Seasonal Diet Composition of Gray Wolves (*Canis lupus*) in Northeastern Minnesota Determined by Scat Analysis

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Yvette Chenaux-Ibrahim

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Dedication

This thesis is dedicated to Tamer Sayed Ibrahim, my wonderful husband, whose endless support, love, and encouragement allowed me to complete this project. Without his dedication, which involved driving countless hours so we could be together while I was in school, listening to presentations, and providing unending encouragement, this project would not have been possible.

Abstract

I determined seasonal diet composition of gray wolves (*Canis lupus*) in northeastern Minnesota from 2011 to 2013. Average occurrence of prey items was identified in 1,000 scats collected in the Grand Portage Indian Reservation, Voyageurs National Park area, and the 1854 Ceded Territory (greater northeastern Minnesota). Deer *(Odocoileus virginianus*), moose (*Alces alces*), and beaver (*Castor canadensis*) composed the majority of wolf diet, with moose the primary prey in Grand Portage and deer the primary prey in the Ceded Territory and Voyageurs National Park. Beaver were important in spring and summer in Grand Portage and Voyageurs National Park. I performed a sensitivity analysis of expected densities of deer, moose, and beaver to calculate prey preference and determined that at most prey densities, moose were preferred and deer avoided in Grand Portage and the Ceded Territory and beaver were preferred in Voyageurs National Park. Small mammals, black bear (*Ursus americanus*), snowshoe hare (*Lepus americanus*), and canids composed a minor portion of wolf diet. Calves were important prey in spring in the Ceded Territory and fawns were important prey in spring and summer in Grand Portage and in summer in Voyageurs National Park. I estimated that wolves consumed about 30% of calves born each year in Grand Portage. I performed a sensitivity analysis to test how selecting 3, 6, 12, and 25 hairs per scat affects accuracy in determining diet composition. Prey items were occasionally missed when selecting fewer hairs, thus I recommend selecting 12 hairs per scat when using the point-frame method to determine wolf diet.

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Chapter 1: Seasonal Diet Composition of Gray Wolves (*Canis lupus*) in Northeastern Minnesota Determined by Scat Analysis

Introduction

Determining the seasonal composition and proportion of prey species in the diet of gray wolves (*Canis lupus*) in northeastern Minnesota is an important component of our understanding of wolf impact on prey species. Wolves were extirpated across much of their worldwide range due to hunting, trapping, and poisoning (Mech 1995). In the United States, wolves were listed as a federally endangered species in 1973 and relisted as threatened in Minnesota in 1978 (Ruid et al. 2009). The wolf population in Minnesota increased from an estimated low of 350 wolves (31,080 km^2 range) in 1963 (Cahalane 1964) to about 2,900 wolves (71,514 km^2 range) in 2008 (Erb 2008). The Minnesota wolf population was considered stable from 1997 to 2008 and decreased from 2008 to 2013 (Erb 2008, Erb and Sampson 2013). However, wolf numbers increased to the highest levels in forty years in at least one area in northeastern Minnesota (Mech and Fieberg 2014). The northeastern Minnesota moose (*Alces alces*) population is declining, and the increasing wolf population in some areas may be partly contributing to the decline (Mech and Fieberg 2014), because adult moose and calves are eaten by wolves.

Wolves and ungulates have lived together for centuries without extirpation of prey (Gasaway et al. 1983). However, wolves can significantly impact prey populations that are already compromised due to other factors. Many factors could be contributing to the declining moose population, including adult moose health, habitat quality, climate, parasites, and predation (Lenarz et al. 2009). Past wolf diet studies in northeastern Minnesota have shown that wolves consume primarily white-tailed deer (*Odocoileus virginianus*), moose, and beaver (*Castor canadensis*) (Van Ballenberghe et al. 1975, Fuller 1989, Gogan et al. 2004). However, as the moose population is declining and with

climate change affecting prey populations, wolf diet composition may be changing. In this study, we evaluated wolf diets in northeastern Minnesota and estimated numbers of prey consumed by wolves each year in the Grand Indian Portage Reservation.

Gray wolves prey primarily on ungulates but may consume animals that range in size from 1 to 1,000 kg (Mech and Boitani 2003). Deer fawns were an important food source for wolves in summer in northeastern Minnesota (Van Ballenberghe et al. 1975). Moose calves may also be an important prey in summer because calves are easier to catch than adults (Peterson 1977, Smith et al. 2004).

