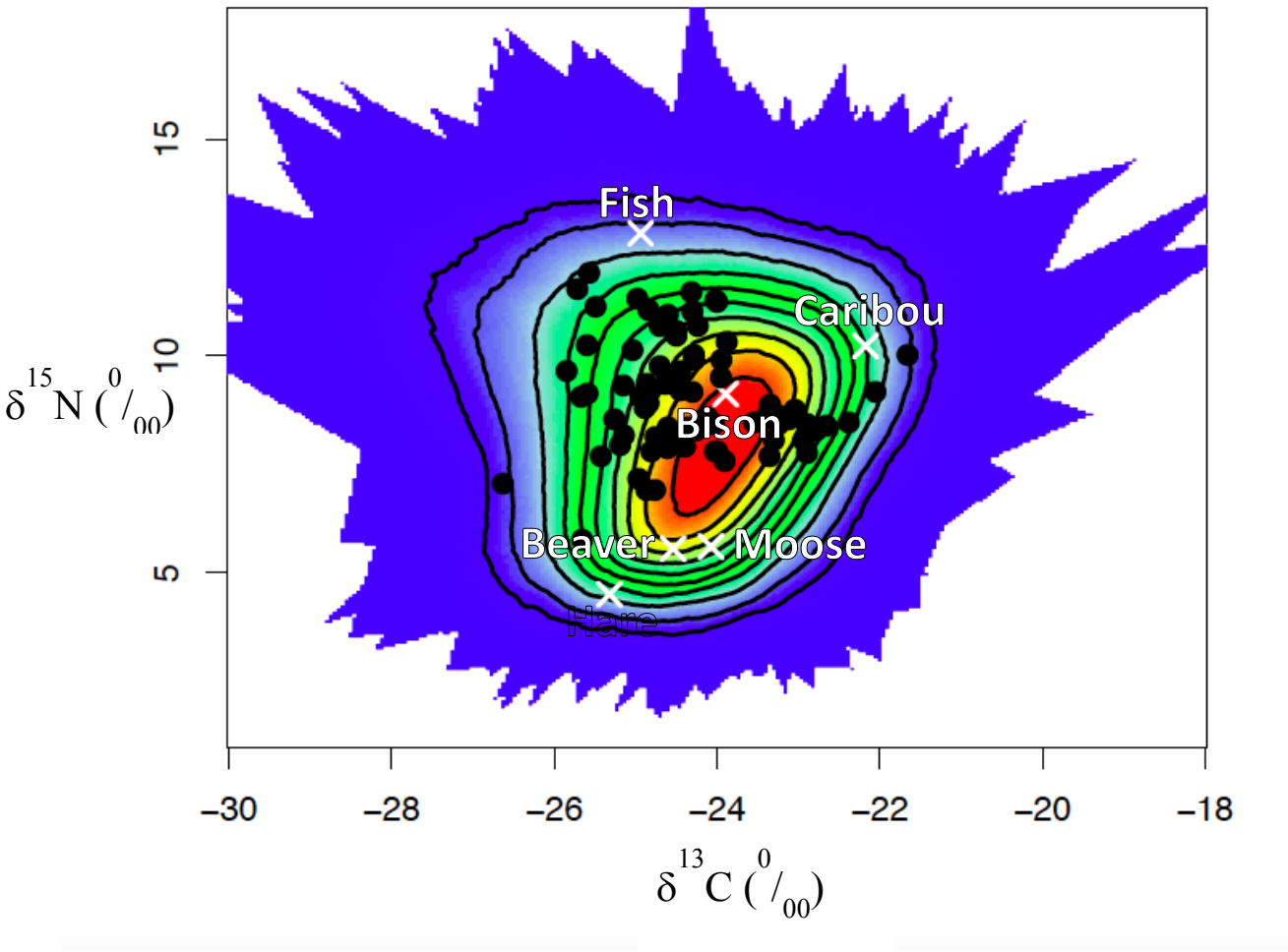
18:3*n*-3	4.8412
22:4*n*-3	3.5067
20:1*n*-9	3.2625
16:1*n*-5	2.7457
18:4*n*-3	2.3218
16:1*n*-11	2.0286
16:0	1.7853
18:1*n*-13	1.7543
22:5*n-3*	1.6867
22:5*n*-6	1.5727
21:5*n*-3	1.3925
20:4*n*-6	1.2264
16:1*n*-7	1.0776
20:3*n*-3	0.7949
24:1*n*-9	0.5924

Appendix Table 2. PERMANOVA results for differences between demographic groups based on combined SI-FA profiles for wolves from southern Northwest Territories harvested during winter between 2012 and 2016. Age classes are juvenile (<1 year old), adult (1 - 5 years old), and old (>5 years old). Because wolf profiles from different harvest regions were significantly different, those wolves were modeled hierarchically to generate diet estimates for each region.

Group	**n**	**df**	**Sum of squares**	**Mean squares**	**Pseudo-*f***	***p***
Age class	74	2	16.37	8.18	1.62	0.13
Sex	74	1	0.94	0.94	0.18	0.95
Harvest region	61	2	48.84	24.42	5.37	0.001*

Appendix Table 3. Summary of wolf stomach content surveys for entire study area and by region. Data shown here indicate the number of times each prey item was found in a wolf stomach.

Prey Species	Study Area (n=64)	Hay River Lowlands (n=15)	Mackenzie (n=10)	Slave River Lowlands (n=13)
Beaver	4	1	1	0
Bison	25	4	9	7
Caribou	10	2	0	0
Fish	10	3	1	0
Hare	5	1	1	1
Moose	4	1	0	1
Willow ptarmigan	2	1	0	0
Vegetation	8	1	1	3
Domestic dog	2	1	0	0
Domestic chicken	6	2	0	0
Lynx	3	1	0	1
Spruce grouse	4	2	0	1
Red squirrel	1	0	0	0
Vole spp.	4	1	0	0
Marten	1	0	0	1
Garbage	16	0	0	3
Mink	1	0	0	0



Appendix Figure 1. Simulated mixing region based on $\delta^{13}C$ and $\delta^{15}N$ profiles for 78 wolves (black dots) sampled in the southern Northwest Territories, Canada, and average source profiles (white crosses). All wolves fell within the 95% mixing region, suggesting the six prey species plotted were appropriate and that fitting a mixing model to the dataset could explain the SI profiles of all wolves.