Using Stable Isotope Analysis to Infer Prey Specialization of Cougars

by

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ABSTRACT

Although typically referred to as a generalist species, individual prey specialization has been documented in cougars (*Puma concolor*). This behaviour has the potential to limit and regulate ungulate dynamics, particularly in cases where ungulates exist in small or isolated populations. Cougars are notoriously difficult to monitor due to their low density, large range-size, and solitary nature. Traditional methods used to quantitatively estimate the diets of these carnivores are often resource limited, labour intensive, and restricted in their resolution. We explored the use of stable isotope analysis to infer prey specialization of 7 cougars in west-central Alberta and compared results to observed specialization as estimated through kill-site analysis. We defined four isotopically distinct ($P < 0.001$) prey sources: bighorn sheep (*Ovis canadensis*), cervids (*Odocoileus* spp., *Cervus elaphus*, and *Alces alces*), small carnivores (canid spp. and *Lynx canadensis*), and snowshoe hare (*Lepus americanus*). Specialization inferred through stable isotope analysis agreed with observed estimates, indicating this method may be an efficient and reliable alternative to traditional approaches for monitoring cougar diets.

KEY WORDS

Prey specialization, stable isotope analysis, cougars, *Puma concolor*, bighorn sheep, *Ovis canadensis*, kill-site analysis, Alberta

PREFACE

This thesis is original work by Samantha L. Widmeyer. Data used for kill-site analyses were collected by Samantha L. Widmeyer, Meghan M. Beale, and Mark S. Boyce between March 2017 and October 2018. These data were collected in accordance with the Canadian Council on Animal Care (CCAC) guidelines and approved by the University of Alberta Animal Care and Use Committee (AUP00002113), and in accordance with Alberta Environment and Parks Research and Collection Permit (2017: #17-264; 2018: #18-011, 2019:#19-101), Alberta Tourism, Parks and Recreation Parks Division Research and Collection Permit (2017: #17-009; 2018: #18-003; 2019: #19-098), and Parks Canada Research and Collection Permit (2017-2019: #JNP-2017-24339). Isotope data were generated via stable isotope analysis conducted on wildlife tissue samples. Study cougar tissue samples were collected during capture and tissue samples from prey species were collected from cougar kill-sites, incidental kill-sites or roadside mortalities, and submitted by local trappers. Provincial cougar tissue samples were submitted by members of the Wild Sheep Foundation Alberta to the Boyce Lab between 2016 and 2018. Stable isotope samples were prepared and analyzed using mass spectrometry at the Great Lakes Institute for Environmental Research Chemical Tracers Lab at the University of Windsor.

Chapter 2 of this thesis will be submitted for publication in the Journal of Wildlife Management and includes M. Beale and M. Boyce as co-authors. For this manuscript, S. Widmeyer and M. Beale collected data, S. Widmeyer conducted analyses and wrote the manuscript, and M. Boyce administered and supervised the research including input on project design and manuscript writing.

DEDICATION

I dedicate this thesis to Myrtle, for her love and support over the ups and downs of my academic endeavours.

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CHAPTER 1 – COUGARS IN WEST-CENTRAL ALBERTA: CURRENT MANAGEMENT, TROPHIC ECOLOGY, AND PROJECT JUSTIFICATION

Historically, cougar populations in Alberta underwent a significant decline and range contraction as a consequence of anthropogenically induced prey and habitat declines as well as direct removal under the pretense of predator management (Alberta Environment & Sustainable Resource Development 2012). Cougars were first declared a big game species in 1971, after which they were managed with regulated hunting seasons. In 1990 a quota system was introduced to ensure sustainable harvests. Currently, the Alberta Government uses adaptive management (Walters 1986) to create and change quotas specific to Cougar Management Areas (CMAs). CMAs are divided into three zones: sink, stable, and source. Each zone has a unique target with respect to the proportion of adult females and males above a certain age harvested over a 3-year period. Quotas are altered when harvest proportions deviate beyond specific thresholds below or above target proportions. Alterations to quotas also are made based on current research on population density and demographics as well as consultation with stakeholders (Alberta Environment & Sustainable Resource Development 2012).

Since first managed as big game, cougar populations have increased in both number and distribution across Alberta (Alberta Environment & Sustainable Resource Development 2012, Knopff et al. 2013). However, quotas in mountainous CMAs remain difficult to achieve due to limited access. Stakeholders have become increasingly concerned that cougar predation in these areas might become a threat to wild ungulates, particularly bighorn sheep (*Ovis canadensis*). These concerns are justified in part by a growing body of publications documenting the significant impact cougars can have on bighorn populations (Harrison and Hebert 1988, Ross et al. 1997, Sawyer and Lindzey 2002, Festa-Bianchet et al. 2006). Although many of these

publications reference the influence of cougars in the United States (Hayes et al. 2000, Logan and Sweanor 2001), predation events in Ram Mountain and Sheep River Alberta have been shown to dominate sheep population dynamics (Ross et al. 1997, Festa-Bianchet et al. 2006, Bourbeau-Lemieux et al. 2011). Understanding predation is considered central to the understanding of both cougar ecology and management within the province (Alberta Environment & Sustainable Resource Development 2012).

Cougars are considered generalists at the population level as they will kill and consume a wide variety of prey (Knopff and Boyce 2007). In west-central Alberta prey include: white-tailed deer (*Odocoileus virginianus*), mule deer (*O. hemionus*), elk (*Cervus elaphus*), moose (*Acles acles*), bighorn sheep, mountain goats (*Oreamnos americanus*), feral horses, black bears (*Ursus americanus*), wolves (*Canus lupus*), coyotes (*C. latrans*), foxes (*Vulpes vulpes*), lagomorphs, rodents, mustelids, and avian species (Knopff et al. 2010). However, as individuals cougars may select and specialize on particular prey types (Knopff and Boyce 2007, Murphy and Ruth 2009, Elbroch and Wittmer 2013). Following Knopff and Boyce (2007) and Elbroch and Wittmer (2013), we distinguish prey selection from prey specialization. Cougars display prey selection when they kill and consume prey disproportionately to availability or the population norm (Elbroch and Wittmer 2013, Lowrey et al. 2016). Cougars are considered to specialize on the prey type contributing the greatest proportion of their diet, measured with respect to total kills or biomass consumed (Knopff and Boyce 2007, Elbroch and Wittmer 2013). For example, Lowery et al. (2016) observed a cougar (P06) that strongly selected beaver in Colorado but ultimately specialized on deer; P06 spent a disproportionate amount of time in, and reduced travel speeds around, beaver habitat though the majority of his diet was composed by deer. In Alberta, most cougars specialize on white-tailed deer (Knopff et al. 2010, Bacon et al. 2011, Alberta

Environment & Sustainable Resource Development 2012) though specialization and selection vary based on many factors including: age, sex, individual behaviour, and prey vulnerability and availability (Knopff et al. 2010, Lowrey et al. 2016, Elbroch et al. 2017, Elbroch and Quigley 2019).

Young, inexperienced, dispersing cougars typically specialize on small bodied prey (Elbroch and Quigley 2019) and they are more likely to initiate attacks on dangerous prey (e.g. porcupine, *Erethizon dorsatum*; Elbroch et al. 2017). Adult females focus on smaller bodied prey compared to adult males with both sexes typically targeting vulnerable individuals (Husseman et al. 2003, Knopff et al. 2010). In west-central Alberta cougars have been observed shifting their predation patterns seasonally to take advantage of different prey sex- and age-classes (Knopff et al. 2010). Cougars primarily killed female ungulates in late-stage pregnancy during early spring; in late spring, after the birthing period, cougars focused on juveniles. Similarly, cougars killed male ungulates just before and after the fall rut. Prey availability influences cougar predation patterns such that it is common for individuals to specialize on the most abundant ungulate within their range (Knopff et al. 2010, Elbroch and Wittmer 2013). However, prey abundance alone does not account for much variation in cougar selection and specialization, individual characteristics (i.e. age, sex, with litter) appear to have greater influence (Knopff et al. 2010).

The selective behaviour of predators can have marked effects on small or isolated populations of prey as a consequence of asymmetric apparent competition (Knopff and Boyce 2007, Johnson et al. 2013). Apparent competition refers to changes in abundance experienced by prey species that share a common predator which can influence interspecific prey dynamics (Holt 1977). Asymmetric apparent competition occurs when one species is more strongly affected than the other, such as when secondary prey exist in small populations relative to

primary prey (Knopff and Boyce 2007). In such a system, predators can exploit secondary prey while subsidizing their diets on more abundant primary prey. This is the mechanism believed to be responsible for cougar-caused declines in small populations of bighorn sheep (Rominger et al. 2004, Rominger 2018). From the available literature, it would appear that only a few cougars specialize on bighorns (Ross et al. 1997, Ernest et al. 2002a). Of five cougars with home ranges shared by sheep range in Sheep River, Alberta, one female was found to prey heavily on bighorns during some study seasons while the others did so infrequently or not at all (Ross et al. 1997). Similarly, after conducting DNA analysis on scat found at bighorn kill sites (n = 39), Ernest et al. (2002) found thirteen genotypes at only one site while two were found at multiple sites.

Cougars have been addressed as the primary cause of mortality for many bighorn sheep populations across North America (Sawyer and Lindzey 2002, Rominger 2018) including Ram Mountain and Sheep River Alberta (Festa-Bianchet et al. 2006). Specialist individuals that select strongly for bighorn sheep are thought to have the greatest influence on bighorn population dynamics (Ross et al. 1997, Festa-Bianchet et al. 2006). Given Alberta is home to 15% of the North American population of bighorn sheep and multiple record rams have been taken in the province (Alberta Environment and Parks 2015), it is no surprise stakeholders are alarmed by unmet cougar quotas in management areas containing both species.

In September 2016 the Government of Alberta introduced an extended experimental "boot" season to achieve greater cougar harvest in mountainous CMAs (Alberta Environment and Parks 2016). This early season relied on opportunistic encounters rather than the use of hounds and, to date, has been minimally successful, possibly as a consequence of insufficient advertisement. The Government of Oregon stopped the use of hunting with hounds entirely in

1994; initially harvest reduced drastically, though the numbers taken returned to previous levels only after a few years as a consequence of the increase of both cougar populations and general tag sales (Oregon Department of Fish and Wildlife 2006). Though harvest achieved during the Alberta "boot" season may eventually increase the total number of cougars taken from mountainous CMAs, this strategy might not mitigate the effects of specialist cougars on vulnerable ungulate populations. Information on the specialization of harvested cougars could be useful for assessing the success of harvest strategies if future management goals include mitigating cougar predation, a possibility given the concern of stakeholders.

Current methods for assessing the composition of individual cougar diets include: kill-site investigation (identified via GPS clustering, snow tracking, or transecting; Anderson and Lindzey 2003, Knopff et al. 2010), prey identification in scat (via microscopic characteristics or DNA; Bacon et al. 2011), or stomach content analysis (Thompson et al. 2009). These methods are laborious, time consuming, and often costly and/or restricted in temporal resolution. Stable isotope (SI) analysis of body tissues has increasingly been used to assess the assimilated diet of a number of mammalian species over the period of tissue growth and/or turnover (Newsome et al. 2009, Milakovic and Parker 2011, Koike et al. 2013). This method allows researchers to investigate animal diets via minimally invasive tissue sampling, drastically reducing time spent in the field and/or identifying prey hair using a microscope. Additionally, SI analysis has the added advantage of allowing inference into long term prey consumption of individuals post mortem. Because of the success achieved using SI analysis to infer diet composition of other carnivores (Milakovic and Parker 2011, Magioli et al. 2014, Moss et al. 2016a), we chose to investigate the use of this method (detailed in Chapter 2) for estimating specialization of cougars in west-central Alberta. We hoped to provide the Government of Alberta with a more efficient

tool for investigating cougar predation and, in particular, for identifying bighorn specialists taken during the harvest season – a potential metric of harvest success.