Scat analysis is an effective, non-invasive, and commonly used method to identify prey items in a carnivore diet (Weaver 1993; Trites and Joy 2005). Prey items in wolf scats are identified by analyzing macroscopic features of hair and cuticular scale patterns. Prey hairs that can be identified in wolf scats include moose, moose calf, white-tailed deer, deer fawn, beaver, snowshoe hare (*Lepus americanus*), various small mammals, black bear (*Ursus americanus*), wolves, and coyotes (*Canis latrans*) (Adorjan and Kolenosky 1969). Adult ungulate hairs can be differentiated from young hairs from birth until late summer (Pimlott et al. 1969, Voigt et al. 1976, Fritts and Mech 1981, Peterson et al. 1984, Gauthier and Theberge 2010). After young ungulates molt in early fall, the hairs are no longer distinguishable from hairs of adults.

The cultural and ecological importance of wolves and their prey needs to be considered for best management of wolf and prey populations. Wolves (ma'iingan in Ojibwe) are valued for the important role they play in ecosystem sustainability. Wolves prey on white-tailed deer (waawaashkeshi) and moose (mooz), which are species of important cultural and subsistence value to the Anishinaabeg of the Grand Portage Band

of Lake Superior Chippewa. Wolves and moose are also culturally and economically important to citizens of Minnesota. Managing both wolf and moose populations within the Grand Portage Reservation and the 1854 Ceded Territory, where the Grand Portage Band has off-reservation treaty rights in northeastern Minnesota, is important for the health and wellness of the Anishinaabeg and the ecosystem they value. Similarly, moose, wolves, and other natural resources are managed by the state of Minnesota for the benefit of all.

Our objective in this study was to use scat collection and analysis to determine seasonal composition of wolf diets in three areas of varying prey densities in northeastern Minnesota. We predicted that: 1) deer and moose will be the primary prey species, but importance of moose and deer will be affected by densities of these species and beaver; 2) diet composition will change among years within each study area; 3) diet composition will vary seasonally, with prey switching occurring between winter and summer; and 4) beaver, deer fawns, and moose calves will compose a major proportion of diets in spring and summer.

Study Area

The study area encompasses the northeastern region of Minnesota (Fig. 1.1), including federal, tribal, state, county, and private land. The three scat collection areas within the study area were the 1854 Ceded Territory, Grand Portage Indian Reservation, and Voyageurs National Park area (Fig. 1.1). Cook, Lake, St. Louis, and part of Koochiching counties are included in the study area. Vegetation is typical of the southern boreal forest, with upland forests dominated by quaking aspen (*Populus*

tremuloides), paper birch (*Betula papyrifera*), balsam fir (*Abies balsamea*), white spruce (*Picea glauca*), and jack pine (*Pinus banksiana*) (Faber-Langendoen et al. 2007).

1854 Ceded Territory

The Grand Portage Band retains off-reservation treaty rights within the 1854 Ceded Territory, an area of nearly 20,234 km^2 covering much of northeastern Minnesota (Fig. 1.1.). Physical features of northeastern Minnesota have been described (Heinselman 1999, Lenarz et al. 2009). Briefly, the landscape is marked with signs of glacial activity, including high ridges, deep ravines, and numerous streams, rivers, lakes, and bogs. Elevation rises from the shoreline along Lake Superior to 700 m above sea level. The winters are long and cold and summers are warm and short. Lake Superior affects temperatures seasonally with inland areas being colder during winter and warmer in the summer than the shoreline. There is moderate precipitation (annual average of 69 cm), and snow depth can reach 127 cm. Most inland lakes and streams are covered with ice from November until mid-April to May. The forest type varies from boreal mix to hardwood.

Average wolf density in Minnesota is 3.1 wolves/100 km^2, with an average pack size of 4.3 wolves (Erb and Sampson 2013). Deer densities range from 0.4 to 7/km^2 (Minnesota Department of Natural Resources [MNDNR] 2011). In winter deer will aggregate along the Lake Superior shoreline, where densities may reach 50/km^2 (Lankester and Peterson 1996). The Minnesota moose population was estimated at 8,160 in 2005 and 3,450 in 2015 (DelGiudice 2015), which is a 58% decrease.

Grand Portage Reservation

The Grand Portage Indian Reservation is located in the extreme northeastern tip of Minnesota in Cook County (Fig. 1.2). The Reservation is bordered on the north by Ontario and on the west by federal, state, and private lands. Lake Superior borders the eastern and southern boundaries of the Reservation, which provides 38 km of rocky, irregular shoreline. The Reservation encompasses approximately 192 km^2 of land, and glacial activity in the area has produced a mixture of steep ridges and valleys, with an elevation that ranges from 183 to 553 m. There are 68 km of year-round streams and 89 km of intermittent streams. Seventeen inland lakes cover 3.3 km^2 within the Reservation boundaries, and there are 29 km^2 of wetlands.

