Fall 2021

What's In That Scat: An Analysis of Canada Lynx Diet and Distribution in the North Cascades Ecosystem

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Abstract:

This research provides critical information on the diet and distribution of the elusive North Cascades lynx population. Canada lynx (*Lynx canadensis*) are considered threatened under the federal Endangered Species Act and are the focus of protection efforts by the state of Washington as a result of climate change, heightened competition, and human interference. I analyzed the diet and distribution of both lynx and coyote (*Canis latrans*) in the North Cascades to determine whether there was an overlap of prey and habitat that could constrain lynx restoration. During the summer of 2020, the hiking trails in the North Cascades National Park in Washington state were surveyed by the Cascades Carnivore Project (CCP) to collect the scats of rare carnivores. 428 scats were sent to the Quantitative Wildlife Ecology and Conservation Laboratory at OSU to be DNA analyzed for predator and prey species. Of these, 276 were Canada lynx; 97 were coyote, a potential prey and habitat competitor for lynx. I constructed the diet of lynx and coyote and compared the proportional representation of prey species using the chi squared test of independence. To analyze lynx distribution, I created visual representation of scat collection elevations and cover-types and compared the elevations of lynx and coyote scats using the variance test and 2-sample T-test. The data suggest that the diet of lynx in Washington is specialized, consisting of 78% snowshoe hare (*Lepus americanus*), similar to diets described for lynx populations in other regions. In contrast, the diets of the coyotes were more general, but the two predators possess a 14-species overlap in diet. Lynx also specialize by using a smaller range of elevations (4000-8000 ft) than the range of the coyote which overlapped and extended wider and more variable elevations (2000-9000 ft) that included areas with less tree cover. Coyote overlap of lynx diet and habitat, compounded by high coyote abundance, suggest coyotes may be a limiting factor in lynx restoration.

Introduction:

Named for its ever-flowing glacial waters, Washington's North Cascades is a region prized for its beautiful geological features, abundant recreational opportunities, and diverse plants and wildlife. Glaciers and snowfields cover the jagged mountain peaks. Forests are dominated by conifers, broadleaf deciduous trees grow along the edges, and woody shrubs and ferns provide understory, while alpine meadows are blanketed in grasses and wildflowers. Ungulates such as mule deer, elk, and mountain goats; small mammals such as hoary marmots and pikas; and predators such as wolves, wolverines, red foxes, and Canada lynx inhabit this rugged terrain. Yet, many of these species are under increasing stress due to climate change.

Average temperatures are increasing, summer droughts are becoming more prevalent, and drier forests are at elevated risk of wildfire leading to diminishing wildlife habitat (Fourth National Climate Assessment, 2018). Climate change impacts further imperils predators, sentinel species that help researchers evaluate the health of the entire ecosystem (Ripple, 2014). However, these elusive animals reside in remote, montane areas and are therefore challenging to study. What is known about their status is based largely on anecdotal evidence (sightings from government agency employees, conservation groups, and recreationists) and a few population estimates and predictive models. The Cascades Carnivore Project (CCP), a science-based conservation organization, focuses on filling some of these data gaps and providing empirical information about Washington's at-risk carnivores. Over the summer of 2020, the CCP collected targeted carnivore scat (Cascade red fox, Pacific marten, fisher, wolverine, gray wolf, and Canada lynx) along high elevation trails in North Cascades National Park, the Pasayten and Chelan-Sawtooth Wilderness areas, and surrounding areas on the Okanogan-Wenatchee National Forest as part of its *Conserving Montane Carnivores in the Face of a Changing Climate Project*. Over 500 samples were collected and 430 were sent to Oregon State University for DNA metabarcoding in order to verify the species and diet prey. 276 of the successfully sequenced samples were identified as Canada lynx (Lynx canadensis), 97 were coyote (Canis latrans), 15 were bobcat (Lynx rufus), 8 were gray wolf (Canis lupus), 6 were marten (Martes americana), and 2 were cougar (Puma concolor); others were identified as undetermined Canis or deemed inconclusive. This study investigates the diet and distribution of the Canada lynx in northern Washington to determine the constraints to their restoration.

Literature Review:

Status of Canada Lynx (Info and present research):

The Canada lynx is a medium sized felid with flared facial ruff, black-tipped ear tufts, arched back, snowshoe-like paws, and a cropped black tail (Figure 1). It measures 75-90 cm in length and 48-56 cm in height, and weighs 6-14 kg. Its dense fur alternates from greyish-brown to brownish-red depending on the season. Long hind legs and large, wide feet make it well-suited to hunting its preferred prey, the snowshoe hare, in deep, powdery snow (U.S. Fish and Wildlife Service, 2017).

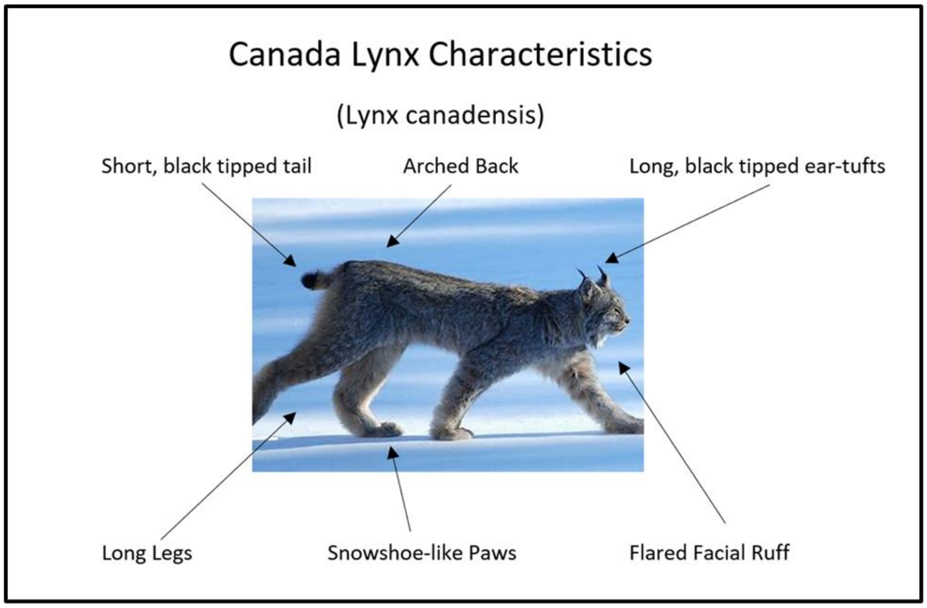

Figure 1: depiction of defining characteristics of Canada lynx. Image credit: Keith Williams (CC-BY-2)

Native to North America, the Canada lynx's range extends throughout Alaska, Canada, and the northern United States in boreal, subboreal, and western montane forests (Figure 2). The northern limit of its range has not changed for two centuries, but the southern limit has been pushed northward over time (Lavoie et al., 2019). While the continental species is currently listed on the IUCN Red List as least concern, the Canada lynx faces competition due to the expanding range of coyotes and habitat fragmentation in the contiguous United States where the effects of Climate Change are more obvious (Vashon, 2016). The United States lists Canada lynx as threatened under the Endangered Species Act. Historically, Canada Lynx were found in 25 states, but now their range is limited to Alaska, Montana, Maine, Minnesota, Wyoming, Colorado and Washington, where they are listed as a State-Endangered species (King et al, 2020). Logging, road-building, and development of forests in Washington has highly fragmented their habitat and led to an increase in snow-packed pathways. Traditionally, Canada lynx were inaccessible to predators like cougar and coyote and competitors like bobcat because of their ability to hunt and survive on fresh snow at high elevations, but packed snow due to human activity has allowed these species access to Canada lynx habitat and prey (King et al., 2020). Of additional concern are the intense forest fires that have substantially reduced lynx habitat. The Tripod fire (2006), for example, is cited as having contributed to Washington state lynx decline from an estimated 87 individuals in the early 2000's to less than 50 today (Washington Department of Fish and Wildlife, 2021). Population-based recovery projects in Washington are difficult to implement because of the lack of knowledge of lynx dynamics in southern boreal forests (Stinson, 2001).

