DNA metabarcoding reveals dietary diversity and prey partitioning in reintroduced fishers in the Washington Cascades

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Abstract

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Diet analyses can play a key role in ecological studies, and particularly in rare carnivore conservation, by revealing trophic interactions. Carnivore populations are often limited by access to prey and so limited knowledge of their foraging ecology can hinder recovery efforts. In recent years, diet analyses and the construction of food webs have been enhanced by DNA metabarcoding, which allows for the identification of prey species at a much finer scale than traditional identification methods. Limited prey availability may be a key factor hindering reintroduction success for small carnivores, thus investigating diet habits with modern techniques in reintroduction sites can provide insights into the role of prey choices in population establishment. Fishers (*Pekania pennanti*) are one of the most frequently reintroduced carnivores in North America, but their reintroduction success is variable. The reintroduction of fishers in Washington state offers an opportunity to assess diet variation across reintroduction areas using high-resolution, DNA metabarcoding methods, to evaluate factors affecting the success of population establishment. I assessed fisher diets in two reintroduction sites in the Washington Cascade Mountains, USA: the South Cascades and the North Cascades. I collected 300 fisher scats and gastrointestinal tracts and 167 scats of sympatric carnivores - bobcat (*Lynx rufus*), coyote (*Canis latrans*), and Pacific marten (*Martes caurina*) - using telemetry and scat detection dog teams. Snowshoe hares (*Lepus americanus*) were the most prevalent prey species in both fisher populations (North Cascades = 9%, South Cascades 68%), but there were substantial compositional differences between diets. Species richness was over three times greater in the North Cascades and no single prey exceeded 10% of sequence counts. I demonstrated that two geographically close and ecologically similar reintroduction sites can yield strikingly different diet profiles in fishers, highlighting the importance of localized diet analyses for carnivores whose foraging strategies can be strongly influenced by environmental factors. Additionally, DNA metabarcoding enabled us to detect important prey species that facilitate resource partitioning among fishers and competing mesocarnivores at a fine scale. I detected relatively high overlap among carnivores that generally increased as pairwise differences in body mass decreased. While dietary overlap was substantial for common prey items, I found strong compositional differences among diets. Consequently, restricted access of fishers to energetically efficient prey may slow recovery efforts in this region, highlighting the importance of investigating the role of competition in species restorations.

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CHAPTER ONE

General Introduction

Species reintroductions are a critical tool for restoring wildlife that cannot recolonize their historical landscapes (Seddon 2010), but these efforts may be unsuccessful if modern species assemblages make habitat that was previously occupied less viable for restored species (e.g., Moseby et al. 2021). Over the last century, there has been unprecedented anthropogenic restructuring of animal communities, where habitat is modified and trophic dynamics are altered as species are extirpated or introduced (Gonzalez et al. 2011, Dirzo et al. 2014). Thus, a fundamental question in reintroduction ecology is how native species will interact with their historical environment following restorations. Translocations have variable success, with many failing to establish self-sustaining populations for many reasons, including poor habitat quality (Griffith et al. 1989, Wolf et al. 1996, Fischer & Lindenmayer 2000). Ultimately, the trophic interactions in which a translocated species engages (e.g., predator-prey or predator-competitor) will be key determinants of reintroduction outcomes and should be monitored closely. This is especially true for carnivores, whose populations are closely linked to prey densities (Hatton 2015), therefore, knowledge of the quality of foraging opportunities can improve reintroduction success.

Fishers (*Pekania pennanti;* Mustelidae) are mesocarnivores native to northern forests in North America (Powell 1993). Fishers experienced a large-scale range contraction following Euro-American settlement and subsequent overexploitation (Lewis, Powell & Zielinski 2012). As one of the most commonly reintroduced carnivores, fishers have undergone numerous translocations to portions of their former range (Lewis, Powell & Zielinski 2012). However, these reintroduction attempts have had variable success: while translocations in their eastern distribution have largely led to population establishment and subsequent range expansions, reintroductions in their western distribution have been half as likely to succeed (Lewis, Powell & Zielinski 2012). Inadequate access to prey is hypothesized to contribute to variability in population establishment (Lewis, Powell & Zielinski 2012, LaPoint et al. 2015). Carnivore populations can be strongly influenced by access to the most profitable prey, which are prey that provide the highest amount of net energy gain per unit time (Wolf & Ripple 2017). Fishers are forest habitat specialists with diverse feeding habits, often consuming small and mid-sized mammals, birds, fruits, and carrion (LaPoint et al. 2015, Powell 1993). Despite their generalist diet, medium-bodied mammals, such as the snowshoe hare (*Lepus americanus*; 1-2 kg), are considered their most energy efficient prey (Powell 1979). Indeed, the presence of hares in fisher diets has been linked to stable or expanding populations (Bowman et al. 2006, Bulmer 1974), and snowshoe hare and porcupine declines have been implicated in declines in fisher body condition over time (Kirby et al. 2018). These findings suggest that access to medium-bodied, high-value prey can influence the degree of success of fisher reintroductions. Decreasing body conditions in response to inadequate resource acquisition can lead to reduced reproductive output and lower survival rates (e.g., Williams et al. 2013, Renton et al. 2015), thus population vital rates of fishers may be affected by persistent restrictions from medium-bodied prey.

Access to prey can be influenced simply by the temporal and spatial abundance of prey on a landscape, but even when resources are abundant, access can also be shaped by competitive pressure (Hardin 1970, Schoener 1983). Competition among species is an important ecological process as it shapes how organisms access key resources (Hutchinson 1957; MacArthur 1972). Competition can be a major limiting factor in carnivore restorations, whether it be exploitative (the use of shared resources) or interference (aggressive displacement or predation; Birch 1957, Miller 1967). Competition theory predicts that in a limited resource space, species must mitigate competitive pressure through niche differentiation to coexist (Hardin 1970, Schoener 1983). This process is typically hierarchical, whereby subordinate competitors are forced to adapt to altered niche spaces whether through fine-scale selection of microhabitats, temporal activity patterns, or alternate prey selection (Cozzi et al. 2012, Kamler et al. 2012, Remonti et al. 2012). Such was the case with San Joaquin kit foxes (*Vulpes macrotis mutica*), which partitioned space and foraging strategies to mitigate predation by coyotes (*Canis latrans*) in their shared habitat (Nelson et al. 2007). Whereas species with broad environmental tolerances ("generalists") can mitigate competitive pressure through flexibility in their resource selection (Wilson & Yoshimura 1994), persistent restriction of optimal food resources may have bioenergetic consequences (Pretrov et al. 2020).

Here, I examined prey selection in reintroduced fisher populations in the Washington Cascades, USA to quantify dietary variation between populations and overlap among sympatric competitors. I assessed the implications of these findings for fisher reintroductions and their effects on prey populations and subordinate competitors. Fishers experienced population losses throughout the Pacific coastal states (California, Oregon, and Washington) following extensive European settlement and increased harvest pressures (Lewis, Powell & Zielinski 2012). These declines included an apparent extirpation from Washington (Hayes & Lewis 2006). In 2008, an effort to re-establish self-sustaining fisher populations throughout their historical range in Washington was initiated (Lewis 2014). Fishers were translocated from populations in British Columbia and Alberta, Canada to three distinct recovery zones: the Olympic Peninsula (2008 - 2013), the South Cascades (2015 - 2020), and the North Cascades (2018 - 2020; Hayes & Lewis 2006; Lewis et al. 2022). The variability in the success of fisher reintroductions in their western distributions necessitates that we evaluate factors that may influence population establishment, such as prey selection, among reintroduction sites. As prior research has demonstrated a key link between the use of small-bodied prey and reduced body condition (Bowman et al. 2006), detailed diet profiles of fishers can help us monitor population establishment.

DNA metabarcoding has emerged as a powerful molecular-based tool in diet assessments for its abilities to simultaneously identify many taxa within a single diet sample, often with finer taxonomic resolution than morphology-based approaches (Shehzd et al. 2012, De Barba et al. 2014, Massey et al. 2021). The application of DNA metabarcoding has led to a more detailed understanding of trophic systems (Lu et al. 2023, Waggershauser et al. 2022) and predator-prey relationships (Roffler et al. 2021, Tosa et al. 2023, Hacker et al. 2022), and has increased the breadth of prey species able to be identified in carnivore diets (Monterosso 2019).

In chapter two, I used DNA metabarcoding to analyze the diet of reintroduced fishers in the Washington Cascades to assess dietary variation between two ecologically similar reintroduction areas, thereby expanding the current knowledge on alternative prey use in fishers. I expected that fisher diet composition between the sites would be largely similar, owing to the general similarity of the habitat and prey compositions. Additionally, as fundamental questions remain regarding the ability of DNA metabarcoding sequencing outputs to reflect biomass consumed by a focal species (Deagle et al. 2019, Lamb et al. 2019), I aimed to better understand the relationship between the prey biomass consumption and relative read abundance of sequence reads.

In chapter three, I compared the diet composition of North Cascades fishers to sympatric competitors: coyotes, bobcats (*Lynx rufus*), and Pacific martens (*Martes caurina*). I tested theoretical predictions on sized-based hierarchical structures of competitive carnivore systems that may impact fishers' access to optimally sized prey, and I identified resource axes that facilitated prey partitioning to better understand the role of interspecific competition in fisher recovery. I predicted that all species would overlap in common abundant prey, such as snowshoe hares and carrion, but the degrees of overlap would be the lowest between carnivores of greatest body size differences (e.g., marten and coyote).

This work will contribute to our knowledge of geographic variation in the fisher's diet, while also shedding light on their responses to reduced prey access. Understanding a species' foraging ecology can lead to important insights into habitat selection (Coffin et al. 1997) and population dynamics (Flynn and Schumacher 2009) and is particularly relevant for forming effective management plans for rare species (Gillespie 2013). Ultimately, the knowledge gained from this study can be synthesized with post-release monitoring metrics of population establishment to better understand prey access as a driver of reintroduction success or failure.

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CHAPTER 2

Metabarcoding reveals striking dietary variation of a reintroduced carnivore.

ABSTRACT

Carnivore populations are often limited by prey availability, so knowledge of their foraging ecology is required for successful recovery efforts. Limited prey availability may be a key factor hindering reintroduction success for small carnivores, thus investigating diet habits with modern techniques in reintroduction sites can provide insights into the role of prey choices in population reestablishment. The reintroduction of fishers (*Pekania pennanti*) in Washington state, USA, offers an opportunity to assess diet variation across reintroduction areas using high-resolution, DNA metabarcoding methods. We assessed fisher diet in two reintroduction sites in the Cascade Mountains of Washington: The South Cascades and the North Cascades. First, we validated relative read abundance metrics through feeding trials conducted while translocated fishers were held prior to release and found moderate correlations between relative read abundance and proportional mass of five prey items consumed ($r = .47$). We then collected 300 fisher scats and gastrointestinal tracts using telemetry and scat detection dog teams and characterized diet profiles using relative read abundance through DNA metabarcoding. Snowshoe hares (*Lepus americanus*) were the most prevalent prey species in relative read abundance data in both populations (North Cascades = 9%, South Cascades 68%), but there were significant compositional differences between diets. The South Cascades fisher diet had limited prey diversity ($n = 19$ taxa), whereas 71 taxa were represented in the North Cascades diet. Optimally sized prey (1-2 kg) comprised 82% of South Cascades fisher diets but only 17% of North Cascades diets. Our findings highlight surprisingly high levels of dietary variation among

adjacent reintroduction sites with similar habitat and prey compositions, underscoring the importance of local diet assessments in formulating conservation plans.

INTRODUCTION

Understanding species' foraging ecology can lead to important insights into habitat selection (Coffin et al. 1997) and population dynamics (Flynn & Schumacher 2009) and is particularly relevant for forming effective management plans for endangered or rare species (Gillespie 2013). As carnivore populations are closely linked to adequate densities and biomass of prey (Hatton et al. 2015), limited knowledge of their foraging ecology can hinder recovery efforts. Lack of sufficient prey resources has been implicated in failed population establishment in carnivore reintroductions (Lewis, Powell & Zielinski 2012, Stepkovich et al. 2022), thus understanding how feeding habits are related to translocation success is vital for improving restoration efforts. Furthermore, diet studies can lead to a better understanding of a reintroduced carnivore's impact on local prey species (Klare et al. 2010). This knowledge is especially pertinent for generalist carnivores, whose broad and flexible diets can have wide-reaching and unpredictable trophic effects on their environments (Dickman 1996).

Carnivore diets have traditionally been evaluated through the meticulous manual sorting and morphological identification of indigestible contents in scats and gastrointestinal tracts (Lockie 1959, Leopold and Krausman 1986, Spaulding et al. 2000, Klare et al. 2011). However, as certain prey lack digestible hard parts or distinguishable remains, misidentification and omission errors can bias ecological inferences garnered from these methods (Massey et al. 2021, Morin et al. 2016, Spaulding et al. 2000, Zeale et al. 2011). Alternatively, DNA metabarcoding is a molecular-based technique increasingly being applied to diet studies for its ability to simultaneously identify many taxa within a single diet sample, often with finer taxonomic resolution than morphology-based approaches (Shehzd et al. 2012, De Barba et al. 2014, Massey et al. 2021). The application of DNA metabarcoding has led to a more detailed understanding of trophic systems (Lu et al. 2023, Waggershauser et al. 2022) and predator-prey relationships (Roffler et al. 2021, Tosa et al. 2023, Hacker et al. 2022), and has increased the breadth of prey species able to be identified in carnivore diets (Monterosso et al. 2019). Yet, global trends indicate that morphology-based analysis continues to dominate carnivore diet investigations, having been used in 92% of studies since the advent of molecular alternatives (Monterroso et al. 2019). This has been true in reintroduction ecology, where the initial time and financial investments of translocations can preclude the incorporation of diet evaluations in post-release monitoring (Stepkovich et al. 2022). However, the declining costs of high-throughput sequencing and wider availability of bioinformatic tools are making these powerful techniques increasingly accessible.

Fishers (*Pekania pennanti;* Mustelidae) are mesocarnivores native to northern forests in North America (Powell 1993) that experienced a wide-scale range contraction following Euro-American settlement and subsequent overexploitation (Lewis, Powell & Zielinski 2012). Through restorations, fishers have recovered sizable portions of their historical range (Lewis, Powell & Zielinski 2012). However, these reintroduction attempts have had variable success: whereas translocations in their eastern distribution have largely led to population establishment and subsequent range expansions, reintroductions in their western distribution are half as likely to succeed (Lewis, Powell & Zielinski 2012). Inadequate access to prey is hypothesized to contribute to variability in establishment success (Lewis, Powell & Zielinski 2012, LaPoint et al. 2015). Notably, LaPoint et al. (2015) linked expanding fisher populations to greater dietary proportions of carrion or leporids, which are considered "optimal prey" as they offer fishers the greatest ratio of energy per unit of effort expended (Charnov 1976). When fisher diet profiles are primarily composed of small prey, reproductive output and survival may be reduced (Williams et al. 2013, Yu et al. 2013, Renton et al. 2015).

The relationship between fisher population dynamics and their prey makes them an exemplary species in which to evaluate diet habits in the context of recovery. Additionally, generalist species may be especially vulnerable to biases in morphological scat analysis (Díaz-Ruiz et al. 2013), so DNA metabarcoding may reveal novel insights on the foraging ecology of fishers. Using DNA metabarcoding, we evaluated the diet of fishers in the South Cascades and North Cascades of Washington, USA. Despite generally similar habitats and prey composition among the two reintroduction areas, a preliminary assessment of prey abundance in the Washington Cascades found that potential key prey, snowshoe hares (*Lepus americanus*) and tree squirrels (*Tamiasciurus* and *Glaucomys*), were five times less abundant in the North Cascades than in the South Cascades (Humphries 2021, Parsons et al. 2020). As the spatial and temporal abundance of prey can greatly influence fine-scale foraging habits (Gittleman 1986, Roff 2002), comparisons of localized diet studies can lead to a better understanding of how specific environmental factors, such as prey selection, impact translocation success.