Moose are present across the entire reservation (0.27/km^2), but their core range is inland away from Lake Superior (Grand Portage, unpublished data). White tailed deer are present across the entire reservation, but they congregate near the shore in winter. Beavers are common throughout Grand Portage, with colony density estimated at 0.30 colonies/km^2 (Smith and Peterson 1988). Black bears are common and prey on moose calves and deer fawns in spring (Grand Portage, unpublished data). There are at least three wolf packs, with an average of four wolves per pack, and estimated wolf density is 3-4 wolves/100 km^2 (Isaac et al. 2013).

Voyageurs National Park

Voyageurs National Park is an 883-km^2 protected area along the U.S.-Canada border in Minnesota. Maximum topographic relief in the park is only 90 m, with slopes ranging from flat glaciolacustrine plains to steep cliff faces; therefore, water bodies are prominent throughout the park, including 5 large lakes (>1,250 ha), 26 small lakes (12-

305 ha), and hundreds of natural and beaver-created wetlands (Kallemeyn et al. 2003, Johnston and Windels 2015).

Hunting and trapping have been prohibited in the park since its establishment in 1975. Moose are present on the Kabetogama Peninsula, a 305-km^2 roadless area in the center of the park, at low density (0.13/km^2), but are rare elsewhere in the park (Windels 2014). White-tailed deer are common throughout the park and pre-fawn densities likely exceed 4/km^2 (Voyageurs National Park, unpublished data). Beavers are also abundant throughout the park, with densities of active lodges exceeding 1/km^2 in many areas (Johnston and Windels 2015).

Abundant food resources and the absence of human-caused mortality (e.g., from trapping, poaching, vehicle accidents) within park boundaries have likely contributed to sustained high densities (2-5 wolves/100 km^2) of wolves in and immediately adjacent to the park since at least the late-1980s (Fox et al. 2001, Gogan et al. 2004, Olson and Windels 2015). The number of wolf packs that overlap at least part of Voyageurs National Park area has ranged from 6 to 9 during this same period. Black bears are also common throughout the park (~0.33/km^2) (D. Garshelis, MNDNR, unpublished data).

Methods

Scat Collection

Scats were collected along roads and snowmobile trails from April 2011 to March 2014. Collectors completed a standardized data sheet (Appendix 1). Data recorded included date, Universal Transverse Mercator (UTM) location in the Ceded Territory and Voyageurs National Park area and geographical description in Grand Portage, whether scats were fresh (e.g., strong smell, moist, tracks present, or on top of new snowfall) or

old (e.g., crumbly or white), and if known, date last time scat was collected on the road or trail.

Collection routes were located in areas of low, medium, and high moose density in the Ceded Territory (Fieberg and Lenarz 2012), low moose density in Voyageurs National Park, and high moose density in Grand Portage. Scats were collected opportunistically in Voyageurs National Park area and the Ceded Territory and systematically in Grand Portage. Grand Portage has an interconnected system of snowmobile trails (90 km/100 km^2) and roads (40 km/100 km^2) that allowed for intensive sampling of wolf scat in a concentrated area. Scats were collected in Grand Portage along designated routes at least once per month and at the end of each season. Because of the dense road network and small size of Grand Portage, 96% of scat collection locations were marked on a map and later entered into ArcMap (ArcGIS 10) instead of taking UTM locations with a GPS unit.

Wolf scats were collected on roads and trails and identified by shape and diameter. Scats were stored frozen in plastic bags until laboratory analysis. Scats $\geq$ 24 mm in diameter were used for analysis (Thompson 1952). A minimum diameter of 30 mm has also been recommended for identifying wolf scats (Weaver and Fritts 1979). I compared 386 scats $\leq$ 29 mm to 303 scats $\geq$ 30 mm to determine whether there was a difference in prey occurrence by scat diameter.

We collected scats throughout the year and determined season category by date collected and age of scat. Scats were assigned to three seasons relating to moose and deer parturition and snow cover: winter (October 1-May 10), spring (May 11-June 30), and summer (July 1-September 30). Moose calf hair was first identified in scats collected

on May 11, therefore, scats collected on or after May 11 through June 30 were assigned to the spring season. Mean date of birth of fawns is about May 26, and fawns move more with their mothers within two weeks (Carstensen et al. 2009). Scats collected on or after July 1 were assigned to the summer season when fawns were more likely to be traveling with their mothers and calves were more mobile. Older scats collected at the beginning of a season were assigned to the previous season.