CHAPTER 2 - ESTIMATING PREY SPECIALIZATION THROUGH STABLE ISOTOPE ANALYSIS OF COUGAR HAIR

INTRODUCTION

Top-down forces exerted by predators on prey have the potential to limit or regulate prey dynamics (Festa-Bianchet et al. 2006, Keehner et al. 2015). To ensure viable populations and effectively manage predator-prey systems, wildlife managers need to understand and monitor predator trophic ecology. Conspecific individuals are often considered to have equivalent influence on prey, however individual specialization and selection within generalist populations has been documented in a growing number of taxa (Bolnick et al. 2003). Such is the case for cougars (*Puma concolor*); these apex predators have been observed specializing on prey disproportionately to availability (Réale and Festa-Bianchet 2003, Knopff and Boyce 2007, Elbroch and Wittmer 2013) and modifying their behaviour to target specific prey types (Ross et al. 1997, Lowrey et al. 2016). Several studies have highlighted the potential and/or realized effects of cougar depredation on small or isolated populations of ungulate species (Kinley and Apps 2001, Rominger et al. 2004, Wittmer et al. 2014). This includes declines of bighorn sheep (*Ovis canadensis*) populations, which can be found in isolate groups of fewer than 200 animals (Ross et al. 1997, Festa-Bianchet et al. 2006). Ross et al. (1997) documented a specialist cougar that killed 8.7% of a bighorn population, including 26% of lambs, over a single winter. Where cougars are managed as a game species, harvests are designed to ensure sustainable populations of these predators. Male and female quotas are assigned to management units but harvesting is otherwise indiscriminate (Alberta Environment & Sustainable Resource Development 2012). If only a few individuals specialize on rare prey, a targeted approach might be required to effectively change predation pressure placed on vulnerable ungulate populations (Ernest et al. 2002b), including those of bighorn sheep.

Cryptic carnivores, such as cougars, are notoriously difficult to monitor due to their low density, large range-size, and solitary nature (Balme et al. 2019). Traditional methods used to quantitatively estimate the diets of these carnivores – including GPS relocation clustering, snow tracking, and scat collection (Anderson and Lindzey 2003, Novack et al. 2005, Knopff et al. 2009) – are often resource limited, labour intensive, and restricted in their temporal resolution (Bacon et al. 2011, Martínez-Gutiérrez et al. 2015). More recently, an increasing number of studies have used chemical tracing to investigate predator diets (Milakovic and Parker 2011, Magioli et al. 2014, Moss et al. 2016b). Stable isotope (SI) analysis, a form of chemical tracing, exploits naturally occurring differences in SI ratios between consumer and source tissues. SI values of predator tissues are the product of the ratios of heavy to light isotopes in digested prey tissue and the physiological processes involved in the assimilation of that tissue (Wolf et al. 2009, Ben-David and Flaherty 2012). Therefore, the contribution of assimilated SIs in predator tissues is proportional to the biomass of prey consumed relative to the metabolic activity of that tissue. Metabolically inert tissues, such as hair, store diet information over their growth period (Hénaux et al. 2011, Robertson et al. 2013). On cougars, dorsal guard hairs grow to a maximum length of 30 – 40 mm (Adorjan and Kolenosky 1969, Moore et al. 1974) and mammalian hair is estimated to grow at a rate between 0.39 – 1.06 mm/day (Schwertl et al. 2003, Ayliffe et al. 2004, Cerling et al. 2006), thus SI analysis of cougar hair should reveal specialization over a period of approximately 1-3 months. Additionally, cougars molt in spring and hair growth continues into fall growing slowest over the winter months (Parng et al. 2014), therefore hair is expected to primarily represent diet during snow-free months.

Analysis of C and N isotopes frequently has been employed in diet studies (Kelly 2000). The ratio of ^{12}C:^{13}C reflects the source of forage consumed by omnivores and herbivores plus

some trophic enrichment (or discrimination) factor. Whereas the ratio of ^{14}N:^{15}N reflects trophic level such that predators have enriched nitrogen values when compared to their prey (DeNiro and Epstein 1978, 1981, Fry 2006, Ben-David and Flaherty 2012). Although it has become evident that many processes influence the SI values of consumer tissues (Tieszen and Fagre 1993, Caut et al. 2009, Martínez del Rio and Carleton 2012, Thomas and Crowther 2015, Rode et al. 2016, Hughes et al. 2018), SI analysis has been shown to be a reliable diet proxy in many studies where results were compared with alternative diet analyses (Darimont et al. 2008, Newsome et al. 2009, Milakovic and Parker 2011). Further, SI analysis has been used to infer prey specialization by predators, defined as the prey contributing the greatest proportion of total diet, in a number of studies (Newsome et al. 2009, Brickner et al. 2014, Kernaléguen et al. 2016).

Mixing models are used to relate prey (source) SI values to consumer (mixture) SI values and output quantitative diet estimates, *i.e.* the proportion of diet each prey type contributes to a consumer's diet (Stock et al. 2018). With the increase in application of stable isotopes to ecological studies, mixing models continue to be improved upon in an effort to incorporate greater complexity representative of ecological systems. To accommodate this complexity, the newest models operate within a Bayesian framework and incorporate source and mixture variability as well as uncertainty in the mean and variance of sources (Moore and Semmens 2008, Parnell et al. 2010, 2013, Ward et al. 2010). Mixing models require source values to be significantly different from one another; though this can be an issue when prey species inhabit similar dietary niches, as in the case of cougars feeding on sympatric ungulates. Nonetheless, some studies have achieved reasonable separation among ungulates with stable isotopes alone (Feranec 2007, Milakovic and Parker 2011).

Our objective was to infer prey specialization of cougars in west-central Alberta using stable isotope analysis of cougar hair. Our second objective was to compare SI results for each cougar to observed specialization as determined through kill-site analysis. We predicted prey specialization inferred through SI analysis would agree with observed estimates based on the assumption that adult cougars do not routinely switch prey and instead, consistently prey upon individual-specific primary prey.

STUDY AREA

We studied cougar predation in a 9,577 km^2 area of west-central Alberta, Canada spanning western Yellowhead County to the front ranges of east-central Jasper National Park (Figure 1). Our study area was chosen to increase the likelihood of capturing cougars specializing on different ungulate species, particularly bighorns. The study area was composed of alpine, subalpine, montane, and upper and lower foothill natural subregions. Elevation ranged from 936 – 2,768 m along rolling foothills in the east to rugged mountains in the west, respectfully. The region's climate was characterized by short dry summers and long snowy winters intermittently warmed by Chinook winds. Biophysical environmental factors and anthropogenic development influenced prey availability across the study area.

Vegetation

Vegetation varied based on natural subregion and anthropogenic development. In the Rocky Mountains, the alpine was mostly barren with growth limited to low-lying shrubs and herbs that colonized sites protected from strong winds, the subalpine was composed of herbaceous meadows and open-canopy coniferous stands at higher elevations and closed coniferous stands at lower elevations, and the montane subregion was composed of mixed forest. In the Foothills, the upper foothills primarily contained closed coniferous stands and the lower foothills contained

closed mixed stands. At higher elevations forests were dominated by subalpine fir (*Abies lasiocarpa*) and Engelmann spruce (*Picea engelmannii*). At lower elevations species included lodgepole pine (*Pinus contorta*), white spruce (*Picea glauca*), Douglas fir (*Pseudotsuga menziesii*), aspen (*Populus tremuloides*), and balsam poplar (*P. balsamifera*). Black spruce (*Picea mariana*), tamarack (*Larix laricina*), and willow (*Salix sp.*) were found in wet low-lying areas. Two reclaimed mines in the southern portion of the study area were seeded with non-indigenous graminoids and legumes during the reclamation process (Strong 2002). This unique forage community has attracted several ungulate species to the reclaimed mine including bighorn sheep, elk (*Cervus elaphus*), and mule deer (*Odocoileus hemionus*).

Anthropogenic development

The study area contained approximately 19% human-modified land increasing over a west-east gradient. Residential development was primarily contained within the northeastern quadrant of the study area surrounding the Hinton townsite (population 9,882). Environmental disturbance relating to forestry, natural gas extraction, and mining included: cut blocks and logging roads, well-pads and service roads, and open-pit mines and haul roads, respectfully. Additionally, public trails were found throughout the study area with motorized recreation primarily concentrated in the east (Ladle et al. 2017, 2019).

Prey availability

Along the rolling foothills to the east, cougars had year-round access to white-tailed deer (*Odocoileus virginianus*), mule deer, elk, and moose (*Alces alces*). Transitioning to the front ranges of JNP in the west, cougars had access to bighorn sheep, white-tailed deer, mule deer, elk, and moose. Density of ungulate prey shifted seasonally in the Rocky Mountains relative to elevation and anthropogenic development. In winter, ungulates moved to lower elevations to

avoid snow accumulation at higher elevations. In summer, ungulates often moved to higher elevations following green-up. Bighorn sheep were found year-round on the reclaimed mines; the population of sheep increased during the rut, from fall to early winter, and decreased over late winter into summer. Elk were found year-round on the reclaimed mines with large and small herds occupying open grassland and forest respectfully. Other carnivores in the study area included lynx (*Lynx canadensis*), red fox (*Vulpes vulpes*), wolverine (*Gulo gulo*), coyote (*Canis latrans*), wolf (*C. lupus*), black bear (*Ursus americanus*), and grizzly bear (*U. arctos*). These species were potential prey and/or competition for cougars (Murphy et al. 1998, Elbroch et al. 2015). Non-ungulate prey included, but was not limited to, beaver (*Castor canadensis*), snowshoe hare (*Lepus americanus*), hoary marmot (*Marmota caligata*), and grouse (Phasianidae).

METHODS

Cougar collaring

We captured and attached GPS collars on cougars between March 2017 and January 2018 following protocols approved by the University of Alberta Animal Care and Use Committee (Animal Use Protocol: 00002113) and authorized by several provincial and federal research and collection permits (see: *Preface*). We used trained hounds to track and tree cougars and, once treed, we chemically immobilized cougars with 2.5 mg/kg zolazepam-tiletamine (Telazol®) and 0.075 mg/kg medetomidine. Cougars were sexed, aged, weighed and hair samples were taken for stable isotope (SI) analysis. We estimated age using both tooth colour and wear (Shaw et al. 2007), gum-line recession (Laundré et al. 2000), and pelage spotting (Shaw et al. 2007). Cougars were classified as either adult (> 2 years) or subadult (1 – 2 years); we did not capture kittens or subadults traveling with their mothers. We outfitted each cougar with Lotek Iridium TrackM 2D

GPS collars (Lotek Wireless, Newmarket, ON, Canada) equipped with automatic drop-off mechanisms. GPS collars acquired location data every 1.5 hours (Beale et al., submitted). Finally, we administered 0.3 mg/kg atipamezole to reverse medetomidine.