Here we used DNA metabarcoding to analyze the diet of reintroduced fishers in the Washington Cascades. The primary goal of this study was to assess dietary variation in a generalist carnivore between two ecologically similar reintroduction areas, thereby expanding the current knowledge on alternative prey use in fishers. We hypothesized that fisher diet composition between the sites would be largely similar, owing to the similar nature of the habitat and prey compositions, but we expected alternative prey to be more prevalent in North Cascade fisher diets because of lower hare and squirrel abundance. Because fundamental questions remain regarding the ability of DNA metabarcoding sequencing outputs to reflect biomass consumed by a focal species (Deagle et al. 2019, Lamb et al. 2019), we additionally aimed to better understand the relationship between prey biomass consumption and relative abundance of sequence reads.

STUDY AREA

Fishers historically occurred throughout coniferous forests in Washington but were likely extirpated in the state during a wide-scale range contraction following European settlement. To restore fishers to their historical range in Washington, translocations occurred in the southern (hereafter South Cascades) and northern (hereafter North Cascades) portions of the Cascades Fisher Recovery Area (Hayes & Lewis 2006, Lewis et al. 2022; Figure 2.1). Translocations occurred from 2015 to 2020 in the South Cascades and from 2018 to 2020 in the North Cascades. The North Cascades area spans ~6000 km^2 in Mount Baker-Snoqualmie and Okanogan-Wenatchee National Forests (3000 km^2), North Cascades National Park Service Complex (2768 km2), Washington Department of Natural Resources land (250 km^2) and surrounding private lands (250 km^2). The South Cascades area spans ~5000 km^2 and comprises federal lands on the Gifford-Pinchot National Forest and Mount Rainier National Park, and a small portion of private lands (~5% km^2).

Dispersing fishers may in time travel between the South and North Cascades, but presently, the reintroduction sites are fragmented by less suitable habitat for 60 km between the boundaries of the reintroduction areas (Lewis et al. 2022). Connectivity between these habitats is further limited by Interstate-90, a high-volume travel corridor that is known to limit wildlife movements (Singleton & Lehmkuhl 1999). The North and South Cascades are similar in physiography and vegetative composition. The Cascades have a maritime climate consisting of warm summers and mild winters with high precipitation amounts (90 – 220 cm per year). Dominant tree species included Douglas fir (*Pseudotsuga menziesii*), silver fir (*Abies amabilis*), noble fir (*Abies procera*), and Western hemlock (*Tsuga heterophylla*). Our sampling efforts focused on low to mid-elevation forestlands and were guided by fisher post-release movements throughout the western Cascades to near the Cascade crest (Lewis et al. 2022).

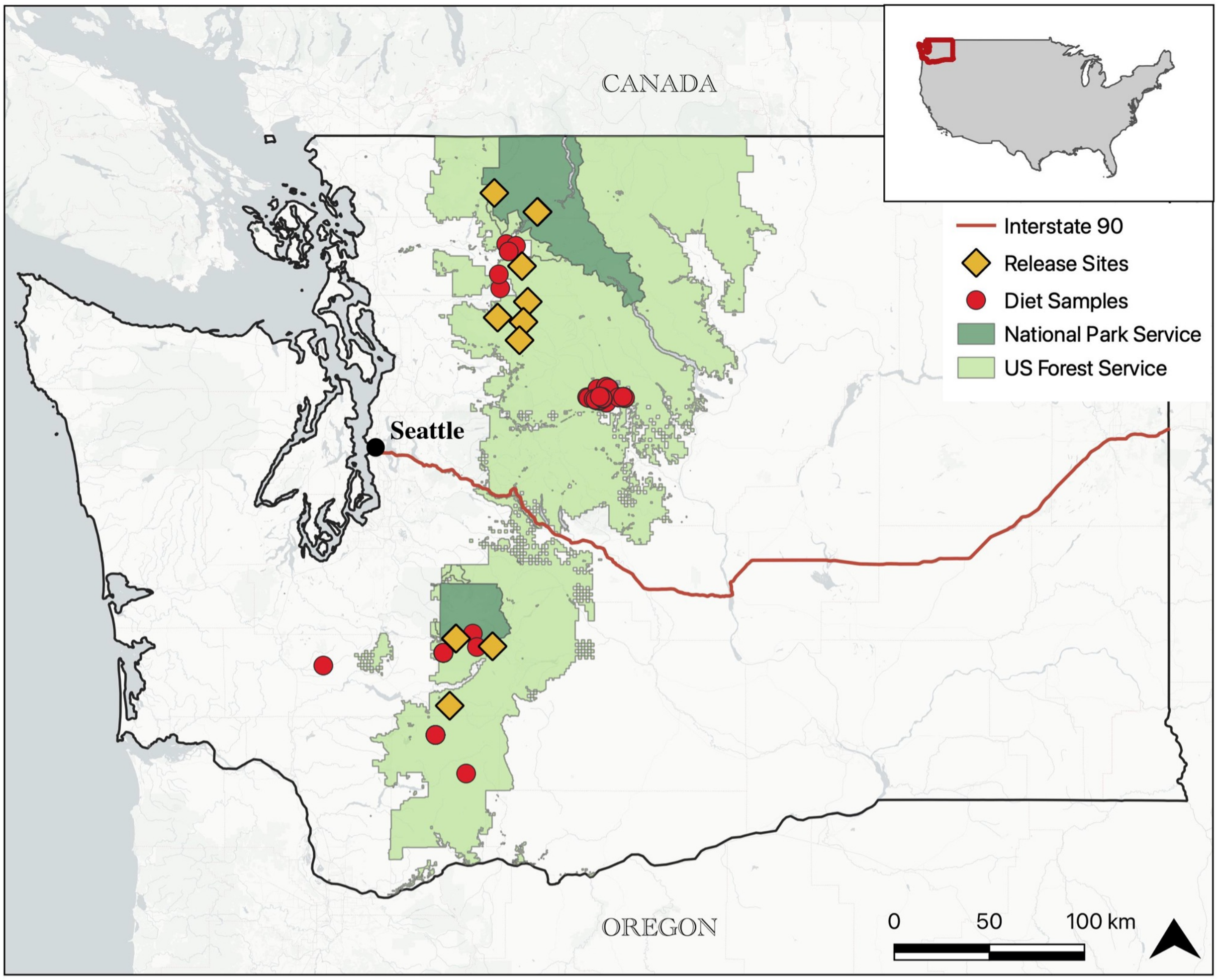

Figure 2.1. Study area map of the North Cascades and South Cascades fisher reintroduction areas in the Washington Cascades, USA. Red points represent diet sample collection sites. Scats were collected from 2016 to 2018 in the South Cascades and 2020 to 2021 in the North Cascades.

METHODS

Sample collection

During translocation, North Cascades fishers were temporarily held at the Calgary Zoo for health assessments and transmitter implantation. During this time, detailed records of fisher food intake were kept for 35 fishers. Food items included chicks (*Gallus gallus*), herring (*Sprattus sprattus*), pacific smelt (*Salanx cuvieri*), horse meat (*Equus caballus*) and house mice (*Mus musculus*). The mass of food items consumed was recorded during daily feedings. Any food not consumed by the following day was removed and the remaining mass was subtracted from the initial measurement. The mass of food items fed was generally consistent across multiple feeding days.

Prior to release, fishers were implanted with very high frequency (VHF) transmitters (Holohil AI-2HM; Carp Ontario K0AIL0, Canada) to allow reintroduction teams to monitor post-release movements and survival using aerial and ground telemetry (Lewis et al. 2022). Fishers were monitored from once per week to once per month. In the South Cascades, scat samples were collected opportunistically at denning and resting sites during post-release monitoring. In the North Cascades, scat detection dog and handler teams conducted surveys near recent telemetry locations, resting sites, and denning sites. During winter months, additional scats from both populations were collected by following fisher tracks in snow. Additionally, gastrointestinal tracts were collected from recovered mortalities.

Laboratory methods

Scats were stored frozen at -80°C until processed. We extracted DNA using Qiagen DNeasy Blood and Tissue kits (Qiagen Inc, USA). To extract prey DNA, scats were broken into multiple pieces and gently rubbed on their interior surfaces with sterile swabs dipped in phosphate-buffered saline solution. We included one negative control extraction with every 29 samples to monitor for contamination. We used a two-step library preparation protocol consistent with Illumina’s 16S Metagenomic Sequencing Library Preparation (CT #: 15044223 Rev. B). Although our focal carnivores are omnivores, vertebrates are their most important prey (Knick et al. 1984, Powell 1993, Pauli et al. 2022, Shi et al. 2021, Wilk & Raphael 2017), so we targeted the 12S rRNA gene V5 region for amplification using 12SV05F/R universal primers for vertebrates (Riaz et al. 2011). We incorporated a base pair modification in the reverse primer to allow for more efficient binding of mountain beavers (*Aplodontia rufa*), a potentially important prey species for fishers in the Pacific Northwest (Happe et al. 2021). We amplified DNA in 3 PCR replicates using Qiagen Multiplex PCR Kit (Qiagen Inc., USA). Each 96-well PCR plate included 3 no-template controls and 3 positive fisher tissue controls. Amplified products were cleaned with a 1.8X SPRI bead solution after both PCR steps (Rohland & Reich 2012). Quantification of libraries was conducted using Qubit dsDNA HS Assay Kits (Invitrogen) and then normalized across plates for pooling. Following final pooling, 150 base pair paired-end sequencing was performed on a NextSeq 300 platform at the Northwest Genomics Center.

Sequence Analysis and Taxonomic Assignment

All reads were automatically demultiplexed using recognized indices during the NextSeq post-run process. Raw sequence read processing was performed in CLC Genomics Workbench 20.0 (QIAGEN). Sequences were clustered by 100% similar to molecular operational taxonomic units (MOTUs) and taxonomically assigned using BLAST (basic local alignment search tool) against the National Center for Biotechnology Information (www.ncvi.nlm.nih.gov/blast) and a custom reference library of Northwest species maintained by the Levi Lab at Oregon State University.

Taxonomic identifications to non-local species were reassigned to closely related native fauna, or removed if no close relatives were present. Additional filtering steps were performed in R (R Core Team 2022) and were similar to those of Tosa et al. (2022), including the removal of the maximum read count of any MOTU occurring in a negative extraction or PCR blank and the removal of scat replicates with <100 total reads. Furthermore, only species occurring in at least 2 of 3 replicates were retained. Sample replicates were merged and MOTUs occurring in fewer than 1% of sequences across a sample were removed. Scats were confirmed to be from fishers if fisher DNA was detected in the metabarcoding data and other carnivores consisted of less than 10% of read counts (Tosa et al. 2022). To limit the effects of contamination, only prey species that consisted of more than 1% of total prey sequences were retained. For quantitative analyses, MOTUs were categorized into prey categories based on taxonomy, behavior, and habitat use (ie., 'waterfowl', 'vole', 'ground squirrel', etc.).

Statistical analyses

Statistical analyses were performed in R (R Core Team, 2013). Diet profiles were summarized using relative read abundance, which is the relative sequence read count of a prey within a sample, that is averaged across the total sequence dataset:

$$RRA_i = \frac{1}{S} \sum_{k=1}^{S} \frac{n_{i,k}}{\sum_{i=1}^{T} n_{i,k}}$$

where $n_{i,k}$ is the number of sequences of prey i in sample k, S is the total number of diet samples, and T is the number of species. Relative read abundance produces semi-quantitative results and can potentially better approximate relative biomass consumption when compared to occurrence and presence/absence-based metrics (Deagle et al. 2019, Krehenwinkel et al. 2017, Thomas et al. 2016). Still, empirical research testing this relationship is scant (Deagle et al. 2019). With metabarcoded scats from fishers in temporary holding at the Calgary Zoo, we used Pearson's correlation to test the relationship between relative read abundance and the approximate proportional biomass of food items that fishers consumed one day prior to scat collection. Relative read abundances were arcsine-square root transformed prior to analysis.

We produced heat trees using the R package *metacoder* to visualize taxonomic contributions to fisher diets in each reintroduction area (Foster, Sharpton & Grunwald 2017). For each fisher population, we calculated the average prey richness as well as diversity of diet samples using Shannon diversity index (H). Shannon index means were compared using analysis of variance (ANOVA). To determine the degree of dietary overlap between reintroduction areas, we calculated Pianka's dietary niche overlap index (*O* metric) in R package *EcoSimR*, where 0 is no overlap and 1 is complete overlap (Pianka 1973, Gotelli et al. 2015). EcoSim utilizes null models to test whether the extent of niche overlap (observed overlap) is greater than expected by chance (simulated overlap). We generated 1,000 simulated matrices of randomized prey composition (randomization algorithm 3; Gotelli et al. 2015). We considered niche overlap to be significant when observed values were greater than at least 99% of simulated values (Meyer, Honig & Hadly 2022). To further test for variation in diet composition between the North and South Cascades populations, we conducted permutational multivariate analysis of variance between seasons (winter and summer) and study areas (PERMANOVA; function adonis2, R package *vegan*) with the Bray-Curtis distance matrix and 999 permutations. To evaluate which taxa contributed the most to diet dissimilarity, we used similarity percentage (SIMPER) tests, with 999 permutations and p-values adjusted with Bonferroni correction for multiple comparisons.

To compare the average prey mass between reintroduction sites, we extracted the average body mass (g) of prey species from global databases of ecological traits, including AmphiBIO, AVONET, and the Macroecological database of mammalian body mass (Oliveira et al. 2017, Tobias et al. 2022, Smith et al. 2007). For prey taxa identified above the species level (e.g., genus or family), the average body mass of the most common locally occurring species was used. We visualized the differences in body mass of prey using boxplots and compared their distributions with Mann-Whitney tests.

RESULTS

We analyzed 35 scats of fishers that were fed chicks, herring, smelt, horse, and house mice while in captivity. Relative read abundance of food items were moderately correlated with the proportional prey mass consumed ($r = 0.47$, $p < 0.001$, $n = 98$; Figure 2.2).

We collected a total of 328 fisher diet samples in the Cascades from 2015 to 2021. Of these samples, 4 failed to amplify and 24 contained only fisher DNA, leaving 300 samples for analysis: 138 scats and 4 gastrointestinal tracts from the South Cascades, and 153 scats and 5 gastrointestinal tracts from the North Cascades.

Prey from 70 genera within 47 families were detected within the fisher diet (Figure 2.3). Species richness was more than 3 times higher in the diet of North Cascades fishers than in the South Cascades (71 versus 22 taxa detected, respectively). In the South Cascades, prey was largely limited to mammals (99.2%), with amphibians and birds comprising less than 1% of total reads. In the North Cascades, mammals were also the most important prey class (Mammalia; 58.8%), but birds (Aves; 33.6%) were also a substantial component. Fish (Actinopterygii; 5%; Table 2.1), amphibians (Amphibia; 0.4%) and reptiles (Reptilia; 0.2%) were minor contributors. Whereas birds were rare in the diet of South Cascades fishers, 30 bird taxa were identified in the

North Cascades fisher diet, including small passerines (16.1%), waterfowl (7.5%), grouse (4.4%), raptors (2.7%), and woodpeckers (1%).