Collecting enough scats each season is necessary to accurately estimate carnivore diet composition. Other factors such as size of study area and ability and time to collect scat will affect sampling effort and must be considered, so collecting 60 scats per season is a general guideline (Trites and Joy 2005). With fewer prey items, power analysis indicates that lower sample sizes could be used to quantify the proportion of wolf diet attributable to different prey types (Brent Patterson, pers. comm). In northeastern Minnesota the primary diet of wolves consists mostly of deer and moose (Van Ballenberghe et al. 1975, Fritts and Mech 1981) with beaver also an important prey (Van Ballenberghe et al. 1975). For this study, our goal was to collect a minimum of 60 scats each season from each study area. The goal was met except during summer when 22 and 28 scats were collected in the Ceded Territory and Voyageurs National Park area, respectively.

We determined adequacy of sample size by calculating the Brillouin Index (Brillouin 1956, Glen and Dickman 2006), using the equation:

$$H_i = \frac{\ln(N!) - \sum \ln n_i !}{N} \qquad \text{Eq. 1}$$

where H_i represents the diversity of wolf diet, N is the total number of prey items in all samples, and n_i is the number of prey items in the ith category. The cumulative diversity was calculated and resampled randomly for ten repetitions and then plotted against number of scats collected for each location and season.

Laboratory Procedure

Full scats were processed following a Standard Operating Procedure (Appendix 3) and data were entered into a standardized data sheet. Scats were transferred to nylon stockings and boiled for >30 minutes under a fume hood to kill parasites (Patterson et al. 1998, Chavez and Gese 2005, Klare et al. 2011). Scats were then washed in a dishwasher to remove digestible material until only bone and hair remained in the stocking. The scats were rewashed and wrung out by hand to remove remaining digestible material if needed. The undigested remains were air-dried in a fume hood for 24 hours and then weighed.

After washing and drying, scats were spread out on a plate. Prey species were identified from hairs. The point-frame method was used to select the hairs that would be identified in each scat (Chamrad and Box 1964, Ciucci et al. 2004). A grid the same size as the plate was pre-marked with 25 points and placed over each scat. One hair was randomly selected and pulled from each point and identified.

Hairs were classified into nine different prey categories, including moose, moose calf, white-tailed deer, deer fawn, beaver, snowshoe hare, small mammals, black bear, and canid, which could include wolf or coyote. I determined presence of young ungulates in scats during May through August and classified all ungulates after August to

species level only. Birds, vegetation, insects, and trash were recorded as present or absent.

Hair Identification

I examined all hairs with a dissecting microscope to compare color, shape, diameter, and length. I used a compound microscope to analyze the medulla, the innermost part of the hair, which can be observed using a compound microscope with the contrast positioned to the brightest light. The hairs of ungulates and snowshoe hares have distinctive medulla patterns, which allowed for initial classification of the hair as ungulate, snowshoe hair, or other. I identified all beaver hairs and most hairs of other species macroscopically. However, identification of hairs occasionally required additional analysis by examining scale patterns, especially when differentiating between moose and deer and identifying to age class (Appendix 2).

Hair scale patterns were extracted by taking negative impressions of hairs with Duco Cement® (Carrlee and Horelick 2010). A thin layer of Duco Cement® was spread on a microscope slide, and a hair was placed in the cement. After three minutes, the hair was pulled out and taped to the slide. Scale patterns were identified using collected hair samples from the region and a reference manual (Adorjan and Kolenosky 1969). Before performing hair identification in scats, I took a blind test using 100 known hair samples with each expected prey species present, including calves and fawns (Ciucci et al. 1996). Accuracy in identification was 95%.

I could not differentiate between coyote and wolf hair, so these hairs were classified as canid. Single or few canid hairs were found in many of the scats with few scats containing all or mostly canid hairs. The canid hairs were most likely from

grooming (James 1983, Muller 2006), but when a scat contained more than four canid hairs, the hairs were included in analysis.