Kill-site analysis

We visited kill-sites for all cougars beginning one week post capture. We downloaded location data every 1-3 weeks and rarified to 3-hour intervals for cluster analysis (as per Knopff et al. 2009). Clusters were defined as ≥ 2 relocations within 200 m of each other bound by a 6-day temporal window (Anderson and Lindzey 2003, Knopff et al. 2009). Kill-site likelihood was estimated using a logistic regression model developed by Knopff et al. (2009). During months with snow cover, we visited clusters with ≥ 30% likelihood of containing a kill to improve field efficiency and during months without snow cover, we visited clusters with ≥ 10% likelihood to increase detection of prey < 8 kg (Knopff et al. 2009). We programmed cluster centroid and relocations into a handheld GPS and attempted to visit accessible clusters within 7-10 days of the last relocation ($\bar{x}$ = 16, range = 1 – 291 days) between May 2017 and October 2018. Search time at each cluster varied based on whether or not remains from cougar kills were found. We began searching for remains by assessing cluster centroids and relocations, if no remains were found within a 20 m buffer around these points we walked ~ 10 transect lines to form a 100 x 100 m square around the centroid. If prey remains were found, we distinguished between predation and scavenging events by assessing presence of cougar predation sign and feeding behaviour, and age of kill with respect to cluster age.

Where prey remains permitted, we determined species, age, and sex of prey by assessing some combination of skeletal, anatomical, and/or pelage characteristics (Roest 1991, Stelfox 1993, Jensen 2001, Elbroch 2006). We collapsed white-tailed deer and mule deer into a single

group because we were frequently unable to distinguish between the two species. If we could not determine species with certainty (*i.e.* only enough evidence to narrow to family or genus), we sampled hair for DNA analysis. We submitted a subset of hair samples from prey remains to the Molecular Biology Service Unit, University of Alberta for DNA barcoding; mitochondrial gene CO1 was amplified to identify mammalian species. Some closely related species were too similar to distinguish using this method: fox, coyote, and wolf collapsed to Canidae spp. and white-tailed and mule deer collapsed to *Odocoileus* spp. Using both field-collected and DNA data, we estimated biomass (kg; Appendix Table 1) consumed at each kill-site. Individual cougar prey specialization, defined as the prey species contributing the greatest proportion of biomass over the observation period, was then estimated for each cougar.

Stable isotope analysis

During cougar capture, we sampled dorsal guard hairs and underfur from cougar shoulders and hips. Hair samples from prey species were collected primarily from kill-sites, opportunistically from roadside fatalities, and incidental kill sites, and several samples were submitted by local trappers. All samples were submitted to the Great Lakes Institute for Environmental Research Chemical Tracers (GLIER) Lab at the University of Windsor for sample prep and stable isotope (SI) analysis. Some prep was also completed in the Alberta Cooperative Conservation Research Unit (ACCRU) dry lab at the University of Alberta. Hair was cleaned with a solution of 2:1 chloroform:methanol to remove surficial oils then air dried. Dry samples were homogenized, weighed into tin capsules, and injected into an elemental analyzer (Costech, Valencia, CA, USA) coupled to a Thermo Finnigan Delta Plus mass spectrometer (Thermo Finnigan, San Jose, CA, USA) where $\delta^{13}C$ and $\delta^{15}N$ natural abundances (R_{sample}) were quantified. Reported $\delta^{13}C$ and $\delta^{15}N$

values measured in parts per thousand (‰) are standardized relative to Vienna PeeDee Belemnite (VPDB) and Air respectively (R_{std}) using the following equation:

$$\delta X = \frac{R_{sample} - R_{std}}{R_{std}} \times 1{,}000 \quad \text{(Eq. 1)}$$

SI analysis precision, assessed by the standard deviation of replicate (n = 12) analyses of four standards (NIST1577c, tilapia muscle, USGS 40, and urea), measured ±0.19 ‰ and ±0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$ for all standards respectfully.

We used Bayesian mixing models, within the MixSIAR R-package (Stock and Semmens 2016a) in R (R Core Team 2018), to relate prey tissue (source) SI values to cougar tissue (mixture) SI values. We included raw cougar and prey SI values in our models to account for both process and residual error (Stock and Semmens 2016b). After source selection, we compared models with different literature-derived discrimination factors and generalist or informative priors to results from kill-site analysis for each cougar.

Source selection

Results from kill-site analysis conducted on all 7 cougars were used to select prey species (sources) to include in SI modeling. Mixing models require isotopic source values to be significantly different; to accomplish this we took an a priori approach to combine non-distinct sources (Stock et al. 2018). We first qualitatively assessed prey-specific values by creating $\delta^{13}C$ and $\delta^{15}N$ biplots from raw data (Appendix Figure 1). We then tested for significant differences between prey species, under the null hypothesis of no difference, using two techniques: pairwise one-way multivariate analysis of variance (MANOVA; Appendix Table 2) and a K nearest-neighbors (KNN) randomization test (Rosing et al. 1998; Appendix Table 3). We used two tests because MANOVA requires normally distributed data and we did not have a large enough sample size for most species to confirm this requirement; though for species with sufficient

sample size, this was the case (Appendix Figure 2). KNN randomization avoids the requirement of normally distributed data and has high power with small sample sizes (Rosing et al. 1998). We collapsed sources with non-distinct values into biologically relevant groups (*i.e.* groups with similar niches) and removed potential sources if no such group could be made.

Discrimination factors

We assessed choice of discrimination factor using a method that applies the linear mixing model 'point-in-polygon' assumption – the isotopic value(s) of a consumer must be bound within a polygon of source values to establish mass balance – to a Bayesian mixing model framework (Smith et al. 2013). Mixing polygons are iterated based on the distributions of sources and discrimination factors and a 95% mixing region is calculated; if the proposed model fits the data, all consumer values should fall within this region (Smith et al. 2013). We rejected models where > 2 cougar mean SI values fell outside the 95% mixing region. We attempted to use cougar-specific discrimination factors for hair (Parng et al. 2014; Appendix Figure 3), however models including these factors were rejected as per the aforementioned criteria. Instead we applied commonly used red fox discrimination factors for fur, $\Delta^{13}C = 2.6 \pm 0.1‰$ and $\Delta^{15}N = 3.4 \pm 0.1‰$ (Roth and Hobson 2000); these values have been used to correct cougar hair SI data in previous studies (Moss et al. 2016a, 2016b).

Priors

We explored the use of both uninformative and informative priors. Priors within the package MixSIAR take on a Dirichlet distribution when the number of sources is > 2; the sum of the Dirichlet hyperparameters corresponds to the informative strength of the prior (Stock et al. 2018). Uninformative priors were defined using an even distribution with total weight equal to

the number of sources (n = 4; Appendix Figure 4). We scaled informative priors as per Stock and Semmens (2016a) such that both priors had equal strength for comparison.

RESULTS

Observed specialization

We monitored 7 cougars over the study period for an average of 245 days per cougar (range = 48-342 days). In total, we visited and investigated 455 GPS clusters and identified 180 cougar kills. Kills identified per cougar ranged from 9 to 41 ($\bar{x}$ = 26). Prey identified at kill-sites included: bighorn sheep, deer (*Odocoileus* spp.), elk, moose, coyote, red fox, lynx, beaver, snowshoe hare, red squirrel (*Tamiasciurus hudsonicus*), and ruffed grouse (*Bonasa umbellus*). With respect to total biomass consumed over the study period, five cougars (M2, F1, F3, F4, F5; Table 1) were found to specialize on deer and two specialized on bighorn sheep (M1, F2). For all cougars, species < 10.5 kg contributed < 5% of total estimated diet biomass.

Prey SI values and source selection

We collected hair samples (range, n = 3 – 61) for each of the following 8 prey types: bighorn sheep, deer spp., elk, moose, lynx, canids, snowshoe hare, and beaver. Sampling effort per species was directly proportional to the frequency of species found at kill-sites because these were our main source of samples. The C:N ratio of prey hair ranged from $\bar{x}$ = 3.09 (beaver) to $\bar{x}$ = 3.31 (bighorn sheep). Mean $\delta^{13}C$ and $\delta^{15}N$ stable isotope values ranged from -26.80‰ to -24.72‰ and 1.9‰ to 6.27‰ respectfully (Table 2; Appendix Figure 1). SI values for members of the Cervidae family were not significantly different from each other (MANOVA and KNN Randomization: $P > 0.05$; Appendix Table 2 and 3); likewise, canid and lynx values were not significantly different from each other (MANOVA: Pillai's Trace = 0.37, $F_{2\text{-}9} = 2.590$, $P = 0.129$; KNN Randomization: $k = 3$, $P = 0.398$). Beaver values were not significantly different from

bighorn sheep, moose, canids, lynx, or snowshoe hare ($P > 0.05$); subsequently, we removed beaver from further analyses. We collapsed species into 4 biologically relevant source groups (Figure 2): bighorn sheep (BHS; $n = 29$), cervids (CERV; deer, elk, moose; $n = 75$), small carnivores (SC; Canids, lynx; $n = 12$), and snowshoe hare (HARE; $n = 6$). After grouping, all source values were significantly different (MANOVA and KNN Randomization: $P < 0.001$; Appendix Table 4).

Cougar SI values and inferred specialization

For each cougar, 2-4 subsamples of hair were analyzed for isotopic content, depending on how much hair was originally sampled. Stable isotope values of $\delta^{13}C$ and $\delta^{15}N$ for cougar hair ranged from -24.02‰ to -22.16‰ and 5.97‰ to 7.38‰ respectfully and the mean ratio of C:N was 2.97. When we applied uninformed priors, the ungulate specialization of each cougar agreed with observed specialization; however, snowshoe hare contributions were overemphasized. Model output improved when we included ungulate generalist informative priors (Table 3; Appendix Figure 4). The estimated contribution of bighorn sheep to known deer specialist diets was always > 0%.

DISCUSSION

We have shown that stable isotope analysis of cougar hair can be used as an efficient tool for inferring individual prey specialization. This method may be beneficial when longitudinal diet data of individuals is needed and would otherwise be physically difficult and/or costly to obtain. Our results indicate that felid-specific discrimination factors based on controlled feeding studies of captive cougars (Parng et al. 2014) may not be applicable to wild cougars. However, Parng et al. (2014) determined discrimination between muscle composing the diets of felids and felid hair. We used prey hair to create unique dietary sources. Some studies have found no significant

differences between hair and muscle tissues (Hilderbrand et al. 1996), however others found hair to be enriched in ^{13}C compared to muscle. We analyzed a small sample of muscle from several prey types (Appendix Table 5) and compared isotope values to those of hair but did not find significant differences (Appendix Table 6), suggesting the prey tissue used to create source values did not influence mixing space geometry. Discrimination factors are known to vary as a consequence of diet composition and environment, among other influences (Caut et al. 2009), therefore the discrimination factors obtained by Parng et al. (2014) may be specific to captive felids.

We assumed prey isotope values were representative of respective prey populations within the study area. However, our sample locations (Appendix Figure 5) were altitudinally biased such that bighorn sheep were sampled above 1,600 km and most cervids were sampled below, a consequence of where kills were made with respect to species and cougar home ranges. We were unable to test for spatial differences within species but studies have shown $\delta^{13}C$ and $\delta^{15}N$ enrichment is positively correlated with altitude and regional dryness in plant tissues respectfully (Körner et al. 1988, Kelly 2000, Rubenstein and Hobson 2004). Ungulate species within our study area had the capacity to move and forage across a range of elevations such that altitudinal variation could have been diluted, though bighorn sheep typically were more restricted to higher elevations. A study comparing the scat, enamel, and collagen $\delta^{13}C$ values of five sympatric ungulates in Yellowstone National Park found bighorn sheep to be more enriched than both mule and red deer (Feranec 2007), similar to our results. This suggests the observed variability between bighorn sheep and cervid $\delta^{13}C$ values within our study area reflects true population-level variation among the two prey types.