Snowshoe hares were the most prevalent prey species in both populations, but they were nearly seven times more common in the South Cascades than the North (67.8% and 9.8%, respectively). In the South Cascades, mountain beavers (14.2%), chipmunks (*Tamias spp.*; 6.2%), golden-mantled ground squirrels (*Callospermophilus lateralis*; 2.7%), flying squirrels (*Glaucomys sabrinas*; 2.6%), and Douglas squirrels (*Tamiasciurus douglassi*; 1.9%) were the next greatest diet contributors. In the North Cascades, other prey included eastern cottontails (*Sylvilagus floridanus*; 7.5%), flying squirrels (5.5%), striped skunks (*Mephitis mephitis*; 5.2%), coast moles (*Scapanus orarius*; 5.1%), song sparrows (*Melospiza melodia*; 5%), Trowbridge's shrews (*Sorex trowbridgii*; 4.3%), southern red-backed voles (*Myodes gapperi*; 4.1%), mule or black-tailed deer (*Odocoileus hemionus*) (4%), ruffed grouse (*Bonasa umbellus*; 3.7%), thrushes (*Turdus spp.*; 3.3%), Douglas squirrels (3%), veeries (*Catharus fuscescens*; 2.5%), great blue herons (*Ardea herodias*; 2.2%), and sucker fishes (*Catostomus spp.*; 2.1%), with all other species comprising less than 2% of sequence data.

Fisher diet in the North Cascades was richer and more diverse than in the South Cascades (Figure 2.5; $H_{North} = 0.62$, $H_{South} = 0.25$; ANOVA, $F_{1,298}$, $P < 0.001$). While most prey species we detected occur in both reintroduction areas, 58 of the 80 taxa were exclusively found in the diets of North Cascades fishers and comprised 64% of sequences. Consequently, niche overlap between populations was low to moderate ($O = 0.40$, SES = 1.76). No seasonal differences were detected in North Cascades fishers; $F_{1,156} = 0.52$, $R^2 = 0.003$, $P = 0.88$), so our analyses were not stratified by season. We had too few winter samples to assess seasonal prey variation in the South Cascades ($n = 6$). We found that prey composition between the two populations varied

($F_{1,298}$ = 47.1, R^2= 0.14, P = 0.001). The most influential prey contributing to dietary differences were snowshoe hares (SIMPER = 32%) and mountain beavers (SIMPER = 7%), which were more prevalent South Cascade fisher diets, as well as small passerines (SIMPER = 8%), which were more important for North Cascades fishers. Furthermore, North Cascades fishers consumed more smaller-bodied prey (median prey body mass = 78.5g) than South Cascade fishers (median prey body mass = 1710g; Mann-Whitney P = < 0.001; Figure 2.6).

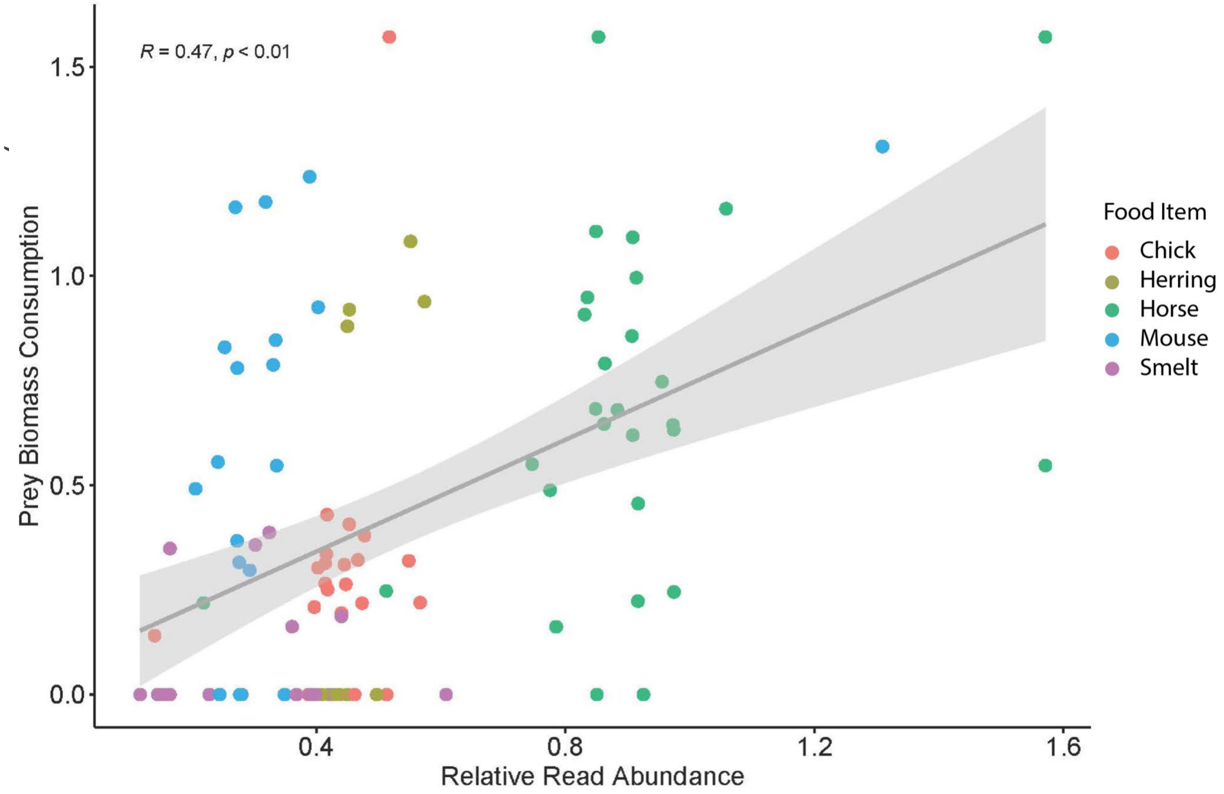

Figure 2.2. Correlation between relative read abundance data from scat metabarcoding and approximate biomass proportions of chicks, herring, horse, mouse, and smelt consumed by fishers in temporary captivity one day prior to scat collection. Relative read abundance is the number of DNA sequences for a food item within a scat divided by the total number of DNA sequences for that scat. Prey biomass consumption is the mass in grams of a food item consumed divided by the total grams of food items consumed. Each data point represents a diet item from the scats (*n* = 35 scats and 98 items total). Prey biomass consumption and relative read abundance were moderately correlated (r = 0.47).

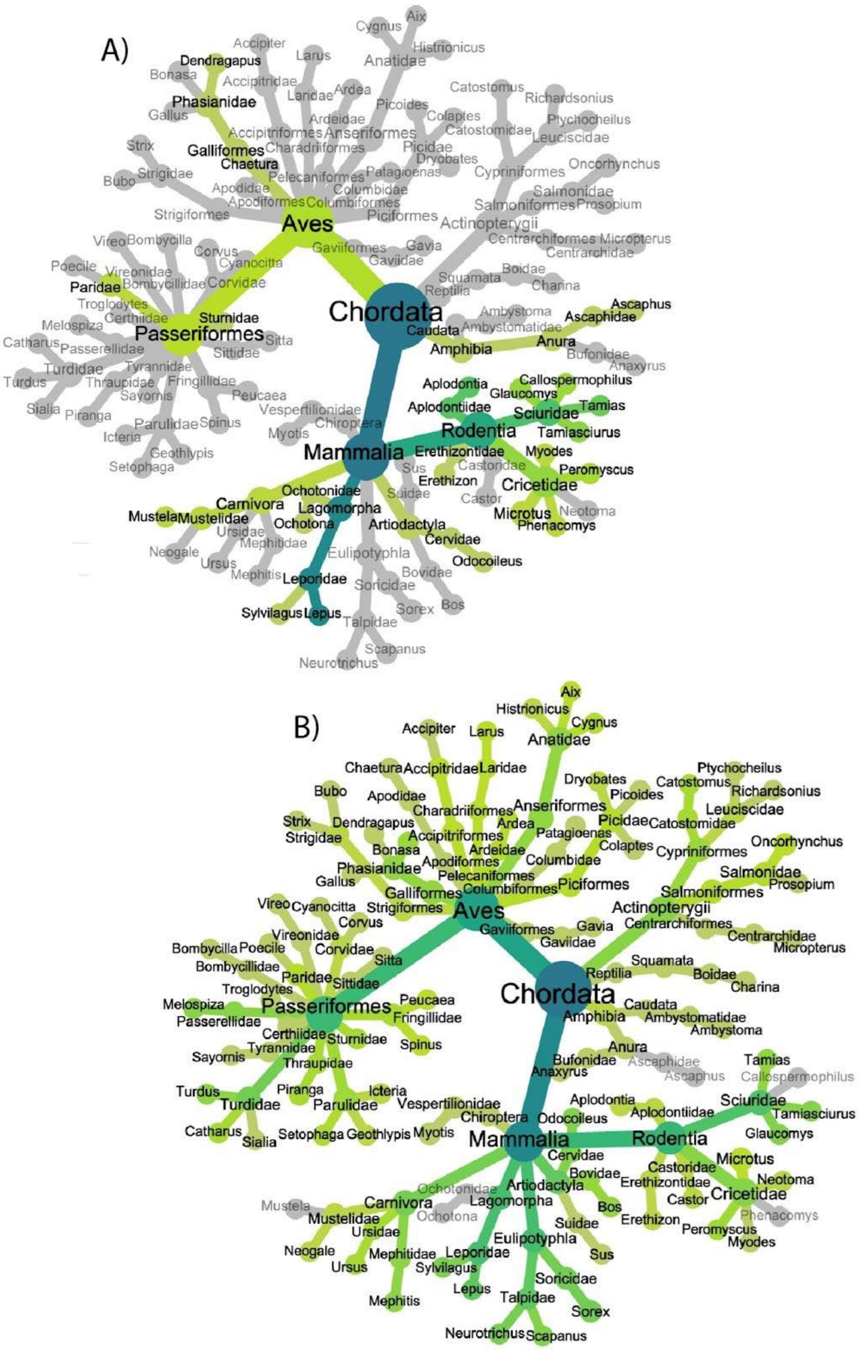

Figure 2.3. Diet summary from the analysis of fisher scats in the (A) South Cascades (*n* = 142 scats) and (B) North Cascades (*n* = 158 scats). For the diet heat tree, the prey community is represented as a taxonomic hierarchy up to the genus level. The abundance of prey DNA in the fisher diet is demonstrated by increasing color and size of the terminal nodes and branches.

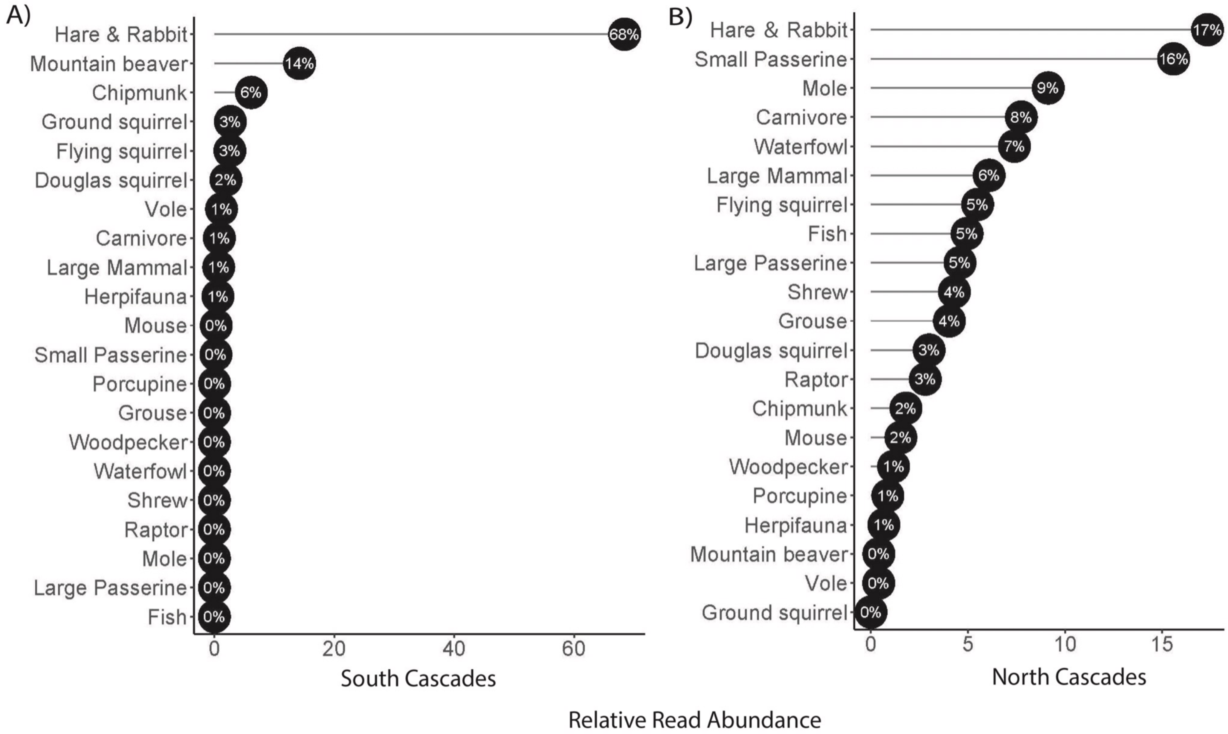

Figure 2.4. Summarized relative read abundance of prey types in fisher diet profiles in (A) the South Cascades reintroduction area (*n* = 142) and (B) the North Cascades reintroduction area (*n* = 158) in Washington, USA.

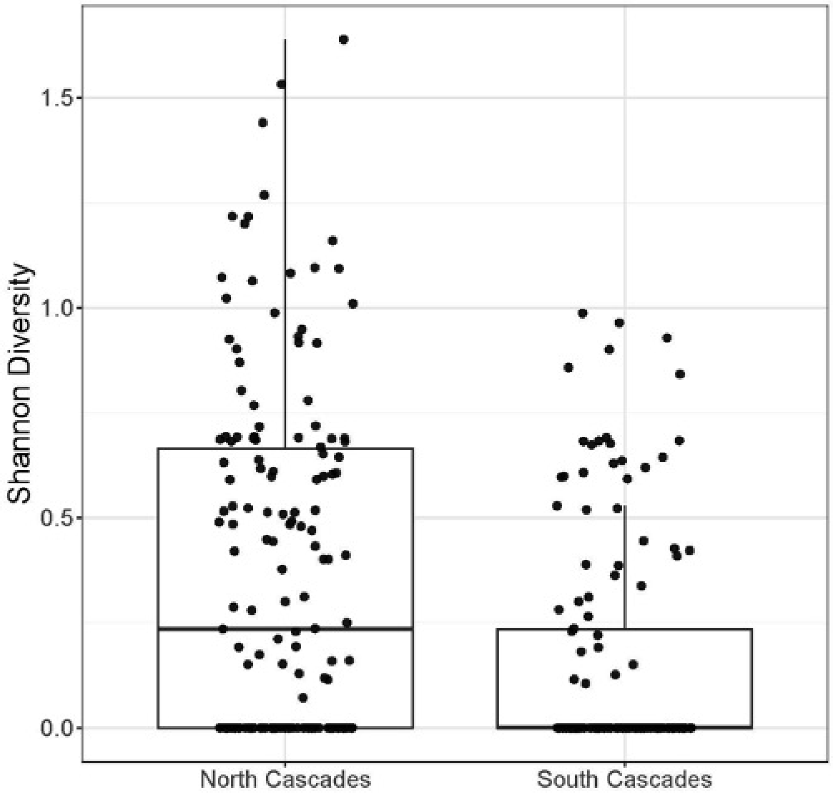

Figure 2.5. Boxplot depicting the Shannon diversity of prey DNA present in scats and gastrointestinal tracts in two reintroduced fisher populations. Scats were collected in the North Cascades and South Cascades of Washington, USA. Zeros on the plot equate to a single prey species present within a scat. Fisher diet was richer and more diverse in the North Cascades than in the South Cascades ($H_{North} = 0.62$, $H_{South} = 0.25$; ANOVA, $F_{1,298}$, $P < 0.001$).