Occurrence and Biomass of Prey Items

To determine the proportion of prey items in the diets of wolves, we calculated the average percent occurrence and percent frequency of occurrence of each prey item per collectable scat. Percent frequency of occurrence is an established method for determining proportions of wolf diet from scat analysis, but bias occurs because smaller prey and younger animals are overrepresented (Floyd et al. 1978, Weaver 1993, Ciucci et al. 1996). Estimating percent biomass reduces bias by accounting for prey species body mass (Van Ballenberghe et al. 1975, Fuller and Keith 1980, Peterson et al. 1984, Messier and Crete 1985, Ballard et al. 1987, Ciucci et al. 1996, Tremblay et al. 2001, Muller 2006, Reed et al. 2006). Equation 2 corrects for bias from prey weight for snowshoe hare to moose by converting average frequency of occurrence to percent biomass ingested (Floyd et al. 1978, Weaver 1993):

$$Y = 0.439 + 0.008 * X \qquad \text{Eq. 2}$$

Where Y represents the correction factor for prey consumed per scat (kg) and X is the estimated live weight of prey (Table 1.1). The correction factor was then multiplied by the frequency of occurrence of each corresponding prey in all scats.

Statistical Analysis

I performed a one-way ANOVA using JMP 10 software (SAS Institute Inc., Cary, NC, USA) at a significance level of $p = 0.05$ to test for statistical differences in diet by percent biomass consumed among the three study areas and among three seasons, within

each area and combining all years. I pooled scats among years due to small sample sizes (Table 1.2).

To determine whether wolves prefer deer, moose, or beaver, Manly's preference index was calculated (Muller 2006):

$$\alpha_i = \frac{r_i}{n_i} \frac{1}{\sum \frac{r_j}{n_j}} \quad \text{Eq. 3}$$

where α_i represents Manly's preference of prey type *i*, r_i, r_j is the proportion of prey type *i* or *j* in the diet by biomass, and n_i, n_j is the proportion of prey type *i* or *j* available in the environment by biomass. There is no preference if $\alpha_i = 1/m$ (m = # total number of prey types). Prey *i* is preferred if α_i is greater than $1/m$ and avoided if α_i is less than $1/m$. Preference was calculated for moose, deer, and beaver in each study area and season.

Actual densities of prey populations are not known, therefore, we performed a sensitivity analysis of expected densities of deer, moose, and beaver across northeastern Minnesota. I multiplied a range of prey densities by prey weight (Table 1.1) to determine a range of kg of prey/km^2 and then divided kg of prey/km^2 by total kg/km^2 to estimate proportions of availability (Table 1.3). Moose density estimates were based on moose population estimates from aerial surveys in Grand Portage and northeastern Minnesota (Grand Portage, unpublished data, DelGiudice 2015). Deer density estimates were based on the MNDNR estimated deer densities across northeastern Minnesota (Dexter 2012). Low and high beaver densities were estimated based on 0.30 colonies/km^2 in Grand Portage (Smith and Peterson 1988) and at least one beaver lodge/km^2 in Voyageurs

National Park (Johnston and Windels 2015) with six beavers per lodge (Jenkins and Busher 1979).

Number of Prey Consumed in Grand Portage

The Grand Portage Reservation wolf population estimate is 9 to 20 wolves (Isaac et al. 2013). We calculated number of prey consumed using a point estimate of 15 wolves in Grand Portage. A 35 kg wolf has an estimated minimum daily food requirement of 3.25 kg/day, or 0.09 kg/kg of wolf/day (Peterson and Ciucci 2003). The average weight of adult wolves in Grand Portage is 28.5 kg (Grand Portage, unpublished data), thus the estimated food requirement in Grand Portage is 2.6 kg/wolf/day. We calculated the number of prey type *i* consumed each season by wolves using the following equation (Kojola et al. 2004):

$$N_i = \frac{2.6WDB_i}{P_i} \qquad \text{Eq. 4}$$

where N_i is the number of prey type *i* consumed per season, *W* is the estimated wolf population, *D* is the number of days per season (parturition is 49 days, summer is 91 days, and winter is 226 days), B_i is the proportion of biomass consumed for prey type *i*, and P_i is the estimated live weight of prey type *i*. The number of prey type *i* consumed during each of the three seasons was summed to estimate the number consumed per year. The number of calves and fawns consumed were estimated only during spring and summer, when the hairs of calves and fawns could be identified in scats.

Results

I examined 1,000 scats, 524 from Grand Portage, 243 from the Ceded Territory, and 233 from Voyageurs National Park area. There was only one food item in 83% of

scats, two food items in 16.5% of scats, three food items in 0.4% of scats, and 1 scat contained four food items. Non-mammal food items including birds, grasshoppers, seeds, vegetation, and trash were occasionally found in scats and were identified as present or absent. There was no difference in prey composition between scats with a diameter ≤ 29 mm and scats with a diameter ≥ 30 mm ($p > 0.05$) (Fig. 1.3). The Brillouin model, H_i, reached an asymptote at 15-50 scats, indicating that sampling effort was adequate (Fig. 1.4).