The majority of bighorn sheep samples were taken from populations inhabiting two reclaimed mines in the southern region of the study area. This anthropogenically modified landscape primarily consisted of dry grassland, potentially increasing the $\delta^{15}N$ values of forage consumed by ungulate species on the mines. This could account for the observed $\delta^{15}N$ enrichment of bighorn values compared to cervids. However, the mines were seeded with non-indigenous graminoids and legumes during the reclamation process, reducing the biodiversity of indigenous grassland species (Strong 2002). Previous studies have found an inverse relationship between the time spent foraging on legumes and $\delta^{15}N$ enrichment (Schoeninger et al. 1997, 1998), so we might expect bighorns, particularly those residing in populations that maintained year-round presence on the mine, to have lower $\delta^{15}N$ values than populations inhabiting unaltered landscapes. If this is the case, the isotopic values of bighorn sheep and cervids might be even more distinctive in unaltered systems (e.g. Milakovic and Parker 2011).

Weak prey resolution was likely the result of a combination of both (1) unbalanced sample sizes and (2) mathematical underdetermination within the mixing system (i.e. number of sources (n) was greater than $n + 1$ tracers). Because most of our samples came from prey remains at kill-sites, prey that were infrequently killed by cougars also were infrequently sampled. Thus, even if differences in population mean isotopic values of related species existed, we were not able to detect them given our data. Mathematical underdetermination is a common problem when only C and N are used to define sources in SI studies of apex predators (Phillips and Gregg 2003, Fry 2013). A recent diet reconstruction study conducted by O'Donovan et al. 2018 on wolves in the North West Territories, Canada successfully resolved all six primary prey types by taking a combined SI and fatty-acid (FA) approach; resolved prey included two cervids (caribou and moose) and beaver. Beaver comprised 6.2% of the biomass consumed by M1 over the study

period, representing a considerable contribution to M1's diet over the spring and summer months; however, because beaver were statistically non-distinct, as found in other studies using only C and N SI data (Milakovic and Parker 2011), we were unable to include these prey as a source in our final model. As a species, cougars kill and consume a broad spectrum of prey – many with overlapping dietary niches. If greater resolution of diet composition is required, future studies should consider using a multi-biomarker approach to increase the number of dietary tracers. That being said, Milakovic and Parker (2011) were able to distinguish between elk, caribou, and moose with bivariate SI data alone, so greater cervid resolution might be achieved simply by increasing sample size.

We assumed observed prey specialization of cougars was the same as when cougar hair was grown, despite sampling hair prior to observation. We tried to control for this by capturing adults with well-established territories, with the exception of M2 – a subadult that had recently left his mother. We assumed adult animals had learned to hunt specific primary prey and maintained their preference between when their coat was grown to when their hair was sampled and kills investigated. However, adult cougars have been documented switching primary prey. Ross et al. (1997) reported that the home range of an adult female had overlapped with bighorn sheep for > 10 years before she first began to depredate, and later specialize, on bighorns. Although possible, we suspect this phenomenon occurs infrequently, such that it would be unlikely for all or most cougars to have switched their primary prey during the study period. In the case of M2, we expected subadult animals to specialize on smaller less-risky prey (Elbroch et al. 2017) and exhibit less fidelity to a single prey type as dispersing subadult cougars travel through unfamiliar terrain with unknown prey distributions (Sweaner and Logan 2010, Ruth et al. 2011, Morrison et al. 2015); thus, we only speculate on the SI results of M2.

Changes in prey abundance and vulnerability have been shown to contribute to seasonal variations in the diets of cougars with established home ranges (Elbroch et al. 2013). Limited data exist on ungulate availability within our study area, though local sources suggested the ungulate prey base was similar year-round (see: *Prey availability*) with the exception of bighorn sheep, which were most abundant on the reclaimed mines during the rut. For those cougars observed for > 200 days, we compared kill-site data over 3-4 seasons and found ungulate specialization (cervid vs bighorn sheep) to be consistent between seasons with the exception of M1 who appeared to focus exclusively on deer between October and January. The reproductive vulnerability hypothesis states that prey vulnerability shifts temporally as a consequence of reproductive physiology and behaviour (Lima and Dill 1990). Cougars in our study and others (Knopff et al. 2010) focused predation on juvenile ungulates in spring. We could not sex most species found at kill sites due to insufficient remains. However, Knopff et al. (2010) found cougars primarily killed female ungulates around the birthing period and males around the rut. We were unable to test for sex-specific or age-specific differences between isotopic values of prey though sex-specific (Kurle et al. 2014) and age-specific differences related to lactation (Jenkins et al. 2001) have been documented in other species. Because SI analysis of cougar hair most likely represents diet during snow-free months between spring and fall (corresponding with annual molt and winter coat growth) future stable isotope studies should consider the potential influence of seasonal prey availability and demographic variability among prey when deciding on sampling periods and/or interpreting results.

Ungulate specialization inferred from SI analysis agreed with observed specialization when uninformative priors were applied, however snowshoe hare was overemphasized such that F1 appeared to be a hare specialist and hare contributed > 30% to several cougar diets (Table 3).

Hare is unlikely to have been such a large portion of diet because prey weighing < 10 kg have been found to contribute < 8% of total biomass consumed by adult cougars (Bacon et al. 2011). To reduce the influence of small prey, we applied an ungulate generalist prior, and after application, the specialization of each cougar agreed with observed specialization.

Due to study design, we were unable to assess the accuracy of the estimated contribution of each prey type as inferred through SI analysis. However, the contribution of bighorn sheep was always > 0% even for cougars that had never been observed killing bighorns. An assumption of mixing models is that all sources included contribute to the mixtures to be solved (Phillips et al. 2014). We included all four prey types when estimating the prey contribution of cougars that did not have access to bighorn sheep with respect to their observed home range. We did this because we wanted to assess SI analysis as a means of inferring specialization of cougars with unknown territories, i.e. harvested cougars. The geometry of the mixing polygon (Appendix Figure 6) was constrained due to the small differences in prey isotope values. Mixing model output is dependent on the position of the mixture within the mixing-space; further, solutions including all sources are more likely when mixtures fall close to the center of a mixing space. Because the bivariate mixing geometry was constrained, model output was quite sensitive to choice of prior and all prey sources appeared to contribute to all cougars – both being well-known limitations associated with mixing-space geometry (Phillips and Gregg 2003, Moore and Semmens 2008, Brett 2014, Phillips et al. 2014).

Specialist cougars have been identified as a concern for the management of bighorn sheep (Wehausen 1996, Bourbeau-Lemieux et al. 2011, Rominger 2018). Identification of specialist individuals is notoriously difficult such that managers often resort to lethal removal of all local cougars when predation is considered a threat to bighorn population objectives (Rominger 2018).

Although intensive removal might be more effective when bighorn populations are at risk of extirpation (Ernest et al. 2002b), culling can have unexpected consequences for cougar demographics (Stoner et al. 2006, Maletzke et al. 2014) and is typically unfavourable with the broader public (Rominger 2007). SI analysis could be used to assess the efficacy of removal strategies by quantifying the number of bighorn specialists removed with respect to different approaches. Where cougars are managed through hunter harvest, such as Alberta, this method could be employed at a greater scale to assess harvest strategies. As the use of chemical tracing becomes ever more prevalent in the study of apex predator trophic ecology, wildlife managers have new opportunities to incorporate these techniques into management practices to avoid the removal of faultless animals and ensure healthy predator-prey dynamics.

Table 1. Proportion of total biomass (% kg) of prey species found at kill-sites. Results exclude prey species contributing < 0.1% or found at < 2 total kill-sites. Cougar prey specialization was defined as the prey-type contributing the greatest proportion of total biomass over the observation period.

Species	Cougar (*n* = no. total kills)						
	M1 (*n* = 38)	**M2** (*n* = 10)	**F1** (*n* = 27)	**F2** (*n* = 9)	**F3** (*n* = 41)	**F4** (*n* = 30)	**F5** (*n* = 25)
Bighorn sheep	56.4	10.5	0.0	91.5	0.0	0.0	0.0
Deer spp.	33.3	81.3	75.9	7.3	92.7	50.9	91.7
Elk	3.5	0.0	22.7	0.0	0.0	41.7	6.0
Moose	0.0	0.0	0.0	0.0	3.3	7.3	0.0
Canid spp.	0.4	4.9	1.4	1.2	2.3	0.0	2.2
Lynx	0.0	2.8	0.0	0.0	1.6	0.0	0.0
Snowshoe hare	0.1	0.4	0.1	0.0	0.1	0.1	0.0
Beaver	6.2	0.0	0.0	0.0	0.0	0.0	0.0

Table 2. Mean C:N and $\delta^{13}C$ and $\delta^{15}N$ stable isotope values (‰) of prey hair. Samples were primarily collected from prey remains at cougar kill-sites within the study area.

Species	*n*	Mean C:N	Mean $\delta^{13}C$	St. Dev.	Mean $\delta^{15}N$	St. Dev.
Bighorn sheep	29	3.31	-24.85	0.45	3.90	1.00
Deer spp.	61	3.27	-26.10	0.82	3.22	0.76
Elk	11	3.27	-26.22	0.76	3.75	0.68
Moose	3	3.21	-26.38	0.88	2.84	1.04
Canid spp.	8	3.30	-24.81	1.08	6.27	0.68
Lynx	4	3.20	-24.92	1.16	5.33	0.58
Snowshoe hare	6	3.14	-26.80	1.41	1.90	1.23
Beaver	4	3.09	-24.72	0.55	5.06	2.49

Table 3. MixSIAR results for all study cougars (n = 7) including mean proportion of diet (%) estimates and 95% credible intervals (CI) with respect to prey-type. Mean values are highlighted with an asterisk to indicate prey specialization and bolded to indicate ungulate specialization. Prey specialization was defined as the prey type contributing the greatest proportion of total diet.