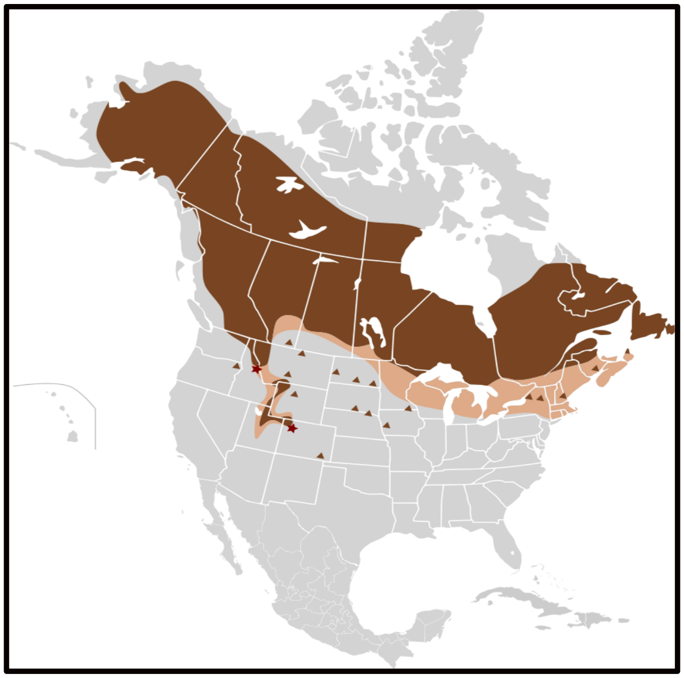

Figure 2: Map of Canada lynx distribution in North America. The dark brown depicts the current range and the light brown shows the probable historical range. Image credit: [File:Lynx canadensis map.svg - Wikimedia Commons]

The Canada lynx's home range varies between 15.5-221 km^2 dependent on sex, age, and prey density. The average lynx range in Washington is 69 km^2 for males and 39 km^2 for females; mean ranges in Montana are considerably higher at 122 km^2 for males and 43.1 km^2 for females. In Washington, Idaho, and Montana, lynx are found at elevations over 1,200 m (4000 ft), whereas in Colorado and Utah they are found above 8000 ft. In Alaska they are found at even lower elevations, 980-3520 ft perhaps due to colder climate (Ulev, 2007). A 10-year study in Colorado of reintroduced, radio-collared lynx found the average elevation for lynx habitat was 10,780 ft and the majority between 9,900-11,620 ft. Researchers also found that 65% of lynx habitat was upper montane forest and the majority of lynx used habitat with at least 20% tree-cover. Grasslands made up 16% of lynx habitat and there was little use of any other habitat types (Theobald & Shenk, 2011). Although they prefer to avoid open areas, Canada lynx have been known to cross long distances and atypical regions when prey are scarce (Schwartz et al., 2002).

Territorial and solitary animals, adult Canada lynx typically avoid each other except during the breeding season in winter. To demarcate home ranges, they use feces, sprayed urine, or anal secretions. They are a strictly carnivorous species, primarily eating snowshoe hare (⅓ of diet) and rodents such as squirrels and mice, birds such as grouse and ducks, fish, and ungulates (eaten as carrion) (Lavoie et al., 2019). To meet their dietary metabolic needs, Canada lynx require 0.4 snowshoe hares per day. According to a study in Minnesota, 76% of lynx scat contained remnants of snowshoe hare (Hanson & Moen, 2008). Because the snowshoe hare

(Lepus americanus) is the primary prey species, lynx diet and snowshoe hare populations are cyclically linked. The lynx population cycle lags about one year behind the hare population cycle. During the summer months, the lynx diet broadens due to higher prey availability (Mowat et al., 2000). In general, young lynx tend to have more variable diets, while older lynx typically stick to snowshoe hare. Yearling lynx may have broader diets because they are forced to adapt when snowshoe hare population density declines (Burstahler et al., 2016). As lynx are considered hare specialists, their geographic range and hunting tactics are consistent with hunting hares.

Using sight and sound, lynx hunt by stalking and bed-ambushing near the trails of prey. Hunting success depends on factors such as length of chase, age of individual, knowledge of area, and season (Lavoie et al., 2019). The reduction in length and intensity of the snowfall season, for example, may result in the inability for lynx to specialize in snowshoe hare. Exploitative competition is also more likely to occur if the lynx's deep snow advantage no longer exists. It is not known whether lynx eat more alternative prey during the summer because of greater abundance or decreased success on hares and competition (Mowat et al., 2000). Unlike sympatric carnivores, lynx prefer to eat fresh kills over scavenged meat and don't cache meat very often (U.S. Fish and Wildlife Service, 2017). They are found in regenerating forest stands and often den in mature forests with a high quantity of wind-felled trees. Logging allows succession to occur which is optimum for hare and lynx habitat, but it can remove cover and thus, lynx denning structures (U.S. Fish and Wildlife Service, 2017). Forest management also creates roads which cut-off habitat and increase human and competing predator access.

Coyotes are a mesopredator of particular concern as they have encroached into the lynx's high elevation habitat and compete for prey, including the snowshoe hare, which is the predominant lynx food source in many regions. Mountain areas were historically off-limits due to deep snow-cover, but coyotes have gained access using groomed trails, snowmobile routes, and forestry roads and could impact the Canada lynx population in the North Cascades (Dowd et al., 2014). Although they were originally adapted to the arid plains and brush areas of the West and Midwest, coyotes now inhabit much of North America including urban areas (Bradford & Pester, 2021). Many attribute the extirpation of wolves as directly responsible for the coyote's spread and wonder how wolf reintroduction efforts will impact the coyote's range (Arjo and Pletscher, 2004). Coyotes are omnivores; their diet varies based on food availability. In areas where resources are scarce, Coyotes will eat fruit (40%), insects (23%), mammals (25%), trash (6%) and birds (3%) (Swingen et al., 2016). In wilderness settings, coyotes are 90% carnivorous. Their prey includes deer, sheep, rabbits, rodents, birds, snakes, fish, and insects, but they will also scavenge on large ungulate carcasses. Although generalized feeders compared to lynx, coyotes not only consume the lynx's preferred prey (snowshoe hare), but are sometimes known to kill lynx. More studies are needed to determine if coyotes are having a detrimental impact on lynx population abundance (Guillaumet et al., 2015).

While there are a few competition studies, most current Canada lynx research focuses on their demographics, distribution, and diet using research methodologies such as radio telemetry equipment, motion camera trapping, and DNA analyses of hair and scat samples. In one study, for example, microphones and accelerometers were attached to GPS collars on 26 Canada lynx to capture hunting and social behavior as well as location. Over the 5-year Yukon study, 14,000 hours of audio recording were collected and kills were identified with 87% accuracy. Notably, there was more social interaction among female lynx than expected; they were recorded sleeping, grooming and hunting together, but not sharing prey (Studd et al, 2021). Another study evaluated use of species distribution models (SDMs) on GPS data collected from radio-collared lynx in 3 geographically distinct locations (Washington n=17, Montana n=66, and Wyoming n=10) over 9 years and concluded range predictions could be applied to other areas (Olson et al., 2021).

A recent, broad-scale, distribution study in lynx probable habitat in Washington used spatially extensive motion-sensitive camera arrays that covered 7,000 km^2. Researchers estimated lynx occupancy and assessed the efficacy of their cameras to monitor lynx. They successfully surveyed a large area with minimal personnel and concluded that motion-sensitive cameras are beneficial to such studies, and revealed that Washington's lynx population is imperiled and does not warrant delisting (King et al., 2020). These findings corroborate an earlier study between the Washington Department of Transportation and the U.S. Fish and Wildlife Service (Aubry et al., 2002) which employed hair-snagging techniques and DNA analysis to detect Canada Lynx along the North Cascades Highway (HWY 20) during Fall 2000 and Summer 2001. Out of 33 transect locations, only 4 total lynx were detected, 3 in 2000 and 1 in 2001 (Aubry et al., 2002). This is not surprising in light of an USFWS assessment that concluded that lynx are 13 times less likely relative to random expectation to cross 4-lane highways and 3 times less likely to cross 2-lane highways (U.S. Fish and Wildlife Service, 2017). Lynx habitat generally occurs an average of 27 km. away from high traffic highways and at least 5.2 km. away from the nearest highway. (Theobald, 2011).