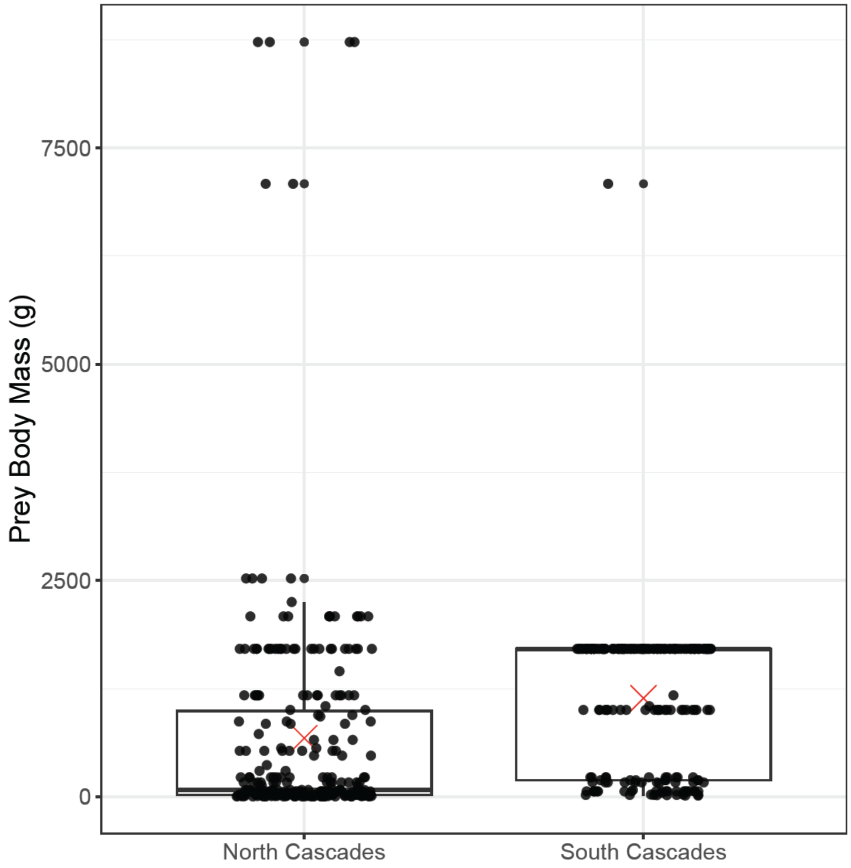

Figure 2.6. Boxplot depicting the average body mass (g) of prey species detected in the scat and gastrointestinal tracts of fishers reintroduced to the North Cascades and South Cascades of Washington, USA using DNA metabarcoding. Mean prey body mass is denoted by red Xs. Fishers in the North Cascades consumed greater proportions of smaller bodied prey than South Cascades fishers (Mann-Whitney *P* = < 0.001).

Table 2.1. Contributions of prey DNA summarized in relative read abundance from DNA metabarcoding in reintroduced fishers in the South Cascades and North Cascades of Washington, USA from 2016 to 2021.

Class	South Cascades	North Cascades
Mammals	99.15%	58.81%
Birds	0.27%	35.57%
Fish	0	4.95%
Amphibians	0.59%	0.42%
Reptiles	0	0.24%

DISCUSSION

Methodological limitations of morphology-based diet analyses have hindered our complete understanding of the foraging ecology of carnivores and sometimes limited the application of findings to conservation plans (Monterroso et al. 2019). Using DNA metabarcoding, our study has described a greater richness of prey in the fisher diet than has previously been reported (LaPoint et al. 2015). We demonstrated that two geographically close and ecologically similar reintroduction sites can yield strikingly different diet profiles in fishers, highlighting the importance of localized diet analyses for small carnivores of conservation concern, whose foraging strategies can be strongly influenced by environmental factors. We validated our use of relative read abundance by incorporating the analysis of known-diet scats, contributing to a small but growing body of evidence supporting the utility of this metric in approximating biomass consumption of vertebrate prey (Shi et al. 2021, Vesterinen et al. 2018, Massey et al. 2021).

We predicted that reintroduced fisher populations in the South and North Cascades would have relatively similar diet profiles consisting primarily of their most energy-efficient prey, which are hypothesized to be medium-bodied mammals, such as hares, rabbits, mountain beavers, squirrels, and porcupines. Due to preliminary evidence that some of these prey are approximately five times less abundant in the North Cascades than in the South Cascades (Humphries 2021, Parsons et al. 2019), we expected that North Cascade fisher diets would be supplemented with higher proportions of less calorie-rich prey, such as birds and small mammals, but we did not anticipate the magnitude by which these prey would contribute to fisher diets. In the North Cascades, fishers consumed birds, mammals, fish, and herpetofauna from a broad range of body sizes, whereas South Cascades fishers predominantly consumed medium-bodied mammals. Carnivore diets can be shaped by environmental factors, such as the temporal and spatial distribution of prey and the densities of competing predators (Rosenzweig 1966, Holt 1984).

In the Cascades reintroduction areas, a number of other factors may have contributed to the variation we observed in fisher feeding habits. Notably, the majority of fishers translocated to the South Cascades were sourced from central British Columbia, Canada, whereas all fishers in the North Cascades were translocated from a spatially distinct population in Alberta, Canada. While most fishers were captured as young animals, learned foraging behaviors from their distinct natal territories may have contributed to different feeding habits. However, it should be noted that the reintroduction project trappers in both British Columbia and Alberta targeted areas with snowshoe hares to maximize fisher capture success. Additionally, fishers in British Columbia were identified as the most suitable source population for Washington fishers from a genetic standpoint, whereas Alberta fishers were not as genetically similar to historic Washington populations (Hayes & Lewis 2004).

Differences in predator densities between the two reintroduction areas may have also contributed to the substantial variation in the proportion of leporids and squirrels between study sites, as these taxa are key prey groups for predators of fishers, such as coyotes (*Canis latrans*), bobcats (*Lynx rufus*), and cougars (*Felis concolor*; Wengert et al. 2014). However, even if predator densities were similar, reduced prey abundance in the North Cascades may intensify agonistic interactions among these carnivores. Parsons et al. (2019) found that fishers in the South Cascades may mitigate bobcat encounters by avoiding forest stands with the highest densities of hares, because these areas were preferred by bobcats. Heterogenous forest stands are important for fisher safety and food procurement (Parsons et al. 2019, Lewis et al. 2022, Sauder and Rachlow 2015), wherein fishers utilize mature stands for denning and protection from predators, but forage in the middle-aged to young stands preferred by tree squirrels, snowshoe hares, and mountain beavers. While the North and South Cascades were subject to similar forest management histories and are each composed of habitat mosaics with remnant old forests and heavily managed, thinned stands, our study did not assess the compositional differences between these study sites that may have influenced fisher diets.

The reproductive status and sex of individual predators may influence dietary choices. For example, Raley et al. (2020) found that kit-rearing female fishers primarily preyed on medium-bodied prey to support their elevated nutritional needs. In the South Cascades, the documentation of female reproduction following their release was a primary goal of the translocation project and thereby facilitated our sampling regime in the South Cascades, where scats were opportunistically collected at denning and resting sites. We acknowledge that our diet findings in the South Cascades may therefore be biased towards denning females and dependent offspring, which may explain the relatively high proportion of larger prey. However, our results were similar to diet profiles constructed via stable isotopes by Parsons et al. (2019), who sampled an even ratio of males and females and found that mountain beavers and snowshoe hares comprised 68% of South Cascade fishers diets.

Ultimately, the diet profiles constructed in the North Cascades support that prey abundance may be relatively low (Humphries 2021), which has implications for fisher recovery in this ecosystem. Optimal foraging theory predicts that species will minimize energy expenditure and maximize energy gained in pursuit of food (Charnov 1976). Accordingly, fisher populations with relatively high proportions of medium to large prey, like snowshoe hares or porcupines, tend to have stable or expanding population dynamics (Bulmer 1974, Bowman et al. 2006, LaPoint et al. 2015). Golightly et al. (2012) estimated that male and female fishers require approximately 205g and 154g of prey for daily energetic needs, respectively. At this rate, a fisher in the South Cascades could feed on a single mountain beaver (~800g) across several days, while a North Cascades fisher would need to procure multiple coast moles (~130g) to meet daily food requirements. The demonstrated flexibility of the fisher's diet suggests a resiliency to periodic lows in prey populations, but prolonged reliance on small prey can negatively impact fisher populations by diminishing body fat stores and reducing fecundity (Martin 2004). Interestingly, North Cascades fishers exhibited diet habits most similar to the endangered Pacific fisher subpopulation in the southern Sierra Nevada in California, which have starkly different diet habits than fishers throughout the rest of their range due to the higher proportion of birds and herpetofauna (Golightly et al. 2012). The substantial proportion of energy-efficient prey in the diet of South Cascades fishers suggests that these fishers are likely meeting the nutritional requirements for population growth (LaPoint et al. 2015). In contrast, the extensive reliance on birds and other small prey by North Cascades fishers raises questions about their ability to meet adequate resource needs for recovery in this region or whether additional management interventions will be required.

Fishers have a demonstrated capacity to suppress prey and subordinate predator populations following their restoration. For example, porcupine abundance declined by 16% within 13 years of fisher reintroductions in the Upper Peninsula of Michigan (Powell & Brander 1977, Earle & Kramm 1982). Similarly, there is extensive documentation of the inverse relationship between the relative abundance of fisher and marten populations throughout regions where they overlap (Fisher et al. 2013, Strickland & Douglas 1987). Whereas novel fisher predation on a few prey species could lead to population declines, North Cascades fishers distributed predation pressure among 71 taxa, with no individual prey contributing greater than 10% to their diet profile. The variety of forage species used reduces the likelihood that fisher restoration will negatively affect native species through the overexploitation of prey or exploitative competition with martens. These findings support prior hypotheses that fisher predation in western coniferous forests is unlikely to have measurable impacts on the population dynamics and community structure of prey (Aubry et al. 2003).

Our DNA metabarcoding approach revealed detailed new insights into the various forage species of fishers in Washington state. In the North Cascades, we described three times more vertebrate taxa in the fisher diet than has previously been reported in studies using traditional methods (Happe et al. 2021, LaPoint et al. 2015) and nearly twice as many taxa as a metabarcoding study conducted in the Southern Sierras of California (Pilgrim et al. 2023). Notably, we detected 30 bird species that would have been indistinguishable with traditional methods, suggesting that the contribution of this prey group to prior diversity estimates has been largely underestimated. However, as fishers are known to consume invertebrates and vegetation (Golightly et al. 2012), our study's focus on vertebrate prey likely obscures the full dietary range of Washington fishers.

We hypothesized that relative read abundance would correlate with the relative proportional biomass of prey consumed by fishers fed a controlled diet in captivity. A lack of empirical research comparing relative read abundance to actual biomass consumption has raised questions on the quantitative value of this metric in diet studies (Deagle et al. 2019). A consequence of this uncertainty is that frequency of occurrence metrics are the default metric for describing constructing diet profiles from sequence data (Deagle et al. 2019). Importantly, Deagle et al. (2019) found that occurrence-based metrics decrease in accuracy as dietary diversity increases, thus using relative read abundance to report fisher diets avoids the potentially more impactful biases associated with occurrence metrics, such as the inflated importance of rare or small prey. Therefore, any knowledge gained through captive feeding trials is informative. We found moderate correlations ($r = 0.48$) between the approximate biomass consumption of known prey items to metabarcoding sequence data. We offer several factors that may have impacted our results. Firstly, variations in tissue cell density, variations in survival rates of DNA during digestion, and PCR bias owing to mismatches in primer-templates have been known to affect sequence data in metabarcoding (Pinol et al. 2014, Pompanon et al. 2011). Next, as fishers were not constantly monitored throughout the feeding trials to minimize stress from human contact, observer bias may have contributed to imperfect correlations. Caching is an important behavioral adaptation for fishers to secure food from other scavengers for future consumption (Powell 1993). In our feeding trials, cached food items missed by observers upon retrieval of uneaten food may have contributed to false negative data points. Additionally, as food was available to fishers for a period of 24 hours before scat collection occurred, the consumption time of different food items is not known. Mustelids have relatively fast passage times (Abou-Madi 2019), but if prey were not consumed until several hours prior to scat collection, then it may not have been present in sequence data for a particular scat. Finally, observers often recorded the approximate mass of food items offered to fisher, whereas exact measurements were recorded for uneaten food to calculate consumption. Because we detected a moderate correlation despite imperfect experimental conditions, we suggest that relative read abundance provides a suitable depiction of vertebrate biomass proportions in fishers. However, further empirical studies are needed to evaluate its utility across other carnivores and forage types, such as vegetation and invertebrates.

Our study supported that fishers are an adaptable predator that can subsidize their diets with an impressive range of vertebrate prey (Golightly et al. 2012). However, fisher recovery should be monitored closely to better understand the relationship between food habits and population metrics, particularly in the North Cascades. Carnivore population numbers are closely tied to food availability (Hatton et al. 2015), and mustelids are particularly vulnerable to changes in key prey abundance (Sundell et al. 2013). As such, post-release monitoring with diet assessments can reveal whether reintroduced populations are obtaining adequate resources that support population establishment. Furthermore, the North Cascades may benefit from management actions to increase foraging habitat for fishers suggested in Parsons et al. (2019), including the creation and maintenance of understory cover that supports moderate to high densities of snowshoe hares and forestry patterns that offer mosaics of mature forest interspersed with managed stands. Finally, the striking differences in diet profiles between North and South

Cascades populations highlight the need for localized diet assessments rather than regional generalizations for best understanding conservation needs and prescribing management interventions for rare carnivores.

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CHAPTER 3

PREY PARTITIONING IN A DIVERSE CARNIVORE COMMUNITY: IMPLICATIONS FOR REINTRODUCED FISHERS IN WASHINGTON

ABSTRACT

Competition for prey resources influences trophic community structure and carnivore population dynamics. Evaluating the impacts of competitive interactions on carnivore reintroductions is increasingly important, as anthropogenic activities have caused widespread restructuring of animal communities and potentially decreased habitat quality for returning carnivores. Here, we used fecal metabarcoding to evaluate the diets of reintroduced fishers (*Pekania pennanti*) and sympatric coyotes (*Canis latrans*), bobcats (*Lynx rufus*), and Pacific marten (*Martes caurina*) in the North Cascade Mountains of Washington. We assessed similarities in diet composition and measured dietary overlap among species pairs. Niche overlap values ranged from relatively low (0.36) to high (0.76) and generally increased as pairwise differences in body mass decreased. Whereas dietary overlap was substantial for common prey items, we found strong compositional differences among carnivore diets. Additionally, DNA metabarcoding enabled us to detect important prey species that facilitate resource partitioning at a fine scale. Our findings suggest that a hierarchical competitive cascade may be occurring, wherein competition with bobcats leads to a dietary shift in fishers, thereby increasing competitive pressure on subordinate martens. Consequently, restricted access of fishers to energetically efficient prey may slow recovery efforts in this region, highlighting the importance of investigating the role of competition in species restorations.