		Cougar													
		M1		**M2**		**F1**		**F2**		**F3**		**F4**		**F5**	
		Mean	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**	**Mean**	**95% CI**
Prey species	**Prior**	**Uninformative Prior**													
Bighorn sheep	1	**88.0***	71.9 - 97.0	17.8	1.6 - 45.6	23.0	9.7 - 39.6	**52.5***	7.4 - 75.7	31.7	4.3 - 60.9	13.9	1.2 - 43.4	20.3	2.3 - 49.1
Cervids	1	4.8	0.3 - 15.6	**37.2***	1.1 - 87.3	**27.4**	2.2 - 61.9	22.4	0.9 - 76.5	**34.4***	1.3 - 80.2	**43.5***	1.1 - 92.2	**38.8***	1.3 - 87.5
Small carnivores	1	2.8	0.1 - 13.0	8.5	0.2 - 29.5	7.0	0.5 - 18.0	5.9	0.2 - 21.9	9.6	0.2 - 30.4	12.5	0.2 - 39.3	9.5	0.2 - 30.6
Snowshoe hare	1	4.4	0.7 - 12.3	36.6	4.7 - 72.1	42.6*	17.8 - 66.3	19.3	2.7 - 42.4	15.2	2.9 - 31.8	30.0	2.2 - 68.4	31.4	3.8 - 66.4
		Ungulate Generalist Prior													
Bighorn sheep	1.8	**87.3***	69.7 - 96.9	13.5	9.0 - 43.1	18.8	6.8 - 37.7	**45.0***	4.1 - 75.0	27.7	2.1 - 58.3	11.8	1.0 - 38.7	16.7	1.2 - 47.6
Cervids	1.8	9.5	1.0 - 25.6	**69.6***	8.0 - 98.7	**62.1***	15.0 - 92.6	44.3	4.0 - 94.1	**59.8***	8.5 - 96.7	**75.0***	9.4 - 98.5	**68.5***	10.2 - 98.2
Small carnivores	0.2	0.8	0.0 - 0.67	1.7	0.0 - 14.3	1.4	0.0 - 8.8	1.5	0.0 - 11.6	2.3	0.0 - 18.7	2.3	0.0 - 22.0	2.0	0.0 - 16.3
Snowshoe hare	0.2	2.4	0.0 - 9.9	15.1	0.0 - 58.9	17.6	0.0 - 52.6	9.2	0.0 - 34.3	10.2	0.0 - 40.8	10.9	0.0 - 52.8	12.8	0.0 - 51.2

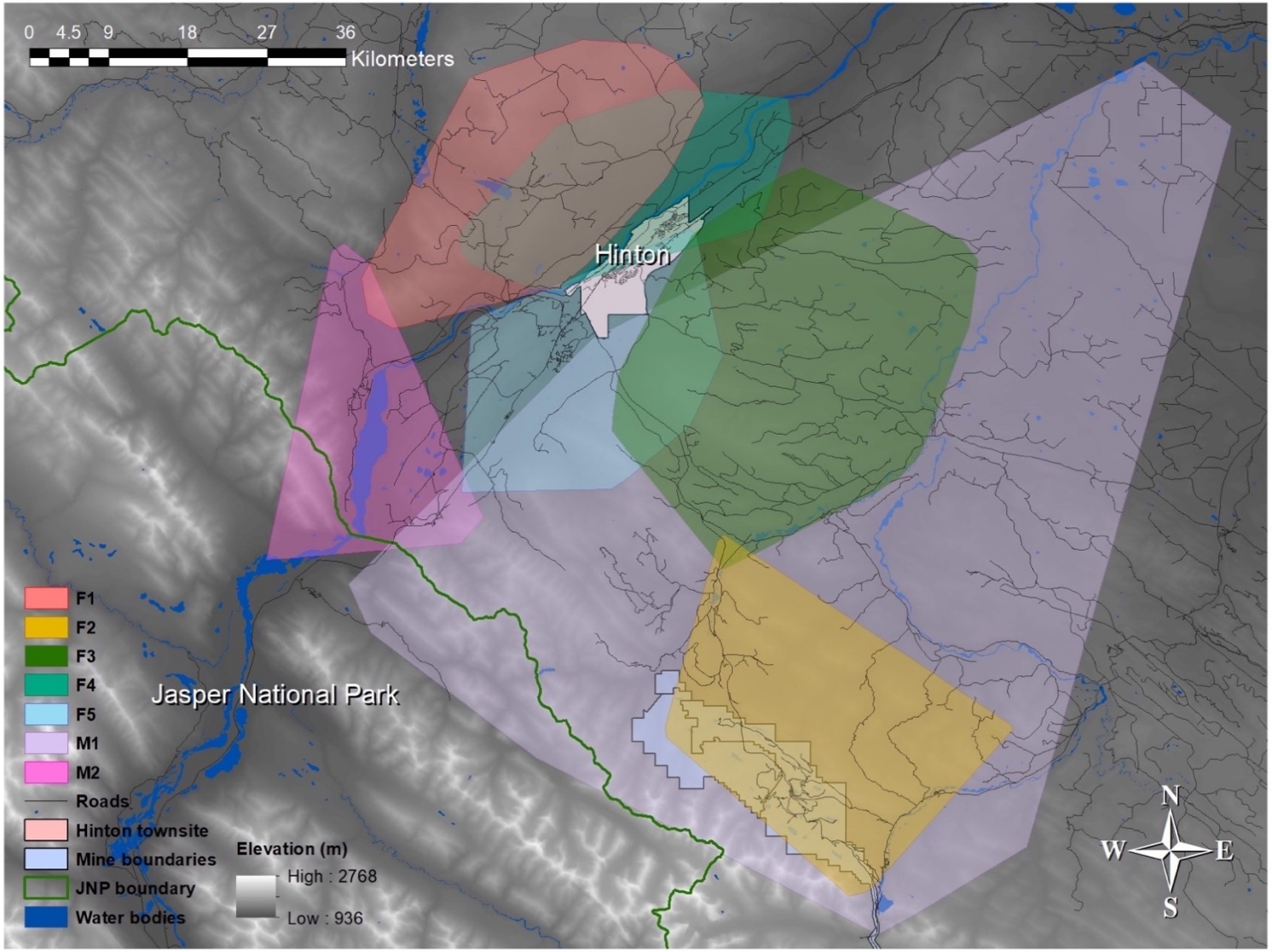

Figure 1. Study area including convex hulls of individual cougar (n = 7) movements acquired by GPS collars between March 2017 – October 2018, in west-central Alberta, Canada. Cougars were lettered by sex and numbered with respect to order of capture.

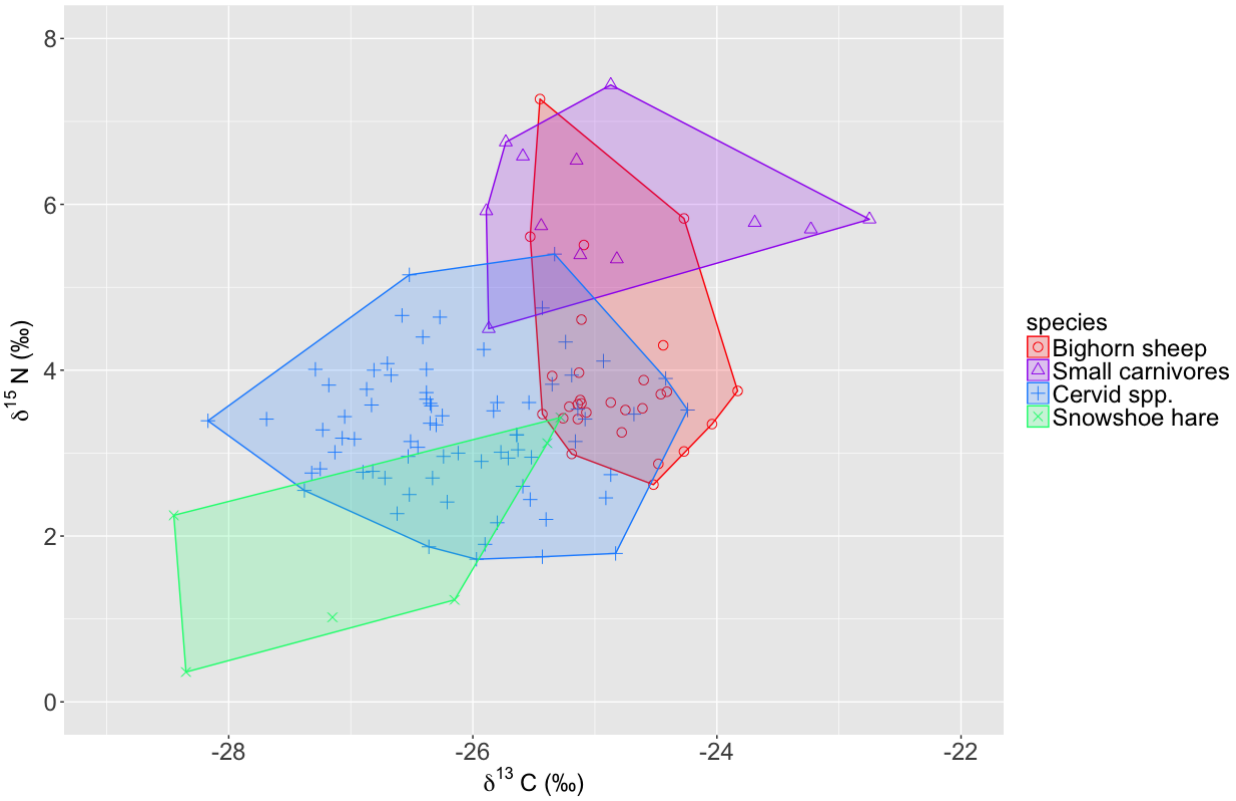

Figure 2. Stable isotope prey profiles post grouping and beaver removal. We collapsed species into 4 biologically relevant source groups: bighorn sheep (n = 29), cervids (deer, elk, moose; n = 75), small carnivores (canid spp., lynx; n = 12), and snowshoe hare (n = 6). After grouping and beaver removal, all source values were significantly different (MANOVA and KNN Randomization: $P < 0.001$).

CHAPTER 3 - APPLICATION OF STABLE ISOTOPE ANALYSIS IN STUDIES OF COUGAR TROPHIC ECOLOGY: ADVANTAGES, LIMITATIONS, AND PRECAUSIONS

Stable isotope (SI) analysis has been successfully applied to a handful of studies exploring cougar (*Puma concolor*) trophic ecology (Allen et al. 2007, Moss et al. 2016a, 2016b). The dietary patterns of Florida panthers (*P. concolor coryi*; $n = 20$) were evaluated using analysis of $\delta^{13}C$ and $\delta^{15}N$ in bone tissue and population-level trends agreed with those derived from kill-site and fecal analysis (Allen et al. 2007). Analysis of $\delta^{13}C$ and $\delta^{15}N$ of hair was used to quantify resource use and demonstrate niche expansion of cougars ($n = 41$) near urban landscapes (Moss et al. 2016a, 2016b) and to explore feeding patterns of cougars ($n = 64$) near agricultural landscapes (Magioli et al. 2014) and we have now shown that such an analysis can be used to estimate prey specialization of individuals (see Chapter 2).

Most methods for determining cougar diets are costly, time-consuming, and often require significant personnel and monetary resources. The above studies confirm SI analysis can be used in place of traditional techniques when researchers are interested in broad scale questions. Under these circumstances, not only will the use of stable isotopes be more efficient and cost-effective, opportunities to explore questions requiring longitudinal data and/or robust sample sizes increase (Ben-David and Flaherty 2012). Such opportunities may include assessing the efficacy of harvest strategies at removing bighorn sheep (*Ovis canadensis*) specialists or exploring sex- and/or age-specific niche width variation over time. Additionally, SI analysis could also be coupled with other techniques to increase confidence when drawing conclusions with respect to cougar-prey relationships. Although many potential applications exist, several limitations inhibit the wide spread use of this method and the specificity of questions that can be addressed using it.

At present, no provincial – let alone range inclusive – database exists containing the inter- and intra-specific variation of stable isotope values among cougar prey. The need for such databases has been addressed by professionals using stable isotopes to investigate questions across a diverse range of fields (Pauli et al. 2017). The stable isotope composition of organisms is a product of the composition of the substrates they assimilate and the physiological processes involved in assimilation and waste production (Ben-David and Flaherty 2012). As many different factors influence these processes (Bearhop et al. 2002, Robbins et al. 2005, Caut et al. 2009, Kurle et al. 2014, Rode et al. 2016), large datasets are crucial for accounting for variation associated with natural regions (Natural Regions Committee 2006). The stable isotope values of cougar prey are within close proximity in bivariate isotopic space (Appendix Figure 1), therefore mixing space geometry is quite sensitive to source variation. Variation in habitat characteristics between ranges of different populations of the same species have been shown to influence regional mean values (Kelly 2000, Semmens et al. 2009, Yang et al. 2015) and temporal variation within the same region associated with anthropogenically induced climate changes has also been documented (Long et al. 2005). To minimize error when estimating specialization, it is crucial that researchers input recent region-specific data into mixing models. Until these data are available across cougar range in Alberta, the extent to which hunting strategies can be assessed by applying SI analysis, among other applications, will be limited to those regions where data exist.