Lynx diet research is varied. Some studies focus on the snowshoe hare as the primary prey of Canada lynx; specifically, what causes the 10-year population cycle which dramatically impacts lynx population. At its peak, hares can reach a density of approximately 1500 per km^2. As a result, there is an increase in hare starvation due to saturated habitat and predation which leads to decline and eventual stabilization. A 45-year, mark-recapture study in the Yukon boreal forest found that the snowshoe hare's average lifespan is 1-year and death is due to predation 95% of the time. The authors conclude that declines in reproduction are due to chronic stress over predation in breeding females which can be inherited through maternal effect maintaining a low phase for 2-4 years (Krebs et al., 2017). Other studies locate the kill sites of radio collared lynx by backtracking and then identify prey through tracks and/or remains as well as hunting success (Ivan & Shenk, 2016). For example, a collaborative study conducted in Washington, Montana, and Minnesota examined the efficacy of DNA analysis of hair and scat collected along putative Canada lynx snow tracks and was able to positively identify 81% of hair and 98% of scat samples collected. The authors conclude that incorporating DNA analysis for species identification should be a high priority (Mckevey et al., 2009).

Methods of Studying Carnivores (Why Scat method?):

As suitable wildlife habitat declines, conservation and protection of carnivores is paramount for these keystone species, but this can only be achieved when there is reliable data concerning the status and health of a species. Biologists and land management must ascertain distribution and abundance over time in order to make appropriate recommendations to policy makers (Gese, 2001). Yet, it is difficult to accurately estimate the size of carnivore populations due to their often nocturnal habits, low densities, wide territories, and elusive natures (Gros et al., 1996). While direct methods of monitoring carnivore populations such as marking and capturing of animals may yield robust estimates of abundance, these methods are resource intensive and often result in high stress to the animal. Fortunately, population size also can be determined through various noninvasive methods. Observational studies like quadrats, line transect, and visual searches have no negative effect on the animals, but are more accurate for smaller species. Observational studies which involve looking for signs can provide better results for larger animals. These include scat counts, track surveys, camera stations, and hair traps (Long et al., 2008). When these techniques are employed, individual animals do not have to be observed directly and results are found efficiently.

Ecologists and conservationists rely on scat collection as a fundamental tool to gather information about a particular species like its diet, distribution, and abundance. As monitoring prey selection through direct observational diet studies is both expensive and logistically difficult to achieve in natural environments, scat analysis is an effective way to determine the diet of terrestrial carnivores (Klare et.al., 2011). Field researchers and volunteers can collect scat in accordance with their study parameters, making selections based on shape (twisted, segmented, pointed ends), size (length and/or diameter), color (black, brown, white), contents (hair, bones, vegetation), and smell. While this non-invasive method provides necessary samples, it is often unreliable due to factors such as scat degradation, carnivore body size, and similarly sized carnivores. Like species identification based on scat appearance, conventional diet studies which examine composition of undigested remains (bones, fur, teeth, feathers, or claws) and classify them based on reference comparisons result in frequent misidentification (Thuo et al., 2019).

DNA metabarcoding of scat takes this a step further by providing a highly accurate picture of prey composition and identifying the carnivore definitively. This method sequences the vast majority of the DNA fragments contained within each scat using high variable universal primers to identify prey (Thuo et al., 2019). Diet data can be obtained from fresh scat as well as older samples (up to 60-days) but becomes less effective with time. Success also varies based on predator and type of prey and duration since consumption. For example, if the carnivore deposits scat less than 8 hours or more than 3 days after ingestion, DNA research may not be able to identify prey. Before 8 hours the scat does not contain the most recently eaten prey species and after 3 days, the prey in the scat is too deteriorated to identify (Thuo et al., 2019). Other limits of DNA metabarcoding include quality of sample collection, field handling, storage, lab contamination, and human error (Liu et al., 2019).

Study area:

The CCP study area contained large portions of the North Cascades National Park, Pasayten Wilderness, and Chelan-Sawtooth Wilderness, extending from Lake Chelan to the Canadian Border. Located in northwestern Washington state, the North Cascades Ecosystem range has an average elevation of 7,000 feet and an average annual rainfall of 80 inches and average annual snowfall of 633 inches. It is the most glaciated area in the contiguous United States with over 300 glaciers and snowfields (US parks, 2021). Warmer summers and decreased precipitation due to global warming, however, have led to dramatic shrinking of North Cascades glaciers (Parks, 2021). The area houses diverse vegetation including Douglas-fir, western red cedar, western hemlock, big leaf maple, mountain hemlock, Pacific silver fir, and yellow cedar forests. The region is also home to alpine heaths, meadows, and fellfields. Due to natural fires, occurring at 90-250 year frequency intervals, a pattern of significant tree replacement occurs. Lynx, gray wolves, wolverines and other wide-ranging and rarely seen carnivores are found here because it is less developed and logged than other Cascade Mountain regions in the south. The adjoining Pasayten Wilderness contains 531,000 acres and lies in the Mount Baker- Snoqualmie National Forest and the Okanogan National Forest. The elevation ranges between 1200 and 9000 feet with over 600 miles of trails to provide access. Lake Chelan-Sawtooth Wilderness encompasses 153,057 acres and ranges in elevation from 1,100 to 9,000 feet.

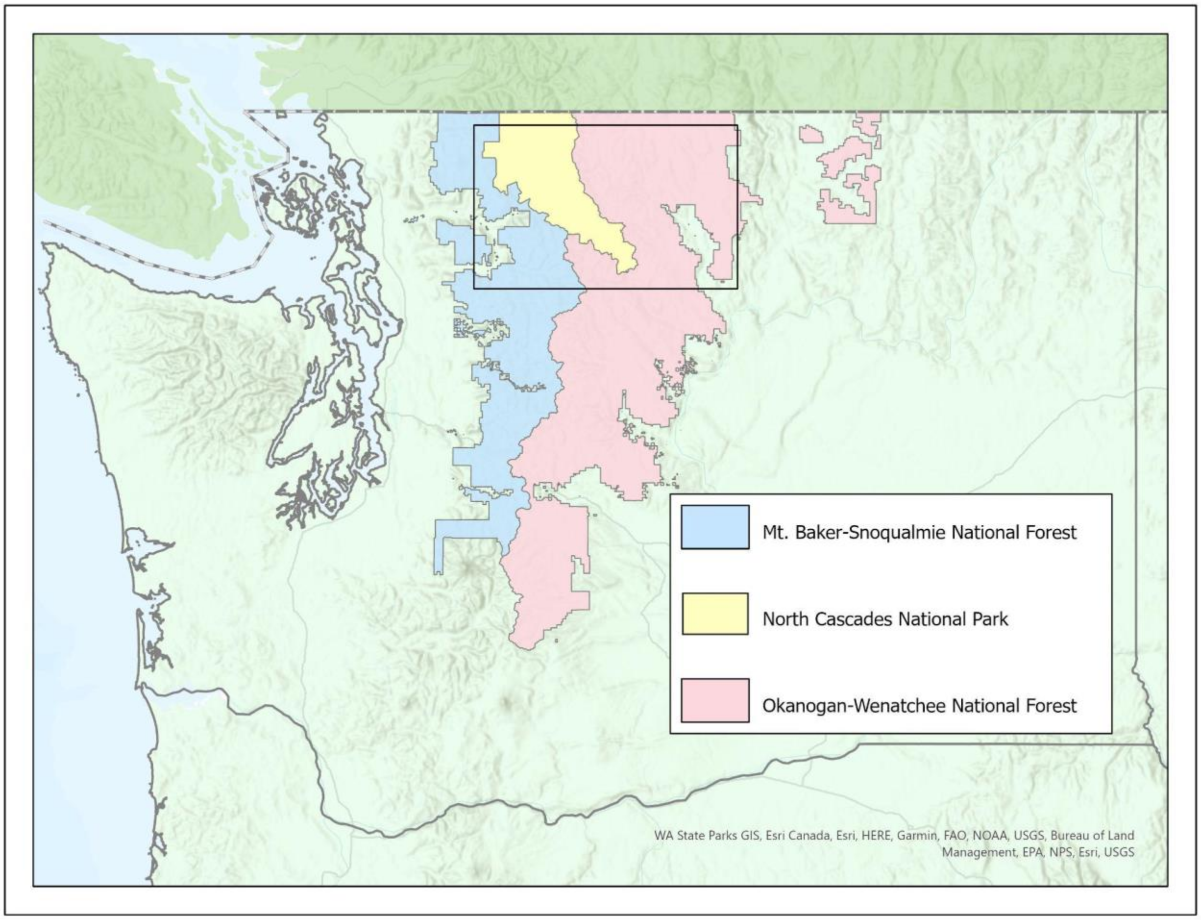

Figure 3: Map of *Conserving Montane Carnivores in the Face of a Changing Climate Project* study area.