INTRODUCTION

A fundamental question in reintroduction ecology is how native species will interact with their historical environment following restorations. Species reintroductions are a critical tool for restoring wildlife that cannot naturally recolonize parts of their ranges from which they have disappeared, but these efforts may be unsuccessful if modern species assemblages make habitat less viable for restored species (e.g., Moseby et al. 2021). Over the last century, there has been unprecedented anthropogenic restructuring of animal communities where trophic dynamics have been altered as species are extirpated or introduced and habitat is modified (Gonzalez et al. 2011, Dirzo et al. 2014, Munguía et al. 2016). Carnivore communities specifically have undergone widespread transformations (Schaller 1996, Ripple et al. 2014, Berger et al. 2001). The removal of large carnivores from landscapes has triggered cascades that affect lower trophic levels, such as herbivore prey and plant communities and smaller predator assemblages (Ripple et al. 2014, Ripple et al. 2015, Estes et al. 2011). Mesocarnivore release theory posits that changes to the abundance of apex predators may trigger niche expansion and increased abundance in previously suppressed smaller carnivores (mesocarnivores; Soulé et al. 1988, Courchamp et al. 1999, Crooks & Soulé 1999). However, some mesocarnivores can experience heightened competition as a result of these releases. For example, the historical extirpation of gray wolves (*Canis lupus*) in the contiguous United States precipitated widespread population expansions in coyotes (*Canis latrans*), which in turn increased predation pressure on snowshoe hares, the key prey of federally threatened Canada lynx (*Lynx canadensis*; Ripple et al. 2011). Competitive pressure shapes community structure (Birch 1957, Hardin 1960, Rosenzweig 1966), and can greatly impact the demographic and vital rates of carnivore communities (Ritchie and Johnson 2009, Brook et al. 2012). Therefore, the trophic effects of the addition or removal of carnivores to a landscape should be closely monitored. Many carnivore populations have experienced severe range retractions (Ripple et al. 2014),and are now actively recovering through natural recolonization (e.g., Boyd et al. 1994, Mckelvey et al. 2014), or through human intervention (e.g., Smith et al. 2003). However, modern carnivore communities, reshaped by mesocarnivore release and habitat alterations, may create competitive environments that are less hospitable for some recovering species. Thus, there is a need to better understand the role of interspecific competition in the success of carnivore restorations (Hayward & Somers 2009).

Competition can be a major limiting factor in carnivore restorations, whether it be exploitative (the use of shared resources) or interference (aggressive displacement or predation; Birch 1957, Miller 1967). Competition theory predicts that in a limited resource space, species must mitigate competitive pressure through niche differentiation to coexist (Hardin 1970, Schoener 1983). This process is typically hierarchical, whereby inferior competitors are forced to adapt to altered niche spaces through fine-scale selection of microhabitats, temporal activity patterns, or alternate prey selection (Cozzi et al. 2012, Kamler et al. 2012, Remonti et al. 2012). Whereas generalist species can exhibit greater flexibility in their responses to competition (Wilson & Yoshimura 1994), a persistent restriction of prey resources can have negative population level effects. Such was the case with slimy salamanders (*Plethodon spp*.) in the Southeastern United States, which experienced decreasing body condition in response to prolonged competitive pressure (Cunningham, Rissler & Apodaca 2009). In carnivore guilds, an examination of the diet of sympatric species can reveal insights into the hierarchical structure of multi-predator communities (Kitchen et al. 1999). As carnivore populations can be strongly influenced by access to prey (Wolf & Ripple 2017), an understanding of community structure and its influence on resource access can allow managers to predict challenges to carnivore restoration in contemporary ecosystems.

Fishers (*Pekania pennanti*) are an ideal focal species for assessing competitive dynamics among reintroduced and extant carnivore species. Owing to their mid-level trophic position, fishers are affected by competition from larger carnivores, like bobcats (*Lynx rufus*) and coyotes (Wengert et al. 2015), but also exert top-down pressure on closely related martens (*Martes spp.*; Pauli et al. 2022). Fishers experienced precipitous declines throughout their range in North America, likely due to unregulated trapping and habitat loss and fragmentation (Balser & Longley 1966, Petersen et al. 1977, Lewis & Zielinski 1996). As historical threats have largely been alleviated, a number of fisher populations have been successfully reestablished through translocations; however, fisher reintroductions in their eastern distribution are twice as likely to succeed as in the west (Aubry & Lewis 2003, Hayes & Lewis 2006, Callus & Figura 2008, Berg 1982, Lewis et al. 2012). Proposed explanations for this disparity include differing habitat features, such as snow cover and forest characteristics, as well as differences in predator-prey communities (Lewis, Powell & Zielinski 2012, LaPoint et al. 2015), yet there is a paucity of direct investigation into these factors. Primary predators of the fisher, such as bobcats and coyotes, have purportedly benefitted from mesopredator release and increased human development (Hody & Kays 2018, Ritchie & Johnson 2009, Roberts & Crimmins 2010), and their populations may exist in larger numbers in the fisher's western range than they did historically. Predation is recognized as a key issue for fisher translocation (Lewis, Powell & Zielinski 2012, Roy et al. 1991). In a reintroduced fisher population in California, for instance, bobcat predation accounted for 77% of female mortalities, with coyote and cougar (*Puma concolor*) predation occurring in smaller proportions (Wengert et al. 2014). Additionally, Parsons et al. (2019) detected a potential food-safety tradeoff in the spatiotemporal activities of reintroduced fishers in Washington's Southern Cascades, where fishers avoided areas with the highest densities of snowshoe hares (*Lepus americanus*, favored by bobcats) and shared little temporal overlap with the felines. While these findings suggest that fishers may alter their use of niche space in response to competition with larger predators, an evaluation of how fishers partition prey resources with bobcats and coyotes can better define these dynamics.

Fishers are forest habitat specialists with diverse feeding habits, often consuming small and mid-sized mammals, birds, fruits, and carrion (LaPoint et al. 2015, Powell 1993). Despite their generalist diet, medium-bodied mammalian prey, such as the snowshoe hare (1-2 kg), are considered their most energy efficient foods (Powell 1979). Indeed, the presence of hares in fisher diets have been linked to stable or expanding populations (Bowman et al. 2006, Bulmer 1974), and long-term declines in the average body condition in fishers have been linked to hare declines (Kirby et al. 2018). These findings suggest that access to optimally sized prey can influence the success of fisher reintroductions. Furthermore, previous studies have suggested that body size ratios between different carnivore species are the dominant factor influencing the strength of intraguild interactions (Palomares and Caro 1999, Donadio and Buskirk 2006, Harrington et al. 2009, Monterroso et al. 2020). Here, we examine the diet patterns of reintroduced fishers and sympatric carnivores to better understand how competitive pressure and niche segregation may influence fisher restorations and subsequent ecosystem-wide impacts.

We used fecal metabarcoding to construct diet profiles for recently reintroduced fishers and three sympatric carnivores – coyotes, bobcats, and Pacific martens (*M. caurina*) – during the snow-free season in the North Cascades, Washington, USA. Fecal metabarcoding and high-throughput sequencing have the capacity to reveal finer-scale diet information than traditional methods and have been useful in yielding new insights into dietary niche partitioning and coexistence dynamics within generalist consumer systems (Kartzinel et al. 2015). We aimed to (1) evaluate the dietary niche breadth and overlap of these species; (2) test how carnivore body size similarity influences dietary niche overlap and segregation; and (3) identify resource axes that may facilitate partitioning and coexistence. We predicted that carnivores with similar body size would have the highest dietary overlap and potentially the most pronounced interspecific competition. Specifically, we expected that bobcats and coyotes, whose average body size differs by a factor of 1.5x, would exhibit the highest dietary overlap, whereas coyotes and marten, whose body size differs by a factor of over 10x, would have the smallest overlap. We anticipated that all species would overlap in their use of high-value prey (i.e., snowshoe hares and carrion), but that dominant (i.e., larger-bodied) carnivores would consume greater proportions of these prey than subordinate (smaller-bodied) carnivores. Through these analyses, we aimed to better understand how dietary niche and trophic dynamics affect fisher recovery in contemporary carnivore assemblages.

STUDY AREA

Fishers experienced population losses throughout the Pacific coastal states (California, Oregon, and Washington) of the USA following extensive European settlement and increased harvest pressures (Lewis, Powell & Zielinski 2012). These losses included an apparent extirpation from Washington (Lewis 2014). In 2008, an effort to re-establish self-sustaining fisher populations throughout their historical range in Washington was initiated (Lewis 2014). Fishers were translocated from populations in British Columbia and Alberta, Canada to three distinct recovery zones: the Olympic Peninsula (2008 - 2013), the southern Cascades (2015 - 2020), and northern Cascades (2018 - 2020; Hayes & Lewis 2006, Lewis 2018).

Our study area was a ~6500 km^2 region of the North Cascades of Washington comprising the Mount Baker Snoqualmie and Okanogan-Wenatchee National Forests (3000 km^2), North Cascades National Park Service Complex (2768 km^2), Washington Department of Natural Resources land (~250 km^2) and surrounding private lands (~250 km^2; Figure 3.1). Elevations ranged from 13 m to 1588 m with a mean of 649 m. Average July temperatures range from 8.8C and 25.7C and average January temperatures fall between -8.8C and -2.9C. The region is largely composed of conifer forests, with dominant tree species including Douglas fir (*Pseudotsuga menziesii*), Pacific silver fir (*Abies amabilis*), western hemlock (*Tsuga heterophylla*), and western red cedar (*Thuja plicata*).

The North Cascades is home to many carnivores, including black bears (*Ursus americanus*), bobcats, cougars (*Puma concolor*), coyotes, ermine (*Mustela ermina*), Pacific marten, mink (*Neovision vision*), river otters (*Lotra canadensis*), raccoons (*Procyon lotor*), striped skunks (*Mephitis mephitis*), gray wolves, and wolverines (*Gulo gulo*). Whereas all of these species are potential competitors of fishers, only bobcats, coyotes, and martens likely share the highest dietary overlap with fishers, owing to the notable shared presence of small to medium mammals and ungulate carrion in their diets (Knick et al. 1984, Pauli et al. 2022, Shi et al. 2021, Wilk & Raphael 2017). Limited information on the prey use of historical Washington fisher populations exists, but snowshoe hares and mountain beavers (*Aplodontia rufa*) have been reported as important prey species to reintroduced fishers on the Olympic Peninsula and in the South Cascades, accounting for 40% and 68% of fisher diet, respectively (Happe et al. 2021, Parsons et al. 2020). Potential prey species in the North Cascades include Douglas squirrels (*Tamiasciurus douglasii*), Northern flying squirrels (*Glaucomys sabrinas*), snowshoe hares, mountain beavers, porcupines (*Erethizon dorsatum*), ruffed grouse (*Bonasa umbellus*), and numerous small mammals like shrews (*Sorex*), voles (*Microtus* and *Myodes*), moles (*Scapanus*), and mice (*Peromyscus* and *Zapus*). Across the fisher's range, an increased presence of small mammals and birds in their diet has been linked to the decreased availability of larger prey such as snowshoe hares, porcupines, and squirrels (Bowman et al. 2006, Golightly et al. 2012).

METHODS

Scat Collection

Prior to release, translocated fishers were equipped with very high frequency (VHF) transmitters (Holohil AI-2HM; Carp Ontario K0AIL0, Canada) to allow post-release monitoring of movements and survival using aerial and ground telemetry. Fishers were monitored from once per week to once per month for two years following release (Lewis et al. 2022). Over one year following releases, scat detection dog teams (Rogue Detection Teams) surveyed for fisher scats on 10 days in October 2020 and 16 days in July and August 2021. Surveys occurred in 16 sites with actively monitored fishers (7 males and 8 females, plus 5 of unknown sex). Search sites were ~10 km^2 and searches lasted from 4-6 hours per day. Detection teams conducted focused searches near recent telemetry locations, resting sites, and denning sites, and along habitat features likely to be used by fishers, such as downed logs, along creeks, and near brush piles. Teams collected all putative fisher scats and all likely bobcat, coyote, and marten scats that were encountered opportunistically.

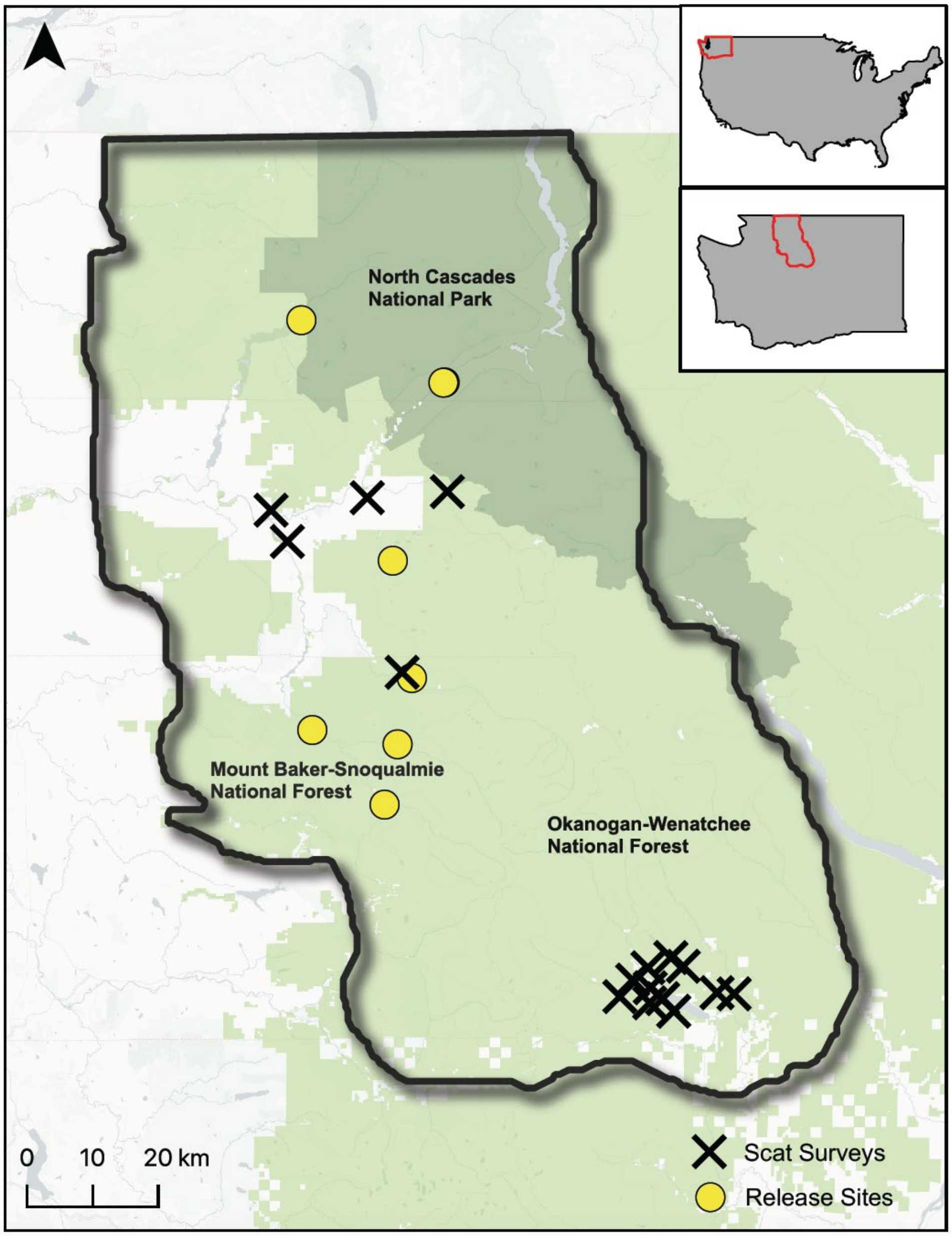

Figure 3.1. Fisher translocation release sites (represented by yellow circles) and scat survey sites (represented by crosses) in the North Cascades Mountains of Washington state, USA. A total of 89 fishers were released in the North Cascades from December 2018 to February 2020 and tracked with telemetry. Scat detection dogs surveyed for fisher, bobcat, coyote, and marten scat near fisher locations during October 2020 and from July through August 2021.