Cougars are apex predators existing among the highest trophic levels of the food chains they occupy. Because they are a generalist species, they have a diverse sweep of potential prey (Knopff et al. 2010). Many of these species share trophic niches and, as a consequence, overlap in bivariate isotopic space. Although the results of our study suggest agreement between

specialization as estimated using stable isotope and kill-site analysis, conclusions drawn are limited by our small sample size of cougars (n = 7) assessed. Mixing model output (Chapter 2 Table 3) suggested bighorn sheep contributed to all cougar diets – even for those individuals whose ranges did not overlap sheep range during the study period. As mentioned in Chapter 2, when constructing mixing models all prey sources included are assumed to contribute to the final "mixture" or consumer isotope values (Phillips et al. 2014). Thus, when using mixing models to estimate the specialization of unknown cougars, researchers may draw incorrect conclusions if sources included do not reflect true availability. To mitigate this issue, a third variable could be introduced to further distinguish sources, either an additional isotope where trophic enrichment patterns are predictable and well known (potentially ^{34}S; Felicetti et al. 2003, McCutchan Jr et al. 2003) or unique fatty-acids (Neubauer and Jensen 2015, O'Donovan et al. 2018). However, including additional variables may be costly (as in the case of fatty-acid analysis where a single sample can cost ~ $60 USD) or limited by sample composition. While carbon and nitrogen typically exists in a ratio around 10:1 in most animal tissues (Nardi et al. 2002), other elements are not as common. Therefore, larger amounts of sample are required in order to obtain the isotopic composition of specific elements, a potential limitation when such quantities are not available. However, as mass spectrometers become increasingly sensitive (Wieser and Schwieters 2005) this constraint may be minimal.

Although SI analysis affords researchers a new method for exploring the trophic relationships of cougars and their prey, like other methods, these analyses are subject to both physical and resource-based limitations. Many of these limitations are well known and may be associated with insufficient data (Pauli et al. 2017), discrimination factors (Bond and Diamond 2011), mixing space geometry (Brett 2014), mathematical underdetermination of models

(Phillips and Gregg 2003), and/or inadequate funding. When using SI analysis to address questions related to the trophic ecology of cougars, researchers should follow the general rules associated with any application of this method in food-web studies (Ben-David and Flaherty 2012, Phillips et al. 2014, Stock et al. 2018). For cougars, these involve having a thorough understanding of the ecology of their ranges, including prey availability, and considering potential sources of variation and/or bias associated with environmental factors, sampling procedure, and sample preparation – though this list is not exhaustive. When carefully and appropriately applied, SI analysis may provide insights into the longitudinal dynamics of cougar trophic ecology that would otherwise be difficult or practically impossible to reveal using alternative methods.

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APPENDIX

Appendix Table 1. (A) Ungulate weights (kg) used to calculate prey composition (biomass) for study cougars in west central Alberta, Canada during 2017-2018 (Knopff et al. 2010). (B) Non-ungulate prey weights (kg) used to calculate prey composition (biomass) for study cougars in west central Alberta, Canada during 2017-2018 (Soper 1970).

(A)

	Species biomass (kg)			
Age and sex class	**Deer spp.**	**Elk**	**Bighorn sheep**	**Moose**
Adult M	95	320	117	450
Adult F	70	230	65	418
Adult Unknown	82.5	275	91	434
Subadult M/F	55	181	51	330
YOY (6-12 mo)	38	124	35	226
YOY (3-6 mo)	21	68	19	123
YOY (0-3 mo)	10	33	9	60

(B)

Species	**Biomass (kg)**
Lynx	9.3
Canid spp.	8.2
Beaver	19.5
Snowshoe hare	1.4
Red Squirrel	0.2

Appendix Table 2. Pairwise multivariate analysis of variance (MANOVA) results comparing isotopic values of prey species (n = 8) under the null hypothesis of no difference.

Species A	Species B	Pillai trace	F value	*P* value	Significance
Bighorn sheep	Deer spp.	0.4579	36.736	0.000	***
Bighorn sheep	Elk	0.5771	25.247	0.000	***
Bighorn sheep	Moose	0.5772	19.799	0.000	***
Bighorn sheep	Canid spp.	0.5512	20.880	0.000	***
Bighorn sheep	Lynx	0.2004	3.758	0.035	*
Bighorn sheep	Snowshoe hare	0.6177	25.848	0.000	***
Bighorn sheep	Beaver	0.1003	1.671	0.205	
Deer spp.	Elk	0.0672	2.484	0.091	
Deer spp.	Moose	0.0166	0.514	0.601	
Deer spp.	Canid spp.	0.6703	67.097	0.000	***
Deer spp.	Lynx	0.3636	17.711	0.000	***
Deer spp.	Snowshoe hare	0.2021	8.106	0.001	***
Deer spp.	Beaver	0.2760	11.840	0.000	***
Elk	Moose	0.2428	1.764	0.217	
Elk	Canid spp.	0.8292	38.850	0.000	***
Elk	Lynx	0.5996	8.986	0.004	**
Elk	Snowshoe hare	0.5358	8.080	0.005	**
Elk	Beaver	0.5022	6.053	0.015	*
Moose	Canid spp.	0.9058	38.456	0.000	***
Moose	Lynx	0.8207	9.156	0.032	*
Moose	Snowshoe hare	0.1547	0.549	0.604	
Moose	Beaver	0.6710	4.079	0.108	
Canid spp.	Lynx	0.3652	2.589	0.129	
Canid spp.	Snowshoe hare	0.8598	33.721	0.000	***
Canid spp.	Beaver	0.1574	0.841	0.463	
Lynx	Snowshoe hare	0.9138	10.603	0.086	
Lynx	Beaver	0.0390	0.101	0.905	
Snowshoe hare	Beaver	0.6035	7.612	0.010	**

Appendix Table 3. K Nearest Neighbor (KNN) randomization test results comparing prey (n = 8) isotopic values under the null hypothesis of no difference between species. P-values are shown, k = 3; $P < 0.05$ was considered significant.

	Bighorn sheep (n = 29)	**Elk** (n = 11)	**Deer spp.** (n = 61)	**Moose** (n = 3)	**Snowshoe hare** (n = 6)	**Beaver** (n = 4)	**Canid spp.** (n = 8)	**Lynx** (n = 4)
Bighorn sheep	–	0.000	0.000	0.007	0.001	0.034	0.000	0.002
Elk	0.000	–	0.488	0.252	0.009	0.020	0.000	0.016
Deer spp.	0.000	0.488	–	0.325	0.001	0.002	0.000	0.002
Moose	0.007	0.252	0.325	–	0.630	0.424	0.009	0.128
Snowshoe hare	0.001	0.009	0.001	0.630	–	0.314	0.000	0.082
Beaver	0.034	0.020	0.002	0.424	0.314	–	0.082	0.205
Canid spp.	0.000	0.000	0.000	0.009	0.000	0.082	–	0.379
Lynx	0.002	0.016	0.002	0.128	0.082	0.205	0.379	–

Appendix Table 4. Results of significance tests post prey grouping (n = 4). (A) Pairwise MANOVA results and (B) KNN randomization test results (k = 3).

(A)

Species A	Species B	Pillai trace	F value	*P* value	Significance
Bighorn sheep	Cervids	0.4390	39.523	0.000	***
Bighorn sheep	Small carnivores	0.5171	20.345	0.000	***
Bighorn sheep	Snowshoe hare	0.8249	35.330	0.000	***
Cervids	Small carnivores	0.6381	74.064	0.000	***
Cervids	Snowshoe hare	0.1879	9.025	0.000	***
Snowshoe hare	Small carnivores	0.6177	25.848	0.000	***

(B)

	Bighorn sheep (n = 29)	**Cervids** (n = 75)	**Snowshoe hare** (n = 6)	**Small carnivores** (n = 12)
Bighorn sheep	–	0.000	0.002	0.000
Cervids	0.000	–	0.000	0.000
Snowshoe hare	0.002	0.000	–	0.000
Small carnivores	0.000	0.000	0.000	–

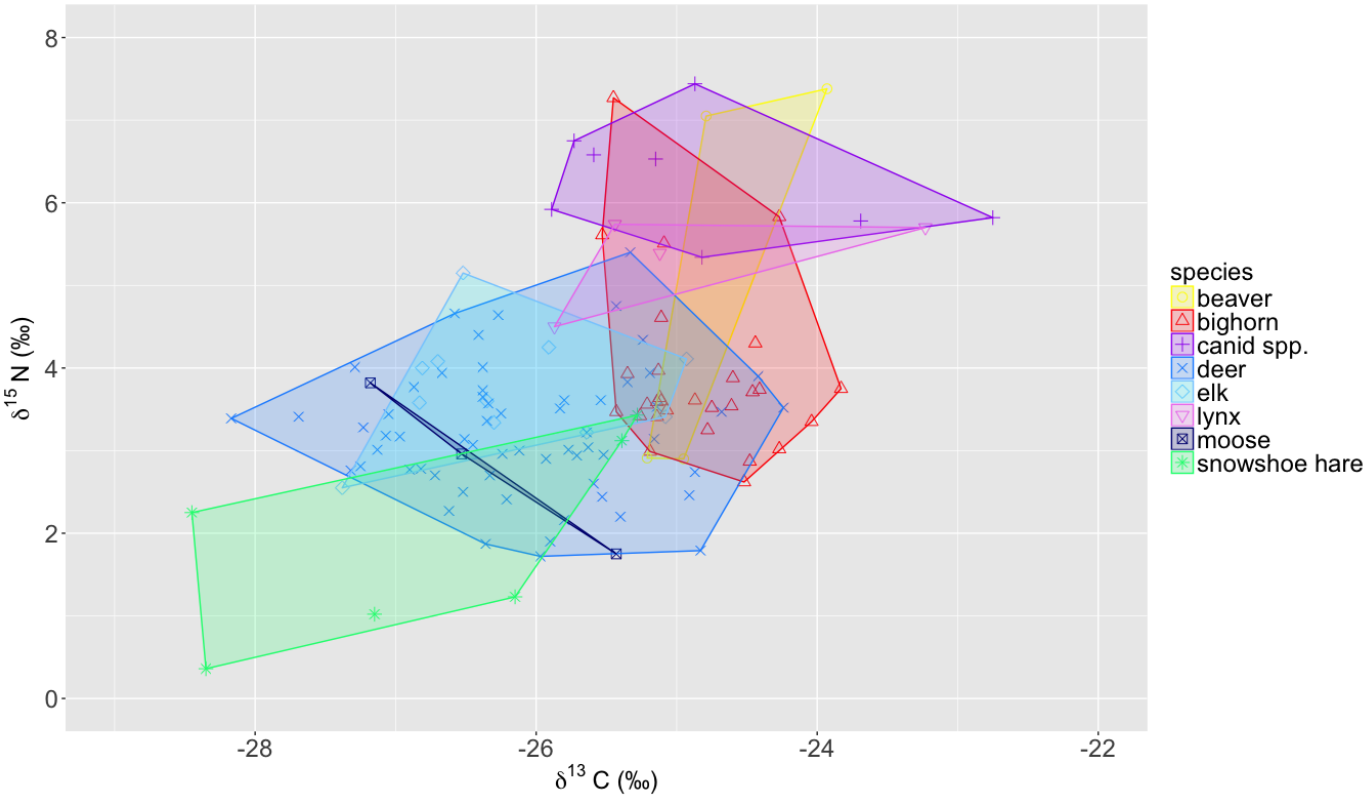
Appendix Table 5. Mean C:N and $\delta^{13}C$ and $\delta^{15}N$ stable isotope values (‰) of prey muscle. Samples were primarily collected from prey remains at cougar kill-sites within the study area.