Methods and Materials:

Survey design:

My analysis used data collected by our team of field technicians working in groups of 2 or individually to collect carnivore scats from June 30 to September 5, 2020. Trails near previous carnivore sightings were surveyed every 2 weeks for up to 6 repeat visits (Appendix A). At the beginning of the field season, the snow had recently melted and much of the scat collected was deposited during the winter months. Later in the summer, the scats were mostly fresh. To find scat, we scanned the trail 10-20 ft ahead and focused on trail edges and high points along the trail like tree stumps and boulders. The mountain pass areas including ridgelines were intensively searched as the 4 focal carnivores (lynx, fox, marten, and wolverine) are known to deposit scat where terrain funnels movement. Scat morphological parameters were provided to guide identification of species. We were assigned trails and specific ending destinations each day and these hikes were recorded as GPX files on the GaiaGPS app. Scat and sometimes hair samples were photographed and retrieved for genotyping and dietary analysis and a waypoint was created with the GPS location and elevation. Waypoints were also created for detections (vocalization and visualizations) of at-risk prey including hoary marmot (*Marmota caligata*), grouse (*Dendragapus obscurus, Falcipennis canadensis, Bonasa umbellus*), American pika (*Ochotona princeps*), and white-tailed ptarmigan (*Lagopus lecura*).

Sample collection and preservation:

For each sample, we recorded the waypoints using the WGS84 datum and labeled them with the Field ID (yymmddXXS0n). We then labeled 2 brown bags and 2 vials with the FieldID, latitude and longitude, elevation, species guess, and study area with a ballpoint pen. To indicate each species guess, we used a specific genus-species code (Cascade Red fox=VUVU, wolverine= GUGU, fisher= PEPE, lynx= LYCA, coyote= CALA, Pacific marten= MAAM, grey wolf= CALU). Before handling the sample, the scat was photographed with a tracking ruler for scale reference. We also filled out a descriptive data form including the field ID, latitude and longitude, elevation, species guess, fresh/dry, and further scat description. Typical scat description indicated whether there were berries, hair, or bone fragments within the scat, the color of the scat, and where on the trail the scat was located. A 1-2ml sample from each end of the scat was collected using sticks as chopsticks and placed into the two ethanol-filled vials. Scat ends were selected because they contain the most epithelial cells from the predator. The vials were then sealed with parafilm wrap and both put into a ziplock bag to prevent spillage. The remainder of the scat sample was placed into the two brown bags which were folded into thirds and then placed into a third brown bag. Any hair samples were placed in small orange envelopes and labeled with the field ID, waypoint location, elevation, and species guess. Once out of the field, we put folded brown bag samples into another unfolded brown bag and allowed them to dry out for a few days to prevent degradation and mold. Once dry, the samples were labeled with the Field ID and stored in separate cardboard boxes with other samples. The data forms, sample photos, and GaiaGPS tracks were uploaded to a shared drive to be reviewed later. Finally, the collected scat was handed over to Dr. Jocelyn Akins (founder of CCP) who assessed the samples and decided which were worthy to send to OSU for metabarcoding (e.g. old/moldy samples).

Laboratory analyses:

Potential target carnivore scat samples of good quality were sent to the Quantitative Wildlife Ecology and Conservation Laboratory at Oregon State University for DNA extraction, amplification, sequencing, and genotyping. Carnivore scat was divided into 3 subsamples and analyzed with DNA metabarcoding and high-throughput sequencing primers were utilized to determine vertebrate prey (McInnes et al. 2017). DNA was sequenced at the 12s locus for identification. Based on their shape and contents, scats were visually identified as one of the carnivore species. The success of the visual identification (Appendix B) was evaluated as part of the DNA data analysis.

Data analysis

Diet:

The predator/scavenger species and the prey/carcass species were separated by analyzing the DNA read count. The read count is lowest for the predator while the prey species display larger counts. More of the prey DNA can be uncovered because the lab primarily samples the inside of the scat. The DNA results were inputted into an Excel spreadsheet which provided the predator species and prey species (n= 0-5). I analyzed diet data for both lynx and coyote to evaluate possible competition. Prey in lynx scat included 19 distinct species and prey in coyote scat included 24 distinct species. There was an overlap of 14 species between the two predators. I configured the data into bar graphs to visually represent the differences in prey preference between lynx and coyote. To further compare lynx and coyote diets, I created graphs to illustrate the number of prey species in each scat and the break-down of single-prey scats. I also used a Chi-Square Test of Independence to determine the relationship between the diets.

I quality assured/quality controlled (QA/QC) the diet data by confirming that each prey species was found in the study area by referencing the North Cascades National Park Species Checklist. If the original assigned species was not found in the study area, I reentered their DNA sequences into the GenBank database using the Basic Local Alignment Search Tool (BLAST) to find a more correct match. In the case that no match was found to a local species, I removed the prey species from the dataset.

Distribution:

I saved GPS locations and elevations of lynx and coyote scat as .csv files and converted them into x-y points which were plotted on Arc GIS Pro. In order to analyze elevation, I plotted lynx and coyote scat points with graduated symbols. The 4 colors of dots represent the 4 bins (< 5000 ft., < 6000 ft., < 7000 ft., <8000 ft.). I graphed elevations of both species on a bar chart to indicate differences in range. To compare elevations of lynx and coyote scat samples, I completed a variance test as well as a 2-sample T-test. I also plotted the points over an ESRI image layer of land cover-types in Washington to determine which are preferred for lynx versus coyote. I counted the points and summarized them with a bar graph to depict land cover-types preferred by each species.

Results:

Out of 428 scat samples sent to OSU, 276 were identified as Canada lynx, 97 as coyote, 15 as bobcat, 13 as undetermined Canis species, 9 as amplification failures, 7 as wolf, 6 as marten, 3 as non-carnivore species, and 2 as mountain lion. Lynx and coyote represented the majority, 87%, of the samples. Of these, 325 were visually identified to be lynx, 60 fox, 20 wolverine, 15 mountain lion, 5 fisher, 2 wolf, and 1 was thought to be coyote. DNA results revealed that about 64% of the samples were lynx and about 23% of the samples were coyote. Out of the 325 predicted to be lynx, 239 of the guesses were correct, making lynx visual identification accuracy about 74%. Approximately 11% of the confirmed lynx scat were predicted to be fox and the other 3% were predicted to be either mountain lion, wolverine, or fisher. On the other hand, none of the confirmed coyote scats had an initial guess of coyote. The coyote was not a targeted carnivore so presumed coyote scats were not intended for lab analysis. Of the 97 confirmed coyote scats, 61% were predicted lynx, 10% mountain lion, 15% fox, 12% wolverine, and 2% wolf. This suggests that coyote scat looks similar to most other carnivore scat, while lynx scat is more distinctive.

Diet:

The 19 prey species found in lynx scat included snowshoe hare, multiple species of squirrel, vole, and birds (Figure 3, Table 1). Only about 1.5 percent of the total scats contained deer, coyote, marmot, pika, or frog. The majority (about 78%) of lynx scat contained snowshoe hare. The least prevalent prey species, making up less than 1% of the lynx diet, were found in only 1-2 of the total scats (n=276). The 24 prey species found in coyote scat included snowshoe hare, multiple species of squirrel, mice, chipmunk, bird, voles, deer, and fish (Figure 4, Table 2). Over 70% of the prey in the coyote diet consists of 3 species: snowshoe hare, Columbian ground squirrel (*Urocitellus columbianus*), and mule deer (*Odocoileus hemionus*). There was a significant difference between the prey consumed by the lynx and the coyote ($\chi^2 = 215.6$, df = 29, $p < 0.001$).