Laboratory methods

Scats were stored frozen at -80°C until processed. We extracted DNA using Qiagen DNeasy Blood and Tissue kits (Qiagen Inc, USA). To extract prey DNA, scats were broken into multiple pieces and gently rubbed on their interior surfaces with sterile swabs dipped in phosphate-buffered saline solution. We included one negative control extraction with every 29 samples to monitor for contamination. We used a two-step library preparation protocol consistent with Illumina's 16S Metagenomic Sequencing Library Preparation (CT #: 15044223 Rev. B). Although our focal carnivores are omnivores, vertebrates are their most important prey (Knick et al. 1984, Powell 1993, Pauli et al. 2022, Shi et al. 2021, Wilk & Raphael 2017), so we targeted the 12S rRNA gene V5 region for amplification using 12SV05F/R universal primers for vertebrates (Riaz et al. 2011). We incorporated a base pair modification in the reverse primer to allow for more efficient binding of mountain beavers, a potentially important prey species for fishers in the Pacific Northwest (Happe et al. 2021). We amplified DNA in 3 PCR replicates using Qiagen Multiplex PCR Kit (Qiagen Inc., USA). Each 96-well PCR plate included 3 no-template controls and 3 positive fisher tissue controls. Amplified products were cleaned with a 1.8X SPRI bead solution after both PCR steps (Rohland & Reich 2012). Quantification of libraries was conducted using Qubit dsDNA HS Assay Kits (Invitrogen) and then normalized across plates for pooling. Following final pooling, 150 base pair paired-end sequencing was performed on a NextSeq 300 platform at the Northwest Genomics Center.

Sequence Analysis and Taxonomic Assignment

All reads were automatically demultiplexed using recognized indices during the NextSeq post-run process. Raw sequence read processing was performed in CLC Genomics Workbench 20.0 (QIAGEN). Sequences were clustered by 100% similar to molecular operational taxonomic units (MOTUs) and taxonomically assigned using BLAST (basic local alignment search tool) against the National Center for Biotechnology Information (www.ncvi.nlm.nih.gov/blast) and a custom reference library of Northwest species maintained by the Levi Lab at Oregon State University.

Taxonomic identifications to non-local species were reassigned to closely related native fauna, or removed if no close relatives were present. Additional filtering steps were performed in R (R Core Team 2022) and were similar to those of Tosa et al. (2022), including the removal of the maximum read count of any MOTU occurring in a negative extraction or PCR blank and the removal of scat replicates with <100 total reads. Furthermore, only species occurring in at least 2 of 3 replicates were retained. Sample replicates were merged and MOTUs occurring in fewer than 1% of sequences across a sample were removed. Scats were identified to carnivore species if their DNA was detected in the metabarcoding data and other carnivores consisted of less than 10% of read counts (Tosa et al. 2022). To limit the effects of contamination, only prey species that consisted of more than 1% of total prey sequences were retained.

Statistical analyses

Diet profiles were summarized using relative read abundance, which is calculated by the relative sequence read count of prey within a sample, which is then averaged across the dataset. Frequency of occurrence, or the proportion of diet samples in which a prey species occurs, is the most commonly used metric to summarize diet, but as occurrence summaries can inflate the importance of prey consumed in small quantities and confound dietary comparisons (Deagle et al. 2018), we limited our analyses to relative read abundance. Metabarcoding often allows for greater species-level identification of vertebrate prey compared to traditional macroscopic techniques (Massey et al. 2021), but this increased differentiation may dampen the detection of biologically meaningful trends in prey partitioning in comparative analyses. Metabarcoding has not yet been widely applied to terrestrial predator systems, so we tested the effects of increased resolution of prey taxa on diet comparisons by performing statistical analysis of prey data described at the lowest-taxonomic level (*Ungrouped Prey*; Table 3.1), and on data reclassified into prey groups based on similar taxonomy and habitat use (*Grouped Prey*; Table 3.1).

We estimated sample completeness and expected taxonomic richness of the vertebrate diet composition based on sample size for each carnivore by producing rarefaction curves within the iNEXT package (Chao et al. 2014, Hsieh et al. 2016; function: iNEXT, datatype = "incidence_raw", q = 2). We plotted rarefied and extrapolated Simpson diversity estimates with respect to sample sizes and obtained 95% confidence intervals for each diversity and sample coverage estimate by applying a bootstrapping method. We assessed dietary niche overlap using Pianka's Index, which ranges from 0, indicating no diet overlap, to 1, reflecting complete diet overlap (Pianka 1974). We tested the hypothesis that interspecific dietary niche overlap was greater than expected by calculating Pianka's index relative to randomized null models of RRA data in the R package EcoSimR (Gotelli et al. 2015; function: niche_null_model, algo = "ra3", metric = "pianka", nReps = 10,000). To test the hypothesis that carnivore diet profiles differed among species, we used permutation-based multivariate analysis of variance (PERMANOVA; function *adonis2*, R package *vegan*). To further identify dietary similarities and departures, we determined primary indicator food types for each carnivore and for all carnivore combinations with Indicator Species Analyses, which use indices of a prey's relative abundance and occurrence and randomization tests to estimate its strength of association with carnivores (De Cáceres et al., 2010; function multipatt, R package *indicspecie*s). Lastly, to test if carnivore body size influences niche overlap values, we compared pairwise niche overlap metrics and body size ratios using simple linear regression. Average body mass estimates were acquired from the Macroecological database of mammalian body mass (Smith et al. 2007), and pairwise size ratios were log transformed.

RESULTS

Between October 2020 and August 2021, sampling teams collected 381 carnivore scats near recent fisher locations. We obtained 70,550,834 raw sequences, with 99% of reads from scat samples and 1% from negative controls. Following genetic identification and the removal of non-target species, our final dataset consisted of 301 scats: 130 from fishers, 64 from marten, 58 from coyotes, and 49 from bobcats (Figure 3.2). The read counts of sample PCR products ranged from 168 to 176,948, with a median of 9,552. Following quality control filtering, our final dataset comprised 57.5% sequence reads from carnivores and 42.5% from prey.

A total of 85 prey taxa were identified in scats (Table 3.1). Of these, only 11 were present in the diet of all four carnivores. Mammals (Mammalia) dominated the diets of all species and were most prevalent in bobcat (RRA = 95.8%), marten (RRA = 83%), and coyote diets (RRA = 79.8%), but less so in fisher diets (RRA = 54.9%). Birds (Aves) made up the next most prevalent prey class for each carnivore, contributing greatest to fisher diets (RRA = 39%), then coyote (RRA = 17.1%), marten (RRA = 15.6%), and bobcat diets (4.2%). Pairwise niche overlap comparisons indicated moderate to high overlap among the diets of all carnivores, and all overlap values were higher than would be expected by chance (Figure 3.3). Niche overlap generally decreased as body mass ratios increased among carnivore pairs ($R^2 = 0.67$, $F_{1,4} = 11.31$, $P = 0.03$; Figure 3.4). Niche overlap values were highest between bobcats and coyotes ($O'_{\text{Ungrouped}} = 0.76$, $O'_{\text{Grouped}} = 0.83$), and lowest between coyotes and martens ($O'_{\text{Ungrouped}} = 0.36$, $O'_{\text{Grouped}} = 0.48$). Fishers had high dietary overlap with bobcats ($O'_{\text{Ungrouped}} = 0.63$, $O'_{\text{Grouped}} = 0.6$)

and moderate overlap with coyotes (*O'* $_{\text{Ungrouped}}$ = 0.56, *O'* $_{\text{Grouped}}$ = 0.51) and martens (*O'* $_{\text{Ungrouped}}$ = 0.51, *O'* $_{\text{Grouped}}$ = 0.61; Figure 3.3).

Despite the high degrees of overlap, PERMANOVAs with both Grouped and Ungrouped prey classifications supported that prey composition was significantly different among carnivore diets (Grouped: $F_{3,291}$ = 4.78, *P* = 0.0001; Ungrouped: $F_{3,297}$ = 3.41, *P* = 0.0001; Table 3.2). Pairwise models detected significant differences among nearly all species pairs, apart from bobcats and coyotes. Indicator species analyses revealed associations between carnivores and both broad prey categories and finer-scale taxonomic groups (Table 3.3). For example, small passerines were indicators for both fishers and martens, but an inter-group delineation was detected at genus and species levels, where song sparrows (*Melospiza melodia*), thrushes (*Turdus spp.)* and starlings (*Sturnus vulgaris*) were indicators for fishers (ISA = 0.30, 0.29, 0.22, respectively).

Table 3.1. Prey contributions of bobcats, coyotes, fishers, and martens in the North Cascades of Washington, USA, summarized in terms of relative read abundance from scat DNA metabarcoding.

Class	Grouped Prey	Ungrouped Prey	Bobcat	Coyote	Fisher	Marten
Actinopterygii	Fish		**-**	**2.4**	**4.7**	**-**
		Catostomus spp.	-	0.1	1.3	-
		Micropterus salmoides	-	-	0.6	-
		Oncorhynchus mykiss	-	-	1.2	-
		Oncorhynchus tshawytscha	-	0.7	-	-
		Perca flavescens	-	1.6	-	-
		Prosopium williamsoni	-	-	0.1	-
		Ptychocheilus oregonensis	-	-	0.7	-
		Richardsonius balteatus	-	-	0.7	-
Aves			**4.2**	**17.1**	**39.7**	**15.6**
	Small passerine		3.2	3.4	18.7	10.6
		Catharus fuscescens	-	1.6	3	4.1
		Chaetura spp.	-	-	0.1	-
		Geothlypis spp.	-	-	0.7	-
		Icteria virens	-	1.3	0.2	0
		Junco hyemalis	3.2	0.5	-	2.2
		Melospiza melodia	-	-	6.5	-
		Paridae spp.	-	-	1.2	-
		Unk Passeriformes	-	-	0.4	-
		Peucaea spp.	-	-	0.4	-
		Piranga ludoviciana	-	0.1	0.8	0.3
		Poecile atricapillus	-	-	0.7	-
		Sayornis spp.	-	-	0.6	-
		Setophaga spp.	-	-	1.9	0.1
		Sialia mexicana	-	-	0.1	-
		Sitta spp.	-	-	-	-
		Spinus pinus	-	-	0.6	-
		Troglodytes	-	-	0.7	3
		Vireo	-	-	0.7	0.9
	Large passerine		-	0.5	6.7	2.9
		Bombycilla garrulus	-	-	0	-

		Colaptes auratus	-	0	0.7	2.3
		Corvus spp.	-	0.5	0.7	0.3
		Cyanocitta stelleri	-	-	0.6	-
		Patagioenas fasciata	-	-	0.1	-
		Sturnella spp.	-	0.1	-	-
		Sturnidae	-	-	0.6	-
		Turdidae	-	-	-	0.1
		Turdus	-	-	3.9	0.1
	Grouse		1.1	7.5	4.4	-
		Bonasa umbellus	1.1	6.4	4.4	-
		Dendragapus spp.	-	1.1	0	-
	Raptor		-	2.9	1.8	0.6
		Accipitridae	-	2.9	1.1	-
		Strix varia	-	-	0.7	0.6
	Waterfowl		-	2.7	7.8	-
		Aix sponsa	-	-	0.6	-
		Anatidae spp.	-	-	0.4	-
		Ardea spp.	-	-	2.5	-
		Cygnus spp.	-	1.6	2.6	-
		Histrionicus histrionicus	-	-	0.1	-
		Larus spp.	-	-	1.6	-
		Mergus merganser	-	1.1	-	-
	Woodpecker	*Picoides villosus*	-	-	0.4	1.5
Herpofauna			**-**	**0.7**	**0.8**	**1.5**
	Frog		-	0.7	0.4	-
		Anaxyrus boreas	-	-	0.4	-
		Ascaphus truei	-	0*	-	-
		Rana luteiventris	-	0.6	-	-
	Salamander	*Ambystoma spp.*	-	-	0	-
	Snake	*Charina bottae*	-	-	0.3	1.5
Mammalia			**95.8**	**79.8**	**54.9**	**83**
	Bat	*Myotis volans*	-	1.6	0	-
	Bear	*Ursus americanus*	-	-	1.7	-
	Beaver	*Castor canadensis*	-	3.7	0.3	-
	Chipmunk	*Tamias spp.*	7	0.6	2.1	14
	Domestic spp.		-	6.4	1.7	1.5
		Bos taurus	-	6.3	1.7	1.5
		Ovis aries	-	0.1	-	-
	Douglas squirrel	*Tamiasciurus douglasii*	9.7	3.4	3.5	10.6

Flying squirrel	*Glaucomys sabrinus*	5.4	0.5	3.9	4.1
Ground squirrel	*Callospermophilus lateralis*	2	1.6	-	1.5
Large leporid		28.9	18.6	16.8	7.6
	Lepus americanus	10.7	10.6	8.2	1.5
	Sylvilagus floridanus	18.2	8	8.6	6.1
Small leporid	*Ochotona princeps*	2.4	-	-	-
Marmot	*Marmota caligata*	-	-	-	0.9
Mink	*Neogale vision*	-	-	0.7	-
Mole		1.9	0.8	9	7.6
	Neurotrichus gibbsii	1.9	0.7	4.1	3.5
	Scapanus orarius	0	0.1	4.9	4.1
Mountain beaver	*Aplodontia rufa*	7.9	0.3	0	1.5
Mouse	*Peromyscus keeni*	1.4	3.6	1.3	10.6
Porcupine	*Erethizon dorsatum*	-	-	1	-
Shrew	*Sorex trowbridgii*	2.5	1.6	5.3	6
Skunk	*Mephitis mephitis*	-	2.2	2	-
Woodrat	*Neotoma cinerea*	-	-	0.8	1.5
Ungulate		14.4	27.5	4.2	2.7
	Odocoileus spp.	14.4	27.5	4.1	2.7
	Sus scrofa	-	-	0*	-
Vole		12.2	7.5	0.5	12.6
	Microtus longicus	2	-	-	0.5
	Microtus richarsoni	-	-	-	0
	Microtus townsendii	9.4	4.3	0.3	1.2
	Myodes gapperi	0.4	1.9	0.2	10.3
	Phenacomys intermedius	0.4	1.2	-	0.6
Unk. Cricetid	*Cricetidae*	-	-	-	0.1
Unk. squirid	*Sciuridae*	-	-	-	0.1