Species	*n*	Mean C:N	Mean $\delta^{13}C$	St. Dev.	Mean $\delta^{15}N$	St. Dev.
Bighorn sheep	3	3.14	-24.37	0.25	4.20	1.44
Deer spp.	9	3.14	-26.32	0.88	3.23	0.84
Elk	3	3.15	-26.40	0.56	3.27	0.63
Moose	3	3.12	-25.32	0.58	2.70	1.92
Canid spp.	2	3.04	-24.68	0.39	7.00	0.11
Snowshoe hare	1	3.16	-27.04	NA	-1.16	NA

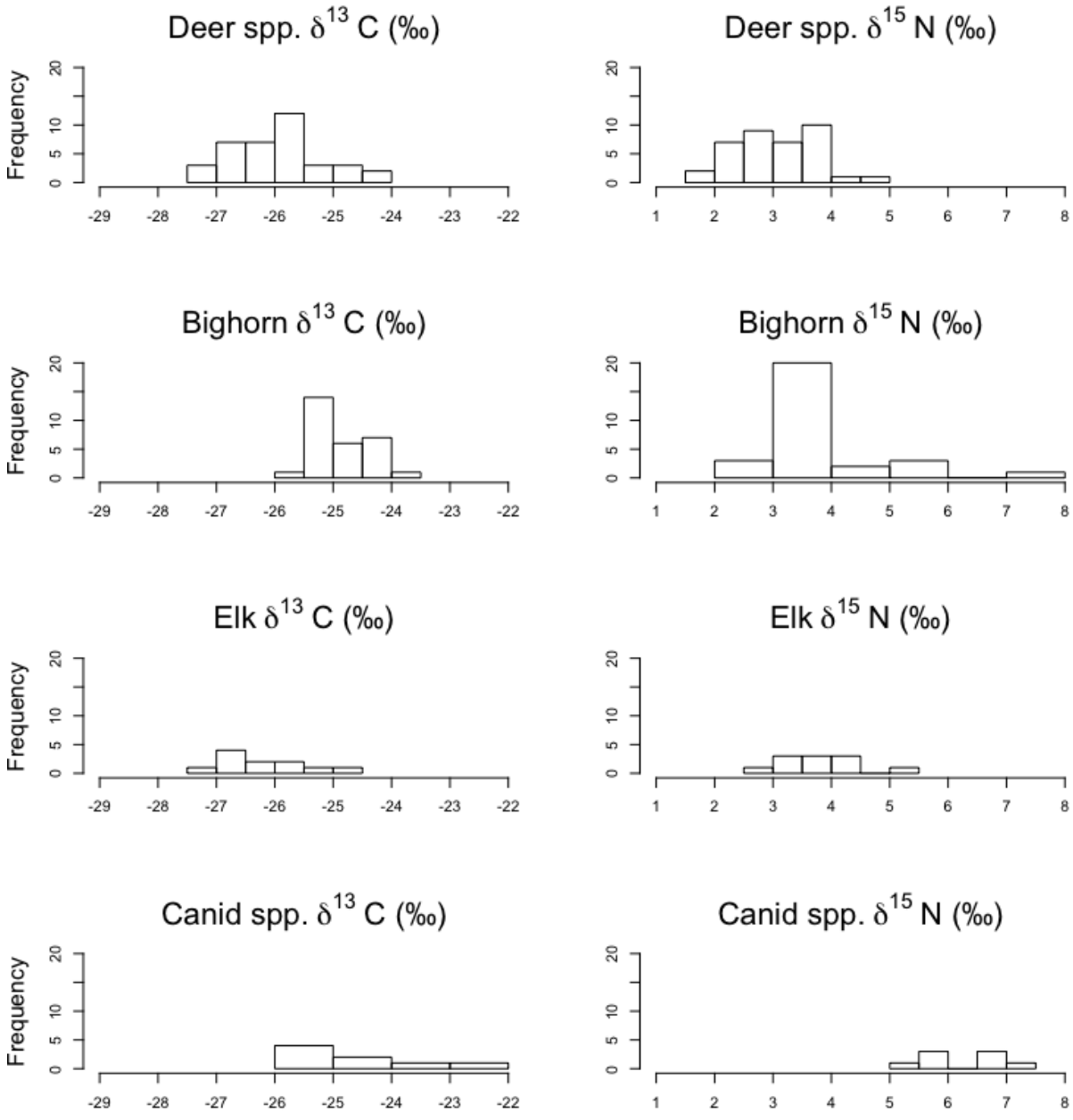
Appendix Table 6. Pairwise multivariate analysis of variance (MANOVA) results comparing intraspecific tissue (hair and muscle) isotopic values under the null hypothesis of no difference. *P* values < 0.05 were considered significant.

Species	Hair (*n*)	Muscle (*n*)	Pillai trace	F value	*P* value	Comparison* type
Bighorn sheep	29	3	0.1255	2.081	0.143	A
Bighorn sheep	26	3	0.1539	2.364	0.114	B
Bighorn sheep	3	3	0.2210	0.425	0.688	C
Deer	61	9	0.0076	0.257	0.774	A
Deer	55	9	0.0125	0.385	0.682	B
Deer	6	6	0.0100	0.045	0.956	C
Elk	11	3	0.1004	0.614	0.559	A
Elk	9	3	0.1137	0.577	0.581	B
Elk	2	2	0.1389	0.081	0.928	C
Moose	3	3	0.6687	3.028	0.191	A
Moose	1	3	0.3996	0.333	0.775	B
Moose	2	2	0.9824	27.892	0.133	C
Canid spp.	8	2	0.2494	1.163	0.366	A
Canid spp.	7	2	0.2680	1.098	0.392	B
Snowshoe hare	6	1	0.6328	3.447	0.135	A

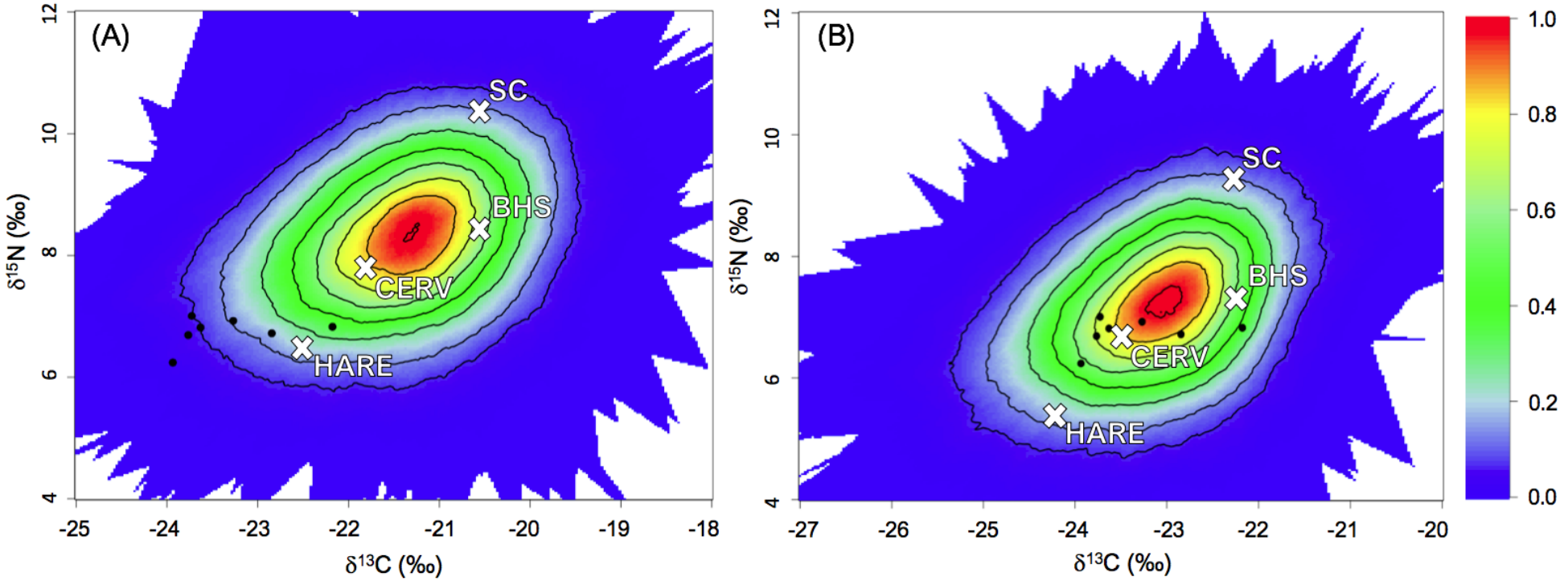
*Comparisons are summarized as follows: (A) comparing all species-specific hair and muscle samples, (B) removed hair samples if muscle was sampled from the same individual, and (C) comparing hair and muscle from the same individual