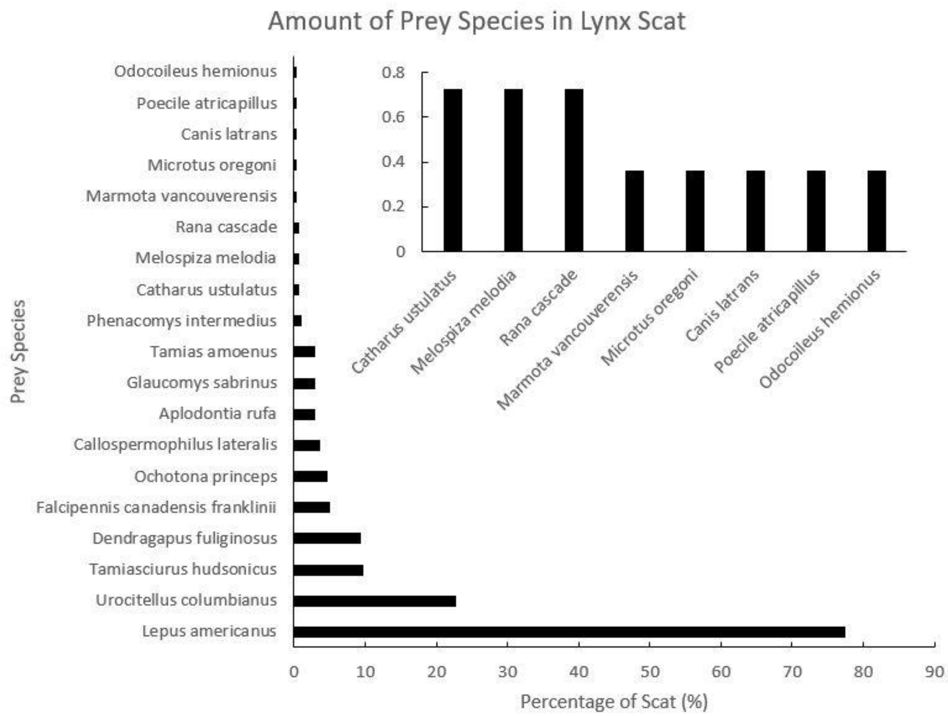

Figure 3: Prey species identified by percentage in Canada lynx scat (n=276). The prey species that ranked under 1% are shown in a separate bar graph to more clearly read their percentages. Totals exceed 100% as some scats contain multiple prey.

Table 1: Listed scientific names and common names of lynx prey species.

Prey Species	
Scientific Name	Common Name
Aplodontia rufa	Mountain beaver
Callospermophilus lateralis	Golden-mantled ground squirrel
Canis latrans	Coyote
Catharus ustulatus	Swainson's thrush
Dendragapus fuliginosus	Sooty grouse
Falcipennis canadensis franklinii	Franklin's grouse
Glaucomys sabrinus	Northern flying squirrel
Lepus americanus	Snowshoe hare
Marmota vancouverensis	Hoary marmot
Melospiza melodia	Song sparrow
Microtus oregoni	Creeping vole
Ochotona princeps	American pika
Odocoileus hemionus	Mule deer
Phenacomys intermedius	Western heather vole

Poecile atricapillus	Black-capped chickadee
Rana cascade	Cascades frog
Tamias amoenus	Yellow-pine chipmunk
Tamiasciurus hudsonicus	American red squirrel
Urocitellus columbianus	Columbian ground squirrel

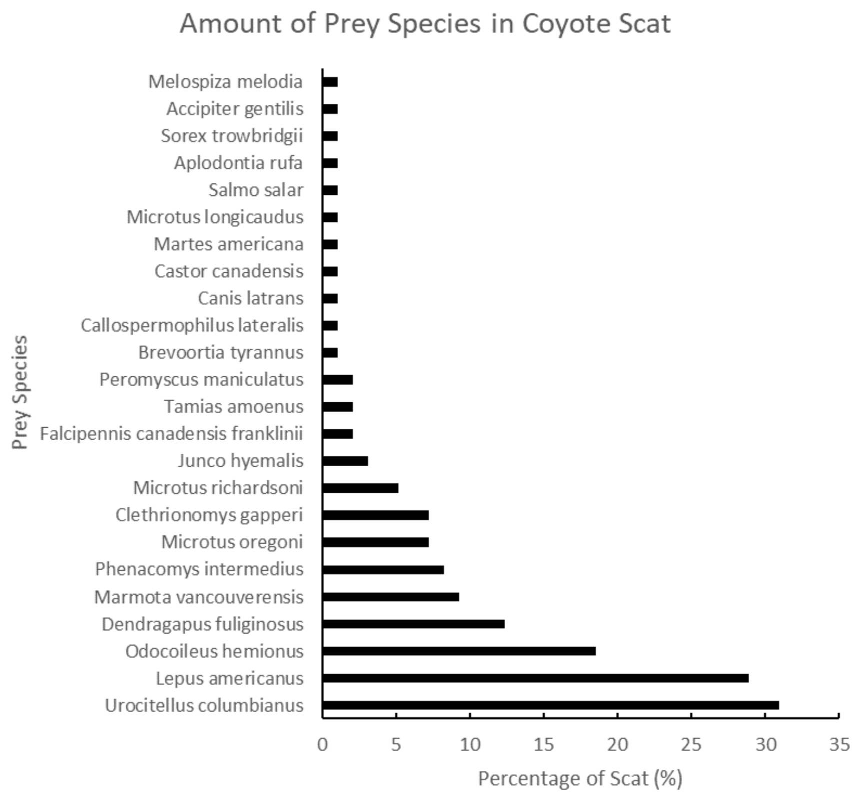

Figure 4: Prey species identified by percentage in coyote scat (n=97). Totals exceed 100% as some scats contain multiple prey.

Table 2: Listed scientific names and common names of coyote prey species.

Prey Species	
Scientific Name	Common Name
Accipiter gentilis	Northern goshawk
Aplodontia rufa	Mountain beaver
Brevoortia tyrannus	Atlantic menhaden
Callospermophilus lateralis	Golden-mantled ground squirrel
Canis latrans	Coyote
Castor canadensis	American beaver
Clethrionomys gapperi	Southern red-backed vole

Dendragapus fuliginosus	Sooty grouse
Falcipennis canadensis franklinii	Franklin's grouse
Junco hyemalis	Dark-eyed junco
Lepus americanus	Snowshoe hare
Marmota vancouverensis	Hoary marmot
Martes americana	American marten
Melospiza melodia	Song sparrow
Microtus longicaudus	Long-tailed vole
Microtus oregoni	Creeping vole
Microtus richardsoni	Water vole
Odocoileus hemionus	Mule deer
Peromyscus maniculatus	Deer mouse
Phenacomys intermedius	Western heather vole
Salmo salar	Atlantic salmon
Sorex trowbridgii	Trowbridge's shrew
Tamias amoenus	Yellow-pine chipmunk
Urocitellus columbianus	Columbian ground squirrel

About 63% of both coyote and lynx scats contained 1 prey species and about 1% of both predators' scat contained 5 prey species. The number of prey in each scat sample is similar between the species, with only slight differences in scats with 0, 2, 3, or 4 prey species (Figure 5). The lynx contained a higher percentage of scats with 2 or 3 prey while the coyote contained a higher percentage of scats with 0 or 4 prey.

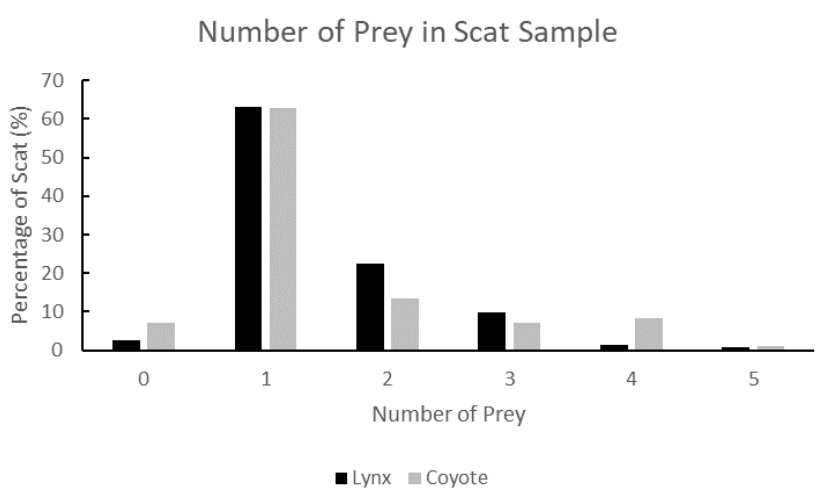

Figure 5: Comparison of number of prey species by percentage in both lynx (n=276) and coyote scat (n=97).