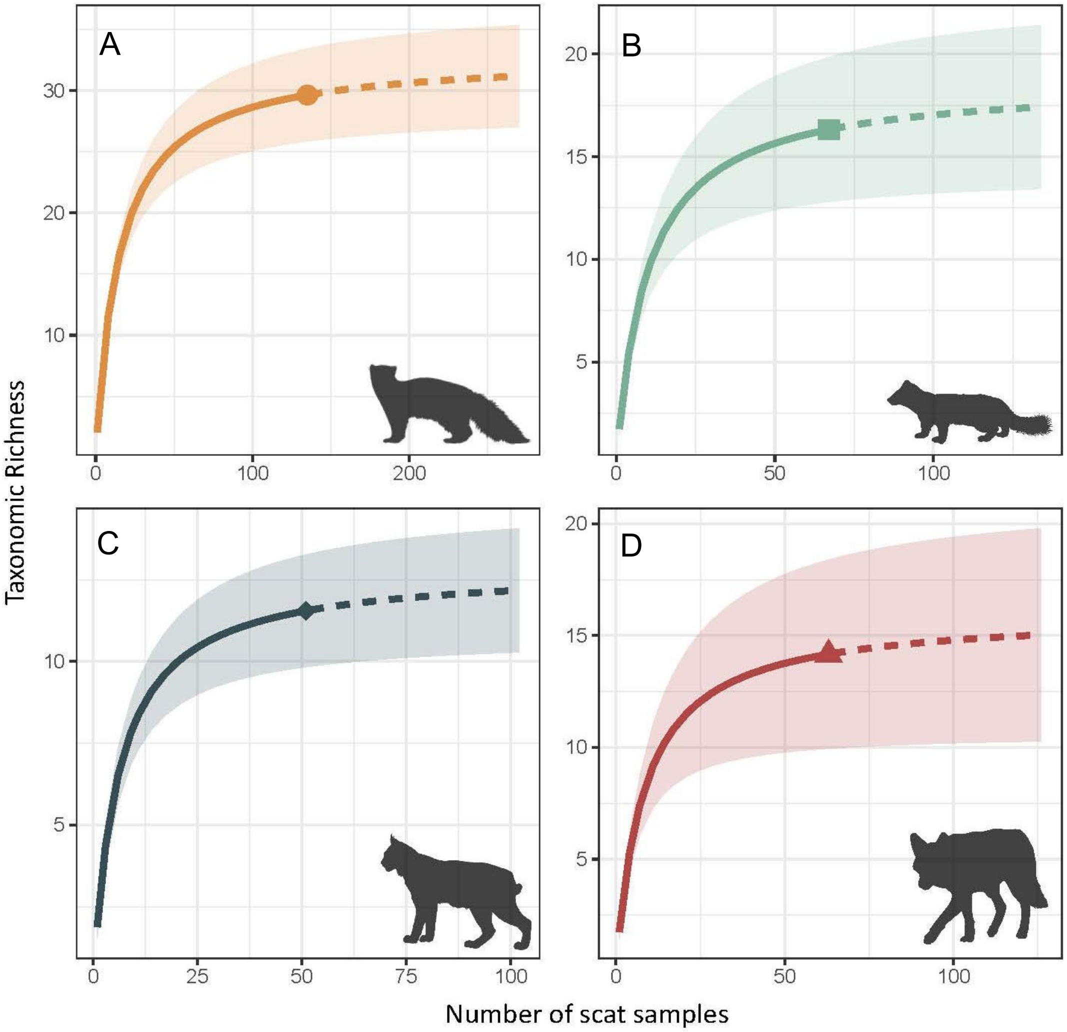

Figure 3.2. Rarefaction curves to evaluate the effects of sample size on prey DNA for carnivore scat samples analyzed with DNA metabarcoding from the North Cascades in Washington, USA. Reintroduced fishers (A) had the most taxonomically rich diet, showing rapid increases in prey detected through the first 25 samples, and continued to grow through over 100 scats, indicating a broad dietary range and rarity of many prey taxa. Sympatric (B) marten, (C) bobcats, and (D) coyotes exhibited similar trends of initiating plateaus in taxonomic richness near 10-15 scat samples.

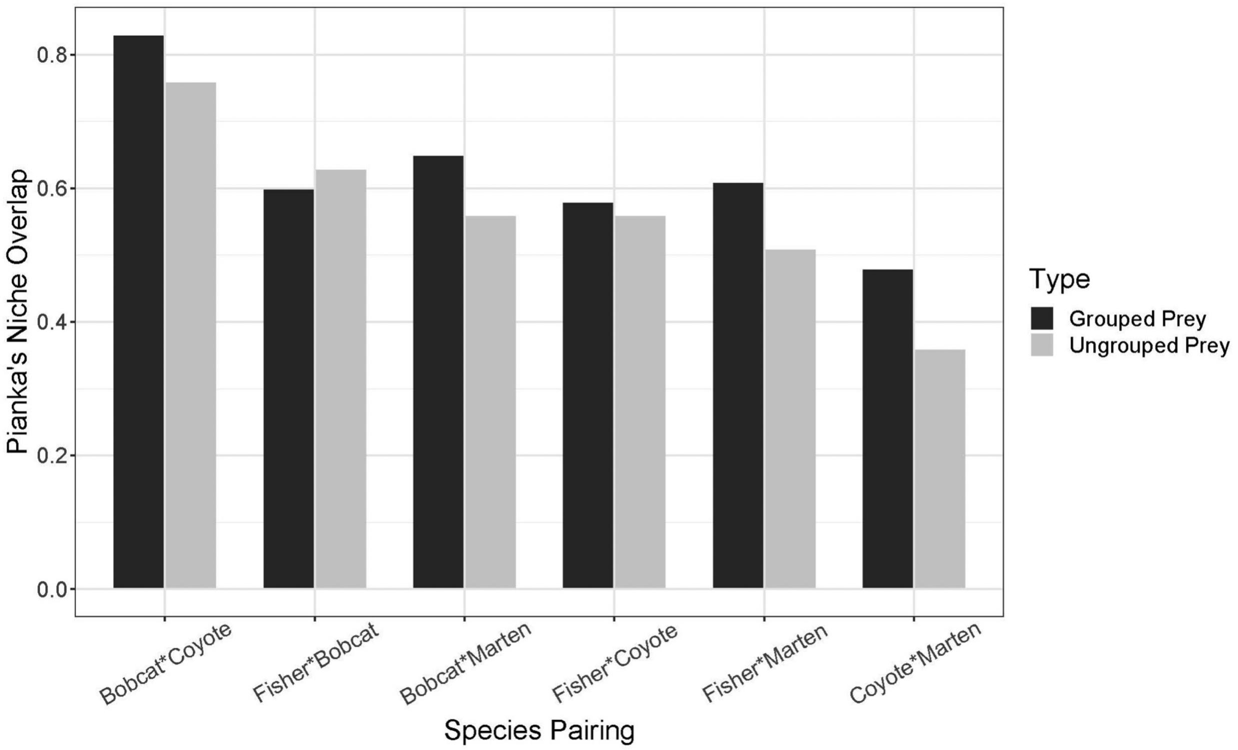

Figure 3.3. Dietary overlap of sympatric carnivores in the North Cascades of Washington, USA, calculated with Pianka's niche overlap index. Complete overlap is indicated by 1 and no overlap by 0. Diet profiles were constructed using scat DNA metabarcoding. Pianka's metric was calculated for diet profiles describing prey assigned to the lowest taxonomic levels (Ungrouped Prey) and for prey grouped by similar taxonomy and habitat type (Grouped Prey). While overlap values ranged from relatively low (<0.4) to high (>0.8), but were moderate-high for all pairs including reintroduced fishers.

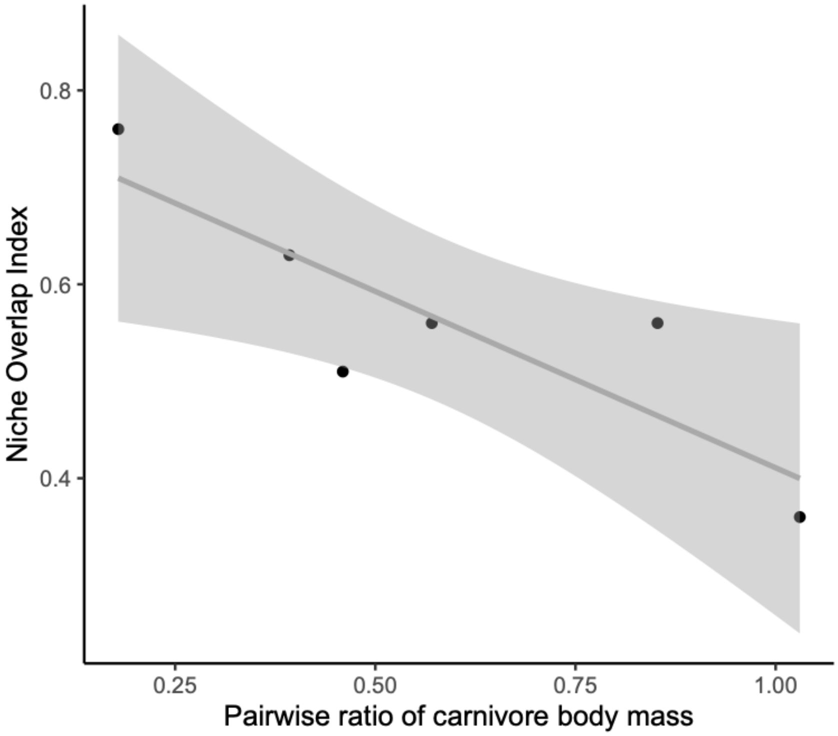

Figure 3.4. Effect of the pairwise ratio of average body mass (g) on Pianka's niche overlap index values in a mesocarnivore community in the North Cascades of Washington, USA. Diet profiles were constructed with prey DNA from scat metabarcoding. Niche overlap decreased as differences in pairwise body mass decreased.

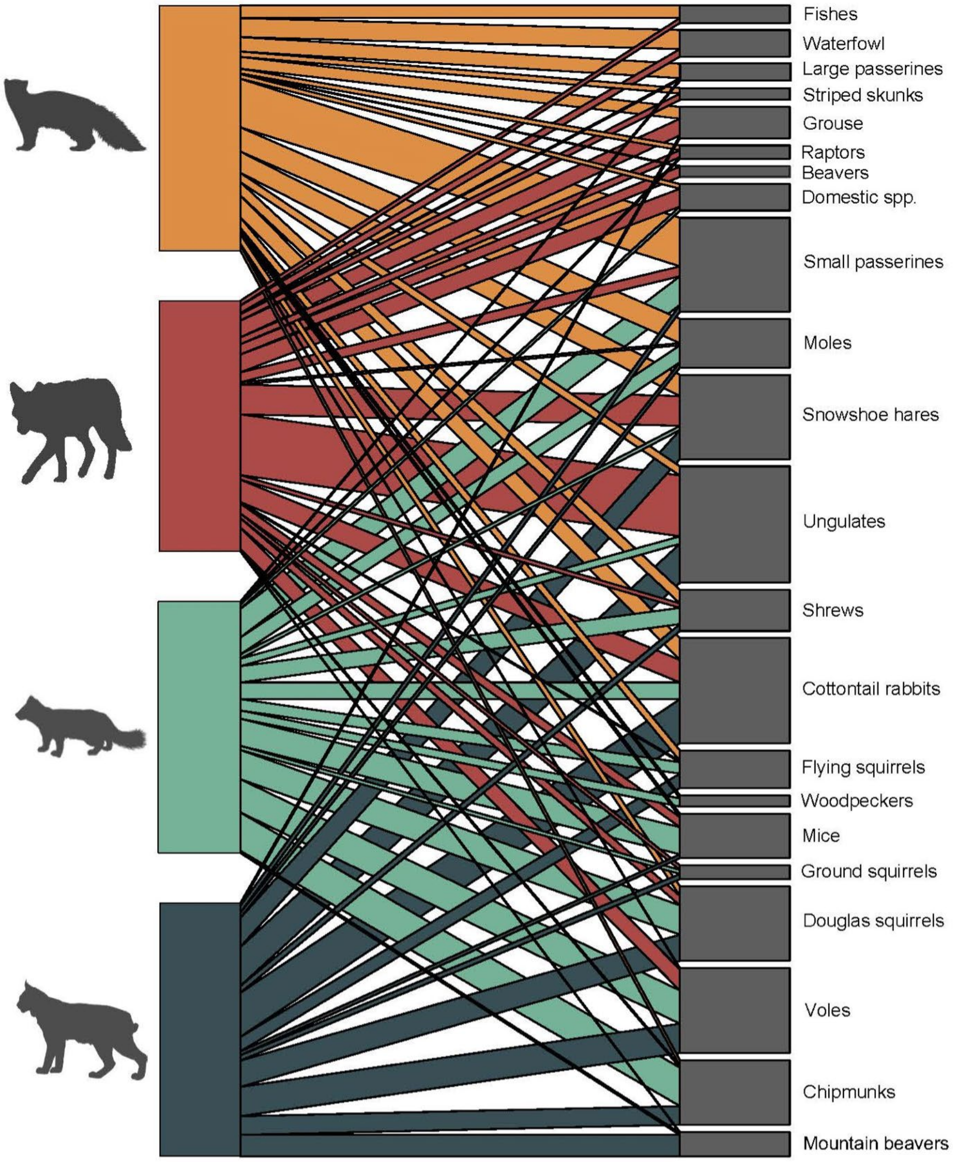

Figure 3.5. Bipartite network diagram representing the predator-prey linkages among reintroduced fishers, coyotes, marten, bobcats (top to bottom, respectively) and their vertebrate prey from scats analyzed with DNA metabarcoding. Prey are grouped by taxonomic similarity and shared habitat use. The width of lines represent dietary contributions of prey to predator diets summarized with relative read abundance. Scats were collected during summer months in 2020 and 2021.

Table 3.2. Permanova analysis results summarizing compositional differences in carnivore diet profiles constructed with relative read abundance data from scat samples analyzed with DNA metabarcoding. Permanova were calculated for diet profiles with prey grouped by similar taxonomy and habitat type (Grouped Prey) and prey assigned at the lowest taxonomic levels (Ungrouped Prey). Prey composition differed significantly (P(perm) < 0.05) with each carnivore assemblage, except for bobcats and coyotes.

PERMANOVA				
	Variable	Pseudo-F	P(perm)	Degrees of Freedom
Grouped Prey				
	All carnivores	4.776	0.0001	300
	Bobcat x Coyote	2.19	0.192	106
	Bobcat x Fisher	5.83	0.006	178
	Bobcat x Marten	4.13	0.006	112
	Coyote x Fisher	5.3	0.006	187
	Coyote x Marten	5.09	0.006	121
	Fisher x Marten	4.84	0.006	193
Ungrouped Prey				
	All carnivores	3.4083	0.0001	300
	Bobcat x Coyote	2.02	0.126	106
	Bobcat x Fisher	3.35	0.006	178
	Bobcat x Marten	3.48	0.006	112
	Coyote x Fisher	3.44	0.006	187
	Coyote x Marten	4.39	0.006	121
	Fisher x Marten	3.52	0.006	193

Table 3.3. Indicator Species Analysis (ISA) values for prey with significantly strong associations ($P < 0.05$) with carnivores calculated from scats analyzed with DNA metabarcoding. Scats were collected during summer months in 2020 and 2021. ISA were conducted for Grouped Prey (prey aggregated by similar taxonomy and habitat types) and Ungrouped Prey (prey assigned to lowest taxonomic levels).

Indicator Species Analysis			
	Prey	Value	P value
Grouped Prey			
Bobcat	Mountain beaver	0.36	0.001
Coyote	Domestic animals	0.29	0.03
	Beaver	0.22	0.03
Fisher	Large passerines	0.31	0.01
Bobcat & Coyote	Ungulates	0.5	0.001
Bobcat & Marten	Chipmunks	0.4	0.001
Coyote & Fisher	Waterfowl	0.3	0.01
Coyote & Marten	Mice	0.39	0.002
Fisher & Marten	Small passerines	0.46	0.01
	Moles	0.36	0.03
Coyote, Bobcat & Fisher	Large leporids	0.49	0.02
Coyote, Bobcat, & Marten	Voles	0.45	0.001
Ungrouped Prey			
Bobcat	Mountain beaver	0.35	0.001
	Pika	0.2	0.03
Coyote	Beaver	0.22	0.02
Fisher	Song sparrow	0.3	0.002
	Thrush spp.	0.29	0.01
	Starling	0.22	0.04
Marten	Southern red backed vole	0.35	0.001
	Wren spp.	0.2	0.05
Bobcat & Coyote	Deer spp.	0.49	0.001
	Townsend's vole	0.32	0.003
Bobcat & Marten	Chipmunk	0.4	0.003
Coyote & Marten	Keen's mouse	0.38	0.003
Fisher & Marten	Coast mole	0.28	0.04
Coyote, Bobcat & Fisher	Snowshoe hare	0.38	0.02

DISCUSSION

Understanding predator diets is vital to evaluating their ecological roles and developing effective conservation plans, particularly in the wake of changing environmental conditions. Our findings suggest that generalist mesocarnivores are subject to considerable dietary overlap, which generally decreases as body-mass ratios increase. However, we documented diverse prey and dietary flexibility among carnivores, with only 11 of the 81 prey taxa shared amongst them all. Whereas the elevated dietary plasticity we observed may allow our focal species to minimize potential intraguild pressure, it may also indicate the availability of larger prey is limited in our study region. Fishers had the most taxonomically rich diet and displayed the greatest dietary flexibility, which potentially increases their resiliency to competitive pressure. However, prolonged niche segregation away from large prey may have negative fitness consequences for carnivores (King & Moors 1979), potentially undermining successful recovery.