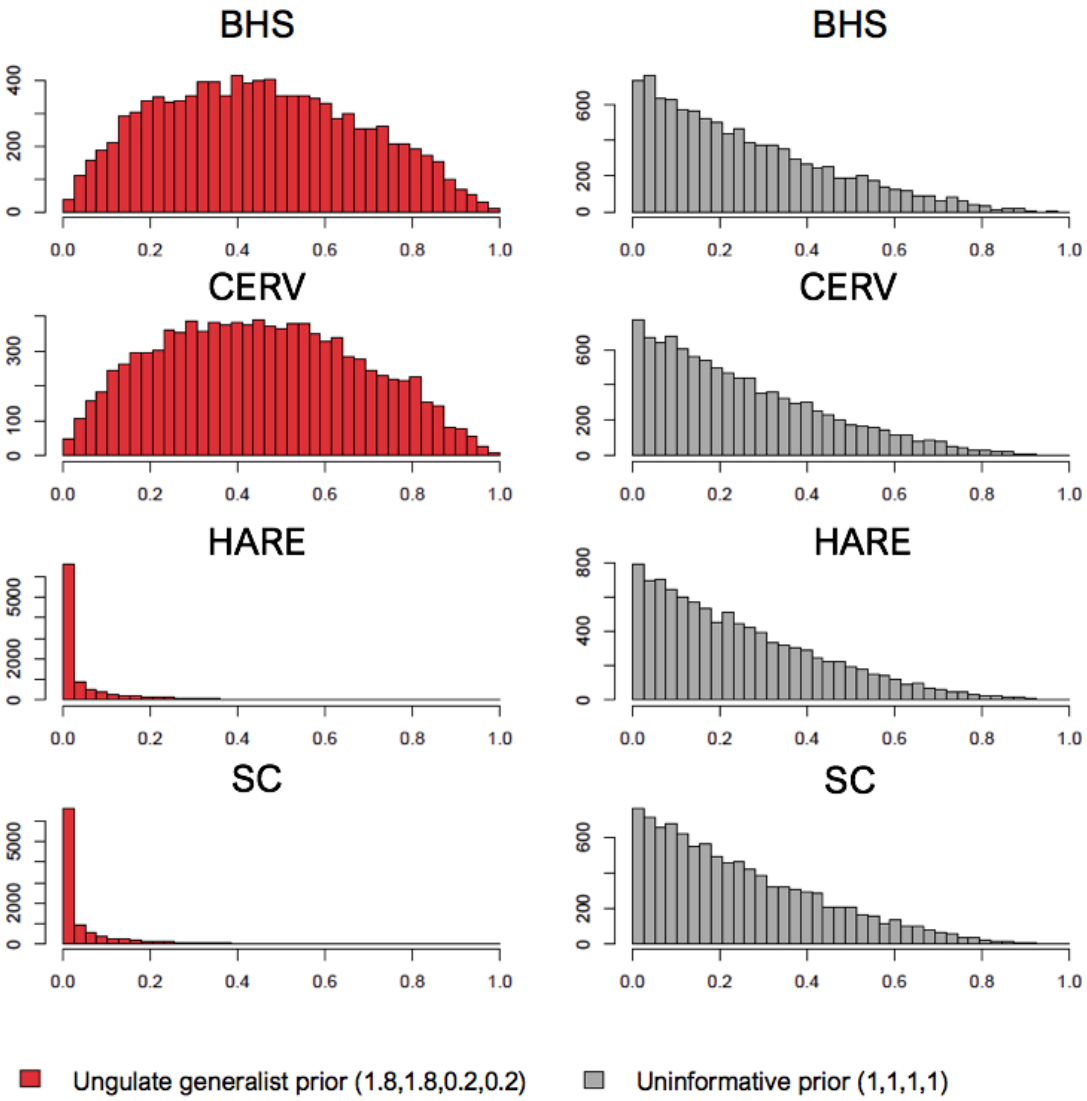
Appendix Figure 1. Stable isotope prey profiles prior to grouping. As seen by the high degree of overlap, cervids (deer, elk, and moose) were not significantly different from each other (MANOVA and KNN Randomization: $P > 0.05$); likewise, canid spp. and lynx values were not significantly different from each other (MANOVA: Pillai's Trace = 0.37, $F_{2\text{-}9} = 2.590$, $P = 0.129$; KNN Randomization: $k = 3$, $P = 0.398$). Beaver values were significantly different from 5/8 species ($P > 0.05$) and beaver was subsequently removed from further analyses.



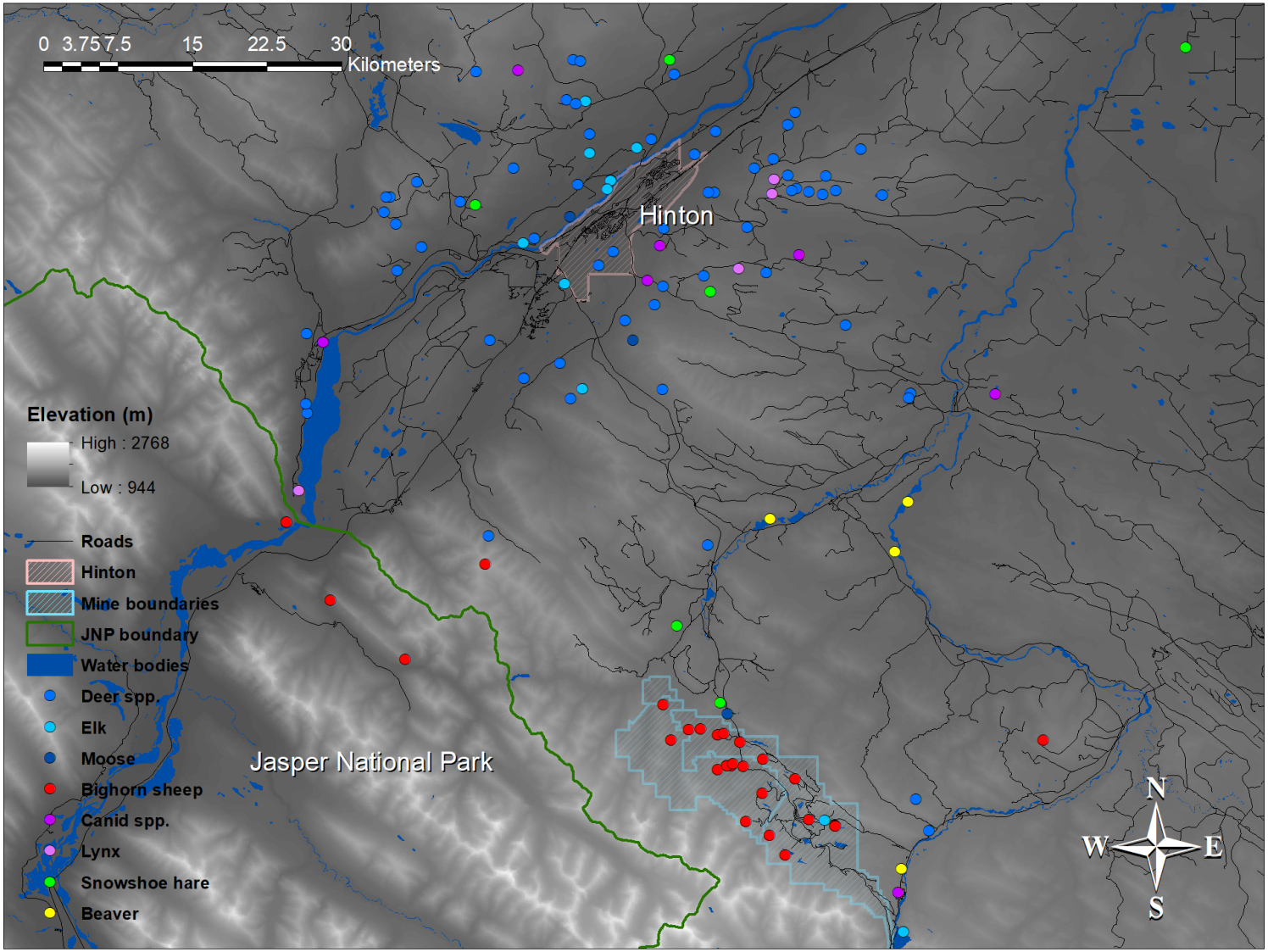
Appendix Figure 2. Hair isotope value histograms for 4 prey species: deer spp. (*n* = 61), bighorn sheep (*n* = 29), elk (*n* = 11), and canid spp. (*n* = 8).



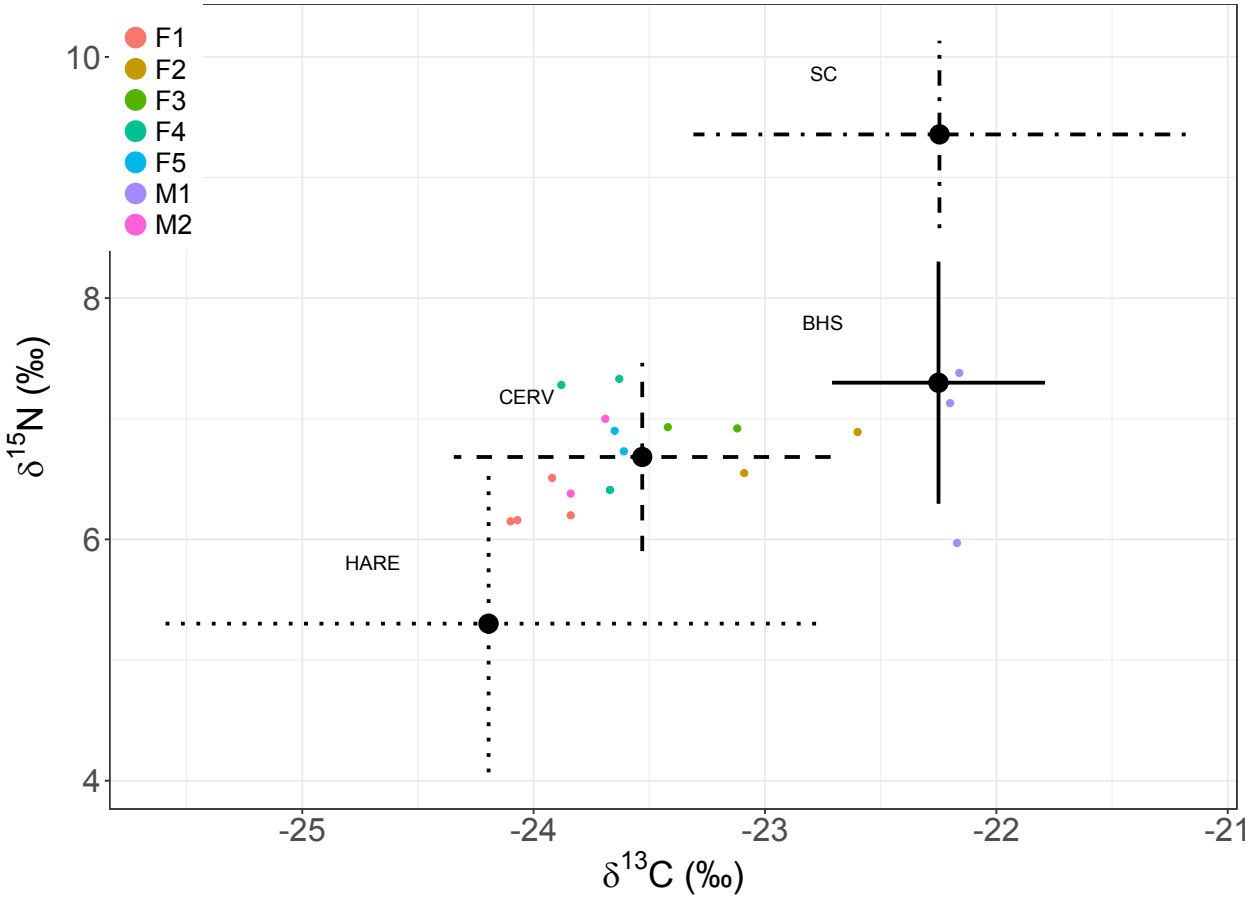
Appendix Figure 3. Simulated mixing region with respect to (A) cougar ($\Delta^{13}C = 4.3 \pm 0.5‰$ and $\Delta^{15}N = 4.5 \pm 0.2‰$; Parng et al. 2014) and (B) red fox discrimination factors ($\Delta^{13}C = 2.6 \pm 0.1‰$ and $\Delta^{15}N = 3.4 \pm 0.1‰$; Roth and Hobson 2000). The positions of mean cougar values (black dots) and the mean source values (white crosses) are shown. Sources include: BHS = bighorn sheep, CERV = cervids, SC = small carnivores and HARE = snowshoe hare. Probability contours begin at the 90% level and every 10% decrease is shown with the exception of the outermost contour at the 5% level. These plots were created using R code from Smith et al. (2013).



Appendix Figure 4. Prior distribution with respect to source (BHS = bighorn sheep, CERV = cervid spp., HARE = snowshoe hare, and SC = small carnivore). This plot was created using R code from the MixSIAR package (Stock and Semmens 2016a).



Appendix Figure 5. Prey hair sampling locations with respect to species. Samples were collected from cougar kill-sites between March 2017 – October 2018. Samples collected by trappers not shown.



Appendix Figure 6. Biplot of stable isotope values including cougar (*n* = 7) hair values (coloured dots) and source means (black dots) and standard deviations (crosses). Sources include: BHS = bighorn sheep, CERV = cervids, SC = small carnivores and HARE = snowshoe hare. This plot was created using R code from the MixSIAR package (Stock and Semmens 2016a).