While lynx and coyote have a similar number of prey species types found in their single-prey scats and snowshoe hare are found in the majority of these scats, the quantities of the prey are different ($\chi^2 = 82.14$, df = 15, $p < 0.001$). In lynx single-prey scats, 79% contained snowshoe hare, 10% contained Columbian ground squirrel, and the remaining 11% contained 9 other species at low quantities (Figure 6). Coyote single-prey scats, however, are more varied. In coyote single-prey scats, 33% contained snowshoe hare, 26% contained Columbian ground squirrel, 15% contained mule deer, 8% contained hoary marmot (*Marmota vancouverensis*), and the remaining 16% contained other species that were found in 1-3 scats (Figure 6).

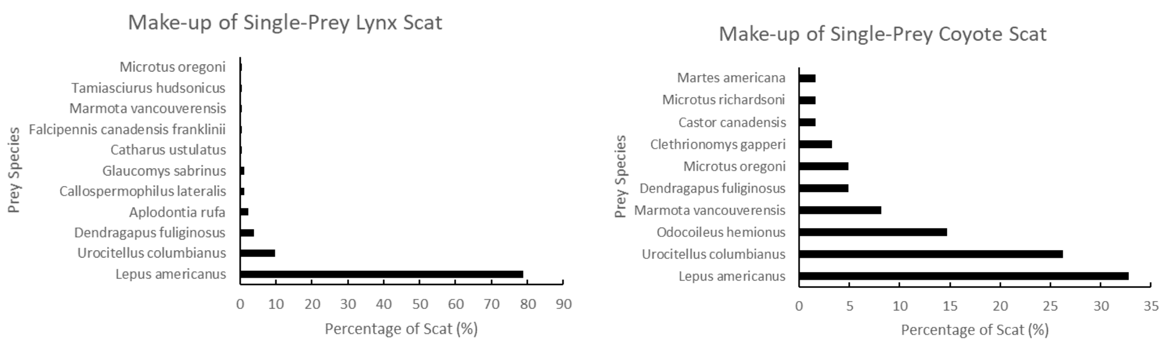

Figure 6: Comparison of prey species break-down in single-prey scats for lynx (n=174) and coyote (n=60).

Distribution:

Lynx scat was collected over a range of 4,348 ft to 7,206 ft with a mean value of 5,667 ft. Lower elevation lynx scats were found mostly on the higher longitude trails while the highest elevation lynx scats were found on medium to high longitude trails (Figure 7). Coyote scat was collected over a range of 2,757 ft to 8,029 ft with a mean value of 5,880 ft. There does not appear to be any pattern explaining the elevation of the scats by the longitude (Figure 8). Most of the lynx and coyote scat were collected at elevations between 4000 and 7000 feet (Figure 9). The variability in elevation for coyote scat is about 2.9 times larger than in elevation of lynx scat ($p < 0.001$). There is not a significant difference between mean elevations, but the lynx mean elevation is lower than the coyote mean elevation ($t = -1.75$, df = 119.69, $p = 0.08$).

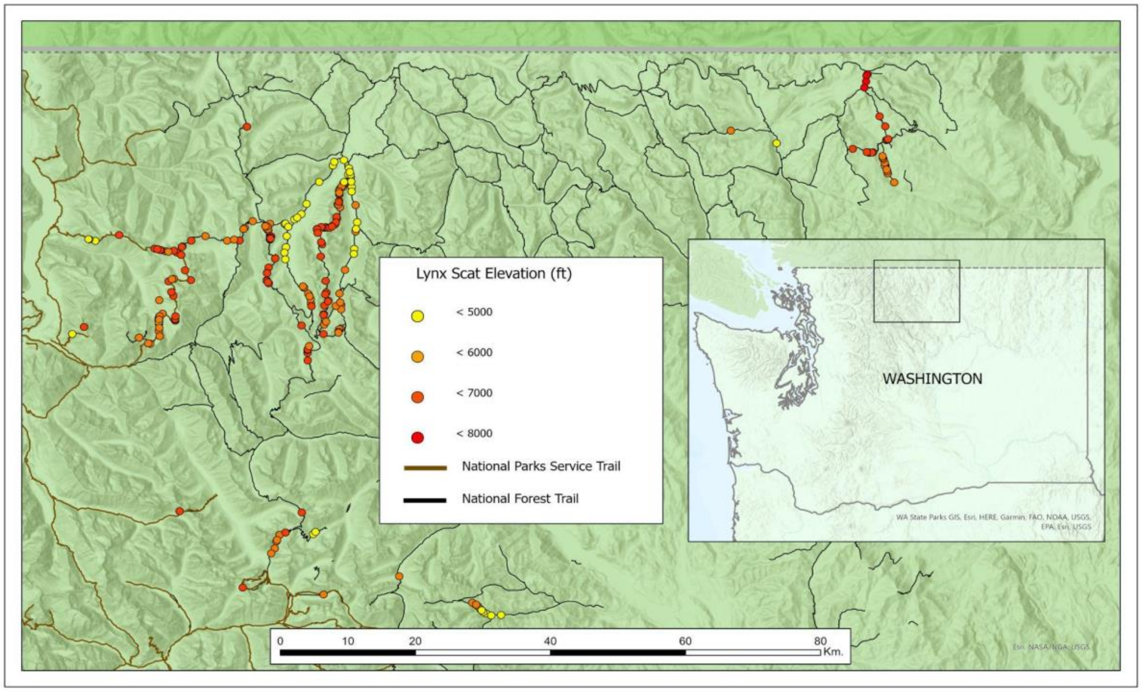

Figure 7: Elevation of lynx scat collection sites organized into 4 classes: 4001-5000, 5001-6000, 6001-7000, 7001-8000 (n=276).

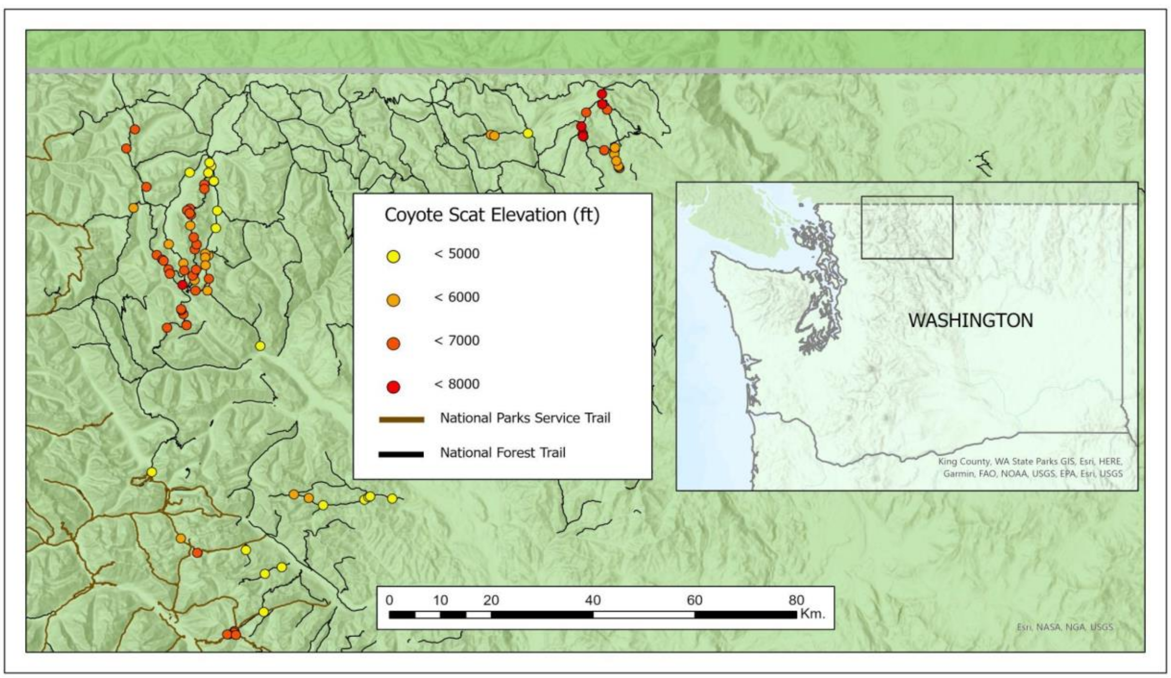

Figure 8: Elevation of coyote scat collection sites organized into 4 classes: 2001-5000, 5001-6000, 6001-7000, 7001-8000 (n=97).