Diet studies play a fundamental role in reintroduction ecology, as dietary habits can be reflections of species' space use, the competitive pressures they face, and the ecological function they perform on a landscape. Our results provide evidence that the ecological niche of fishers can be diverse and shaped by the pressure exerted by dominant conspecifics. Our findings indicate that fishers are exploiting a lower trophic niche in the North Cascades than they do elsewhere in their range (LaPoint et al. 2015). If prey resources are relatively abundant, then dietary overlap does not directly correlate to competitive intensity in a system; however, if prey resources are limited, it can be a useful proxy (Broekhuis et al. 2008, Melero et al. 2008).

Methodological differences complicate the direct comparison of our diet results to prior studies, but compared to prior bobcat diet studies in western Washington, our results found decreased prevalence of key bobcat prey, including snowshoe hares and ungulates, and elevated importance of squirrels (Knick et al. 1984, Nussbaum & Maser 1975). Similarly, across their range, voles are a staple in marten diets and have occurred in 25 – 90% of diet samples in prior studies (Pauli et al. 2022). Yet in our study, marten consumed more varied prey and voles were a less dominant contributor (13%). Additionally, we detected a higher variety of prey species in fisher diets and greater utilization of alternative prey than has previously been documented. Conversely, coyote feeding habitats are typically less sensitive to changes in prey densities (Prugh 2005) and our results were comparable to prior studies in Washington forests, where key large mammal prey (*Odocoileus* or *Cervus spp.*) and snowshoe hares collectively comprise over 65% of prey sequences (Witczuk et al. 2015, Shi et al. 2021). Overall, our focal species were consuming higher amounts of alternative prey, suggesting that prey abundance is relatively low in this system.

We did not directly assess prey availability across our study system, but it is likely to affect the degree of competition and resulting resource selection we detected in this carnivore community, as most agonistic encounters among carnivores are driven by food scarcity (Palomares & Caro 1999). Preliminary prey abundance surveys in portions of the North Cascades reintroduction area and the nearby South Cascades, where fishers were also reintroduced (Lewis et al. 2017), found dissimilarities in the relative abundance of potential fisher prey; snowshoe hares, Douglas squirrels (*Tamiasciurus douglasii*), and northern flying squirrels (*Glaucomys sabrinus*) were detected 5, 4, and 14 times more frequently in the South Cascades than in the North Cascades (Humphries 2022, Parsons et al. 2020). Accordingly, these large prey were four times as prevalent in the diet of South Cascades fishers than North Cascades fishers (Shively 2023). Limited prey resources in the North Cascades may increase the strength of competition among carnivores in this system, which may negatively affect fisher recovery.

Carnivore diet profiles overlapped substantially, yet we found significant variations in the diet compositions of focal carnivores. Partitioning of resources is often compulsory for carnivore communities with distinct dominance hierarchies (Palomares & Caro, 1999), whereas carnivores that differ in body weight by a factor of 2.5 - 10x are likely to be involved in interference competition (Donadio & Buskirk 2006), and the smaller, subordinate species will often alter their niche space to mitigate conflict (Harrington et al. 2009). Whereas most studies investigating dominance hierarchies in carnivore communities have focused on large carnivores, our findings suggest that body-mass ratios similarly drive resource overlap in mesocarnivore communities. We found that as body-mass ratios decreased, dietary niche overlap increased. Accordingly, shared large prey were more prevalent in the diets of larger predators (ie., coyotes and bobcats), than those of smaller predators (ie., fishers and martens).

Whereas partitioning across niche axes (e.g., space, time, and diet) reduces the likelihood of agonistic interactions (Schoener 1974), the long-term partitioning of key resources can have population-level consequences (King and Moors 1979). For example, prey switching and reliance on alternative prey in mustelids have been linked to numerous demographic consequences, such as reductions in population densities, lower reproductive outputs, expanded home ranges, and cannibalism (Thompson & Colgan 1987). As fisher population metrics are closely tied to the availability of large prey (1-2 kg) which provide the greatest ratio of energy per unit effort (Powell 1993, Charnov 1976), we hypothesize that restricted access to these prey resources reduces fisher energy intake efficiency in this landscape and may impede their recovery. We found that close to 60% of the fisher diet consisted of prey with low energetic returns, such as birds, fish, and small cricetids (Powell 1993), whereas larger prey were relatively common in the diets of coyotes and bobcats. We have not provided direct evidence that competition is obstructing fisher access to large prey. However, considering that fishers are particularly vulnerable to bobcat predation and, to a lesser extent, coyote predation (Ewen 2012, Lewis, Powell & Zielinski 2012, Wengert et al. 2014), competition among fishers and larger predators in this system may be acute and warrants further attention.

Similar to the effects larger carnivores may impose on fishers, fisher can likewise suppress smaller carnivores (Pauli et al. 2022, Green et al. 2017). The inclusion of greater proportions of small prey in the fisher diet, like small passerines and cricetids, likely increases their dietary overlap with Pacific martens. Competitive dynamics between fishers and martens have been studied extensively, with martens notably shouldering the consequences of this strong interaction (Pauli et al. 2022). Prey switching in martens has typically been in response to low abundance of voles, their key prey, and involves the greater utilization of smaller species, such as shrews, as well as larger species such as ruffed grouse (*Bonasa umbellus*; Thompson & Colgan 1987, 1990). Alternative prey like chipmunks, tree squirrels, mice, and shrews had proportionally higher dietary importance in our study than previously reported in marten diets in the Pacific west (Wilk & Raphael 2017), indicating that marten populations in the North Cascades may already be limited by vole abundance. While voles were rarely detected in the diet of fishers, they were notably present in bobcat and coyote diets. Fishers and martens in the North Cascades had high dietary overlap, consistent with prior studies in other regions where they co-occur. In midwest states of the USA, fishers and martens exhibited near complete seasonal diet overlap, and subsequently, fishers were the primary source of mortality for marten (Manlick et al. 2017a, 2017b, McCann et al. 2010). Dietary partitioning in fishers and marten is typically facilitated by differences in preferred prey – voles in martens and snowshoe hares in fishers (Pauli et al. 2022) – but trophic niche overlap and competition can increase when these prey are in low abundance (Carlson et al. 2014, Manlick et al. 2017b, Manlick & Pauli 2020). The most notable divergences between fisher and marten diets in this study occurred in martens' use of chipmunks, mice, and voles, and fishers' use of waterfowl, grouse, and leporids. At the time of this study, fishers were in low abundance in the Cascades, but if their reintroduction is successful, the top-down pressure they exert on martens may intensify, particularly during winter months when prey is notably less diverse and abundant.

Collectively, our results reveal the potential for a multi-carnivore cascade, wherein the competitive pressure of bobcats on fishers in a resource-limited environment sparks the subsequent exploitation of a diverse suite of smaller-bodied prey by fishers, thereby increasing competitive pressure on subordinate martens. Multi-carnivore cascades have received considerably less attention compared to classical tri-trophic (predator-herbivore-plant) pathways (Ballard et al. 2003, Thompson & Gese 2007, Brashares et al. 2010), but the restoration of extirpated carnivores provides an opportunity to better understand these trophic dynamics (Thompson & Gese 2007, Berger et al. 2008). As bobcats and fishers share similar prey preferences in Washington state (Happe et al. 2021, Parsons et al. 2019, Knick et al. 1984), we hypothesize that increased competitive pressure imposed by bobcats on fishers can shift fishers' dietary niche toward smaller prey (Gilbert et al. 2000), creating compounded negative effects for marten. However, sufficiently diverse prey resources can facilitate dietary divergence in fishers and marten (Pauli et al. 2022), and the unprecedented distribution of fisher predation pressure across diverse prey groups we observed in this study may weaken this cascade. The assessment of marten-fisher relationships has largely been dyadic and focused on abiotic influences and prey availability (Pauli et al. 2022), so we advocate for a community-level approach that incorporates higher-level interactions.

The restoration of a more complete carnivore guild in Washington may additionally alter species interactions in the coming years. The natural recolonization of wolves in Washington is ongoing, and while no wolf packs were documented in our study system at the time of sampling, two occurred within 50 km (Washington Department of Fish and Wildlife et al. 2021). The restoration of apex predators can have cascading effects on ecosystems and can modify how carnivores, such as bobcats and coyotes, are distributed by increasing interference competition (Ajo & Pletscher 1999, Berger & Gese 2007, Fuller & Keith 1981, Levi & Wilmers 2012). The future restoration of wolves within our study system may benefit fishers by reducing the density of bobcats and coyotes, thereby reducing the predation pressure on shared high-value prey resources and intraguild predation. By contrast, the restoration of wolves could adjust the spatio-temporal habits and diet of coyotes and bobcats, consequently affecting their overlap in niche space with fishers with unknown consequences. However, the addition of wolves to this system may also contribute additional carrion resources to the carnivore guild. The availability of wolf-contributed carrion can facilitate resource partitioning in carnivores (Sivy et al. 2017) and is one example of the ecological benefits of restoring complete carnivore guilds.

Changes in the abundance of important prey species compared to historical times may also affect the competitive resiliency of fishers in our study system. Fishers have been referred to as porcupine specialists (Powell 1993), owing to their specialized behavioral adaptations to effectively prey on adult porcupines (Bowman et al. 2006, Williams 2007). Fisher and porcupine population dynamics have been linked by several studies, with fishers exhibiting numerical responses to changes in porcupine abundance (Bowman et al. 2006), and porcupines experiencing decreases in survival as fisher abundance increases (Mabille et al. 2010, Pokallus & Pauli 2015). Throughout the West, porcupines were aggressively targeted for lethal control by government agencies and forest product companies through poisoning, hunting, and bounties due to the damage their foraging habits caused to forestry products (Hoffer 1967, Woods 1973, Anthony et al. 1986, Borrecco & Black 1990, Witmer & Pipas 1998). Because of the low fecundity and poor dispersal capabilities of the porcupine (Roze 2009), their recovery in the Pacific Northwest has been slow, and they are believed to be in low abundance in forest habitats, particularly in the North Cascades (Appel et al. 2021). As fishers, and to a lesser extent cougars, are considered adult porcupine's main predators (Thompson et al. 2009, Pokallus & Pauli 2015), specialization toward porcupines could have allowed fishers to secure large prey while also facilitating niche segregation from competitors. Considering the close associations between fishers and porcupines throughout much of their range, at their previous abundance, porcupines may have historically influenced community structure in our study system by providing fishers with a near exclusive prey resource; however, in this study, porcupines only comprised 1% of prey sequences in the fisher diet. Limited data are available on current porcupine densities in the northwest, but increased predation pressure by restored fisher populations may also further impede their recovery.

When diet overlap is high, carnivores can adjust their temporal or spatial behaviors to facilitate coexistence (Lovari et al. 2015, Dröge et al. 2017, Karanth et al. 2017). Our study of dietary patterns of co-occurring carnivores did not assess the spatial and temporal component of species interactions, which are integral to fully understanding species coexistence. We also did not evaluate the plant and invertebrate component of our focal species' diets, which may provide important supplements to vertebrate prey during the summer. Additionally, prior studies have demonstrated that niche overlap in sympatric carnivores may increase during winter-time reductions in the diversity and abundance of prey (Manlick et al 2017a, Croose et al. 2019, Palomares & Caro 1999), and as such, our summertime overlap estimates are likely a conservative measure of the potential for competition in this system. While further research is needed to better understand how seasonal and spatiotemporal influence carnivore coexistence, our findings nevertheless suggest that intraguild competition for large prey is contributing to community structure in the North Cascades.

Assessments of prey availability can greatly improve carnivore reintroduction attempts (Steury & Murray 2004, Licht et al. 2016, Jachowski et al. 2011,), but preliminary prey assessments may be ineffective if the densities of prominent competitors are not assessed in tandem (Moseby et al. 2015). Prior research has shown that modeling predator habitat can vastly alter the suitability of translocation sites, yet this strategy is rarely implemented in reintroduction planning (Halsey et al. 2015). While generalist carnivores can effectively partition resources to coexist, prolonged competitive exclusion from prey that provide the most energy-efficient returns will likely have negative impacts on their long-term population health. Our evaluation of sympatric carnivore diets indicates that while fishers may face challenges obtaining adequate resources for population expansion in the North Cascades (ie., large prey), they can effectively supplement their diets with resources sparsely exploited by conspecifics. We recommend that future research should prioritize synthesizing long-term dietary trends with population metrics to better understand the influence of resource competition on species restoration. Additionally, by evaluating the distributions of prey resources and competitors across potential reintroduction sites, the initial success and long-term viability of carnivore reintroductions can be improved.

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CHAPTER 4

General Conclusion

Diet analyses can play a key role in ecological studies and carnivore conservation management by revealing trophic interactions. DNA metabarcoding has advanced the understanding of food webs by allowing the characterization of diet profiles at a much finer scale than traditional, morphology-based methods. As human-caused disturbance continues to restructure animal communities, DNA metabarcoding can non-invasively describe species interactions to provide resource managers with fast, detailed, and efficient methods for monitoring sensitive wildlife populations. Here, I evaluated the dietary resource use of reintroduced fishers and their sympatric competitors on landscapes that have experienced biotic changes since they were last present. I observed substantial differences in fisher diets between the South and North Cascades reintroduction areas, particularly in the quality of forage fishers were accessing. While these landscape-level differences in fisher diet profiles could be attributed to a broad range of biotic or abiotic factors, ultimately my findings highlight an opportunity for a long-term monitoring regime in the Cascades reintroduced fisher populations that link diet habits with correlates of fitness to better understand determining factors in restoration success.

Competition is a strong determinant of where species occur and how they interact (Hardin 1960). Through my evaluation of dietary overlap among fishers and potential competitors with DNA metabarcoding, I demonstrated how fine-scale partitioning of resources by fishers may facilitate their niche differentiation and thereby their ability to coexist with a diverse carnivore community. While the fishers' dynamic diet implies their resiliency to periodic changes in prey populations, questions remain on how prolonged competitive exclusion from key prey will affect their population trajectory in the North Cascades.

Importantly, my study highlighted the need for considering multiple species when investigating the underlying mechanisms of resource partitioning in carnivore communities. I found support for the hierarchical nature of this mesocarnivore community, signifying that the competitive pressure upon one species can impact lower trophic levels. While my investigation was limited to the predator-prey and predator-predator relationships of only four carnivores, the integration of additional prey taxa, such as invertebrates and vegetation, could further enhance our knowledge of community structure and trophic processes in the North Cascades.

A consistent theme emerged from my two chapters evaluating fisher diet: fishers in the North Cascades displayed a uniquely diverse diet compared to their closest competitors, as well as the South Cascades reintroduced population and other fisher populations across their distribution. Environmental factors may be influencing prey abundance in the North Cascades, and subsequent interactions within its carnivore guild, thus future research should explore the potential factors driving the observed low prey abundance to elucidate management activities that promote the recovery of fishers and other rare carnivores in this region.

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