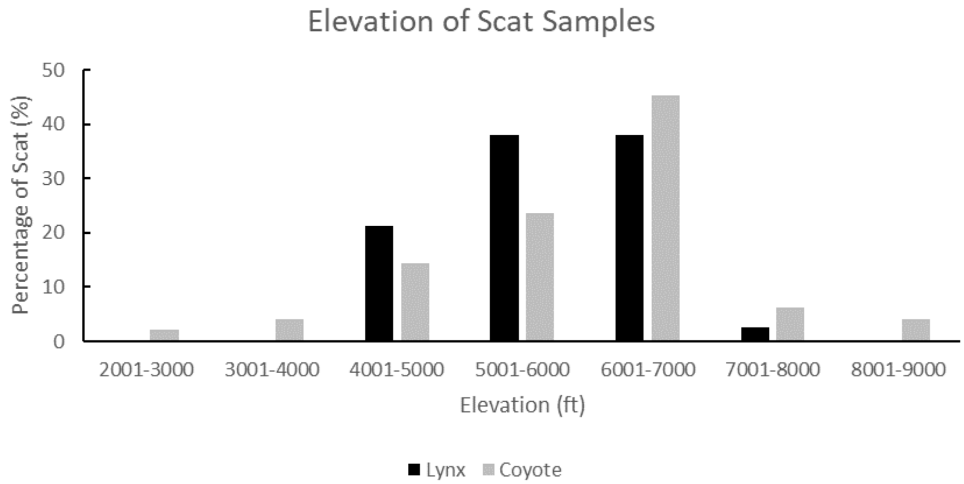

Figure 9: Comparison of percentage of lynx (n=276) and coyote scats (n= 97) found in each elevation class.

In tree covered locations, lynx scat were found 25% more often than coyote scat and in shrubs, lynx scat were found 50% less often than coyote scat (Figure 10). Both lynx and coyote scat were discovered at a higher proportion in tree-cover, but both species utilize tree and shrub-cover. Neither lynx nor coyote scat were found in other land types: water, grass, flooded vegetation, crops, built area, or snow/ice (Figure 11). There was a significant difference in the use of land-cover types by coyote and lynx ($\chi^2 = 14.6$, $p < 0.001$). Lynx heavily prefer tree-cover while coyotes use trees and shrubs more evenly.

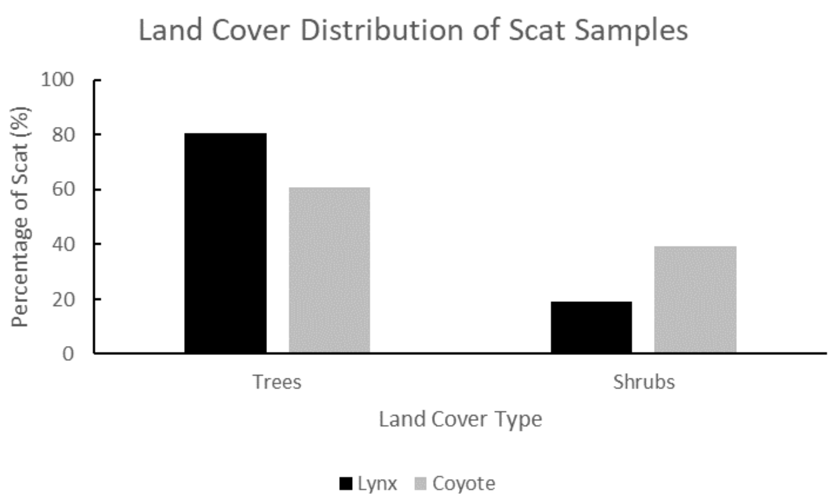

Figure 10: Comparison of percentage of scat samples by land cover-type.

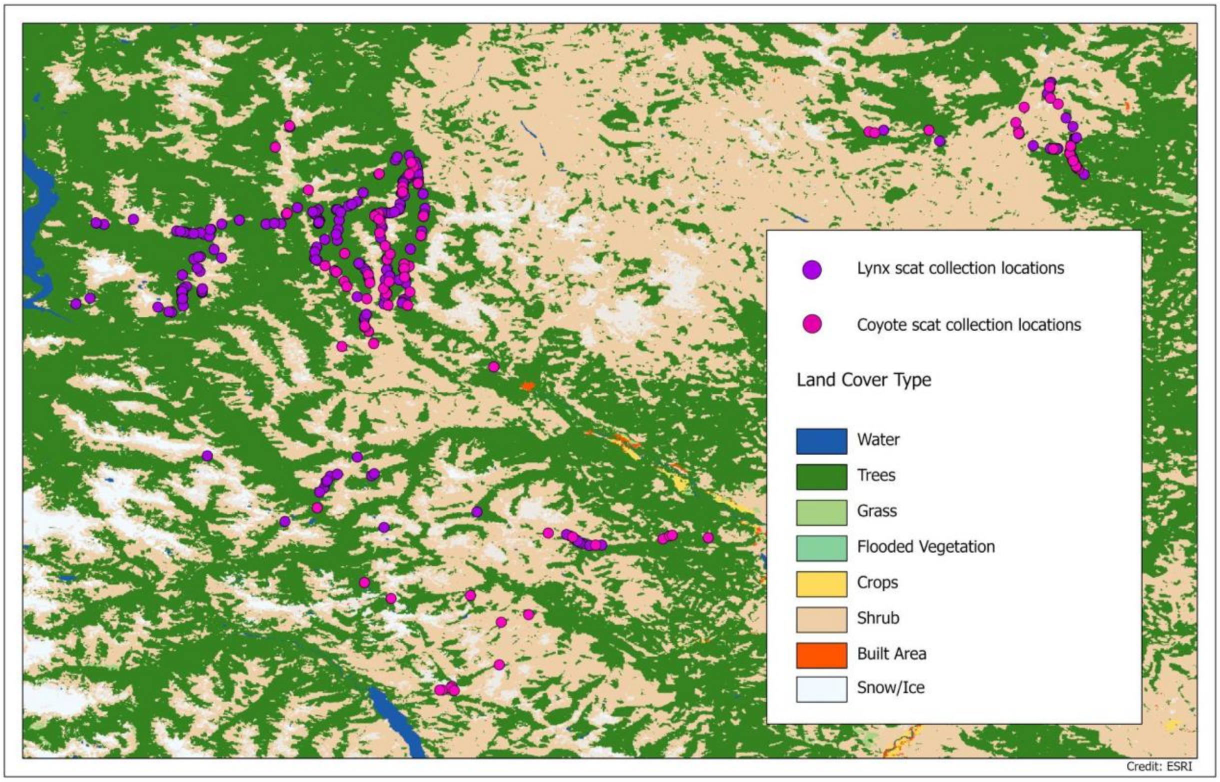

Figure 11: Comparison of lynx scat samples (purple) and coyote scat samples (pink) by land cover-type in collection location.

Discussion:

There were a number of issues in the field and lab settings that may have affected the results. The use of parafilm seals proved insufficient in stopping spillage in the field where vials were carried non-vertically. In some cases, ethanol leakage caused the removal of labels and loss of sample IDs which led to difficulty in identification. Once this problem was recognized, a new protocol was established; dry vials were utilized during scat collection and ethanol and parafilm were added once out of the field. Even after the QA/QC process, the DNA lab results contained 5 prey species that were inconsistent with the study area. 3 of these were the bank vole (*Myodes glareolus*) found in Europe, the biafran palm squirrel (*Epixerus wilsoni*) found in Congo and Cameroon, and the wrentit (*Chamaea fasciata*) found primarily in California. These prey species were therefore removed from the dataset as data entry or laboratory errors. The other 2 species, Atlantic menhaden (*Brevoortia tyrannus*) and Atlantic salmon (*Salmo salar*), are fish species that naturally occur on the Atlantic coast. They were not removed from the dataset as they were most likely introduced into Washington streams by humans.

Diet:

The data maintain that Canada lynx are specialists and coyotes are generalists in diet. There is a greater range of species found in coyote scat than lynx scat. In addition, the bar graphs reveal a much larger proportion of the lynx diet contains snowshoe hare compared to the coyote diet where 80% of the scat is split between 3 prey species. The deer and coyote each found in 1 lynx scat are unusual and were likely the result of a scavenging act. Although lynx typically prefer fresh kills, eating carrion may have been the only option available at the time. Both lynx scat samples containing coyote and deer were collected in August. The samples were either missed during earlier scans of the trails or the lynx consumed carrion during the summer which is unusual. There are 14 species that were found in both lynx and coyote scat and snowshoe hare and Columbian ground squirrel were the top two prey species consumed for both. It is clear that lynx are heavily reliant on snowshoe hare for survival and even though only 29% of the coyote scat held snowshoe hare, this represents a significant competition for prey. A larger sample size of coyote scat may also reveal that snowshoe hare are even more prevalent in their diet than this study shows.

The majority of both lynx and coyote scat contained a single prey species which may indicate that coyote and lynx select prey that provide high nutritional value over other species. Of the singular prey scats, the majority prey type was snowshoe hare. This is likely because the snowshoe hare has a large average mass of 1.49 kg. Snowshoe hares provide enough nutrients that the lynx does not need to hunt for more prey. Coyotes are obvious generalists in their choice for a single prey as they eat a variety of prey species in greater quantities. After snowshoe hare, they also consume mule deer, Columbian ground squirrel, and hoary marmot at high proportions. While mule deer (average mass of 120 kg) and hoary marmot (average mass of 9.05 kg) provide a good source of food, Columbian ground squirrels only have an average mass of 0.58 kg. The Columbian ground squirrel does not seem to provide enough food to sustain a coyote for long but this could be explained by potential scat degradation that didn’t retain other prey species DNA.

Distribution:

The data show coyotes venturing into higher elevations than lynx. Coyote scat was found at a maximum elevation of 8,029 ft and lynx scat was found at a maximum elevation of 7,206 ft. The mean elevation of lynx scat was 5,667 ft while the mean elevation of coyote scat was higher at 5,880. Even though the sample size of coyote scat is smaller than the lynx scat, there is a larger range and variability in elevations where coyote scat was found. The data suggest that both species prefer the elevation range of 4000-7000 feet as this is where the majority of scats were collected. The lynx range is likely defined by the elevation range of their hunting grounds of subalpine forests which are detected at about 5,400 ft to 7,800 ft. Many studies show that coyotes have expanded their range as a result of road building, wolf extirpation, and snowpack reduction so this could explain the large variety of elevations where coyote scat was found (Dowd et al., 2014; Arjo and Pletscher, 2004). The overlap of coyote and lynx scats implies an overlap of hunting and living territory which may lead to competition, negatively impacting the endangered lynx.

The cover-type data suggest that lynx utilize tree-cover more than shrub-cover while coyotes use tree-cover and shrub-cover more evenly with a slight preference for tree-cover. This indicates that lynx have a clear land-type preference while coyotes are more adaptive. Studies show that lynx stalk their prey while hunting instead of engaging in chasing and the data suggest that more hunting may occur under tree-cover (Lavoie et al., 2019). Coyotes may be more likely to use both tree-cover and shrub-cover because they primarily chase small prey which could lead them into many environments. The tree-cover land-type appears to be an important habitat for both species.

Conclusion:

This study corroborates much existing Canada lynx research pertaining to specialized diet and distribution. DNA results confirmed that even during the summer months when more prey species are available, Canada lynx select snowshoe hare 78% of the time. Additionally, Canada lynx scat were found primarily under tree-cover in a limited elevation range of 4000-8000 feet. Because lynx are so prey specific and reside within a limited range and land-type, the species is at high risk for displacement and habitat loss. Early snowmelt, wildfires, and human disturbances not only destroy lynx habitat but also allow sympatric carnivores such as coyotes to compete for limited prey. More studies are required to establish a baseline of lynx activity in North Cascades for future climate studies and to ascertain how changing temperatures, precipitation, and snowpack will impact the imperiled species. Further, more long-term targeted studies investigating the impact of coyotes in lynx habitat should be conducted. While this study begins to analyze the competition between lynx and coyote, it is limited because any coyote scat collection was accidental. In fact, scats thought to be coyote in the field were disregarded. If coyote scats were purposefully collected, a much larger sample size could be reviewed providing a more complete picture of coyotes in the North Cascades ecosystem.

In conjunction with the Confederated Tribes of the Colville Reservation, biologists plan to introduce 50 GPS-collared Canada lynx from British Columbia into the Kettle River Range region over the next five years to help restore the Washington population (Flatt, 2021). Thus far, 5 have been released. This project provides one point of reference in the effort to restore the population in Washington state. Collaring lynx will yield greater information on the movements and more opportunities to use DNA scat analysis to look at the diet of this elusive species in relation to the foraging landscape and their competitors.

Acknowledgements:

I am indebted to Dr. Jocelyn Akins for providing access to the Cascades Carnivore Project data from 2020.

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Appendix A: Scat collection trails

Trail Name	**Distance (miles)**	**Elevation Gain (ft)**	**High Point (ft)**	**Visits**
Buckskin Ridge	**22**	**n/a**	**7580**	**5**
Cascade Pass Horshoe Basin West	**18.5**	**1550**	**7200**	**2**
Cedar Creek	**16.1**	**3809**	**6500**	**2**
Copper Pass - Stiletto Spur	**14**	**2710**	**6720**	**3**
Cutthroat Pass	**10**	**2000**	**6800**	**5**

Easy Pass - Fisher Camp	**12.2**	**2800**	**6500**	**4**
Hidden Lakes Peak	**8**	**3300**	**6900**	**1**
Horshoe Basin East	**12**	**1500**	**7200**	**2**
Jack Mountain	**14.6**	**4858**	**9066**	**2**
Jackita Ridge	**36.4**	**3100**	**7000**	**2**
Libby Lake	**10.2**	**3210**	**7640**	**1**
Louis Lake	**9.8**	**2240**	**5360**	**3**
Maple Loop	**7.2**	**2000**	**6650**	**4**
McAlester South Pass	**17.5**	**3000**	**n/a**	**4**
PCT Harts North	**14.4**	**n/a**	**6900**	**4**
PCT Harts South	**18.6**	**1200**	**7386**	**2**
Rainbow Lake	**11.5**	**n/a**	**n/a**	**2**
Reynolds Creek	**13.2**	**n/a**	**n/a**	**3**
Robinson Pass Loop	**7.9**	**2500**	**6942**	**4**
Stiletto Peak	**14.4**	**3650**	**7660**	**3**
Twisp Pass to Stiletto Lake	**17**	**4091**	**6810**	**3**

War / Purple Pass	**18**	**3840**	**6840**	**4**
Wolf Creek	**21**	**4270**	**5738**	**5**

Appendix B: scat identification

Cascade red fox (Vulpes vulpes cascadensis) scats are generally segmented and have tapered ends. It usually contains hair, berries, small bone fragments, or insect legs. Typically about ¼-¾ inches in diameter and 1.5-4 inches in length, fox scat is smaller than coyote scat.

Coyote (Canis latrans) scat is segmented and similar in shape to fox scat, but larger in diameter, length, and amount. It is quite variable and can be ½-¼ inches in diameter and 4-13 inches in length. Coyote scat often contains a lot of hair and bones, sometimes small mammal skulls.

Pacific marten (Martes caurina) scats are segmented with twisty ends. At ⅛-½ inches in diameter and 1.5-3 inches in length, it is smaller than fox scat. Marten scat is also typically darker in color and contains hair, bone fragments, leaves, and grass.

Fisher (Pekania pennanti) scat is tubular and segmented with typically twisty ends. It is about ½ inch in diameter and 3-5 inches in length. Porcupine quills are often found in fisher scat.

Wolverine (Gulo Gulo) scats are tubular and often have twisty ends. It usually contains a large amount of hair. Wolverine scat is variable and difficult to distinguish from other carnivore scat without contextual information like animal tracks. It is ½-¾ inches in diameter and 4-6 inches in length.

Gray wolf (Canis lupus) scat is very large at 1.25-2.5 inches in diameter and 3-17 inches in length. It generally contains hair and many large bone fragments including small mammal skulls.

Canada lynx (Lynx canadensis) scats are generally segmented with blocky or blunt ends and are indistinguishable from bobcat scat. The segments often look slightly rounded and the scat tends to have less hair and bones than coyote scats of the same size. Lynx and bobcat scat is ½-1 inch in diameter and 2-10 inches in length.