The mean, minimum, and maximum distances between scat collection locations was 7, 0, and 17 km in Grand Portage, 38, 0, and 189 km in the Ceded Territory, and 17, 0, and 95 km in Voyageurs National Park area. The mean number of days between scat collection locations was 1.7, 2.4, and 2.5 days in Grand Portage, the Ceded Territory, and the Voyageurs National Park area, respectively. Scats were collected opportunistically in the Ceded Territory and Voyageurs National Park area, with less than 4% of scats collected within 1 km of another scat in Voyageurs National Park area and less than 2% of scats collected within 1 km of another scat in the Ceded Territory. The number of scats that were collected within 0 km of another scat on the same day was 4 scats in Grand Portage, 2 scats in the Ceded Territory, and 22 scats in Voyageurs National Park area. We collected all wolf scats on designated roads and trails in Grand Portage, thus some scats were collected on the same day within 1 km of another scat. The systematic collection allowed for a complete collection of scats on roads in a small study area.

In Grand Portage, the most important prey items in the wolf diet were moose (35-54% of biomass, including calves), deer (37-46% of biomass, including fawns), and beaver (6-16% of biomass) (Fig. 1.5, Table 1.4). Snowshoe hares, black bears, small

mammals, and canids comprised only 2-6% of diet by biomass. Consumption of adult deer and moose was higher in winter than in spring and summer when beavers, fawns, and calves were available (deer: $F_{2, 521} = 16$, $p < 0.0001$; moose: $F_{2, 521} = 8$, $p = 0.0002$).

In the Ceded Territory, the most important prey items were deer (44-60% of biomass, including fawns) and moose (32-42% of biomass, including calves) (Fig. 1.5, Table 1.4). Beavers, snowshoe hares, black bears, small mammals, and canids comprised 6-14% of diet by biomass. Deer were important prey in summer and winter, with consumption of deer lowest in spring ($F_{2, 240} = 15$, $p < 0.0001$). In spring, consumption of calves was 12 times higher by biomass ($F_{2, 240} = 23$, $p < 0.0001$). There was no difference in consumption of adult moose among the seasons.

In the Voyageurs National Park area, the most important prey items were deer (63-78% of biomass, including fawns), beaver (7-30% of biomass), and moose (3-13% of biomass, including calves) (Fig. 1.5, Table 1.4). Snowshoe hare, small mammals, and canids comprised a minor portion of diet at 2-3% of biomass. Consumption of fawns was 4.75 times higher by biomass in summer than spring ($F_{2, 230} = 22$, $p < 0.0001$). Consumption of adult deer was highest in winter and lowest in summer ($F_{2, 230} = 19$, $p < 0.0001$). Consumption of adult moose by biomass was not significantly different among the seasons.

Differences in Diet among Locations

Adult deer were more important prey on an annual basis in the Voyageurs National Park area and Ceded Territory than in Grand Portage ($F_{2, 997} = 16$, $p < 0.0001$). Adult moose were more important prey in Grand Portage than in the Ceded Territory and Voyageurs National Park area, and additionally, were more important in the Ceded

Territory than in Voyageurs National Park area ($F_{2, 997} = 17$, $p < 0.0001$). Fawns were more important prey in Grand Portage than in the Ceded Territory and Voyageurs National Park area ($F_{2, 997} = 6$, $p = 0.0037$), and calves were more important prey in the Ceded Territory than in Grand Portage and Voyageurs National Park area ($F_{2, 997} = 9$, $p = 0.0002$). Beaver were more important prey in Grand Portage and Voyageurs National Park area than in the Ceded Territory ($F_{2, 997} = 25$, $p < 0.0001$).

Prey Preference

Based on a sensitivity analysis with estimated densities of 2-80 deer/km^2, 0.05-0.50 moose/km^2, and 2-6 beavers/km^2, the proportion of available prey in the environment by biomass would range from 30-99% deer, 0.40-54% moose, and 0.70-41% beaver (Table 1.3). The proportion of each prey species consumed in wolf diet was fixed, with biomass of adult deer combined with fawns and biomass of adult moose combined with calves (Table 1.4). In Grand Portage, at low beaver density (2/km^2) moose would be avoided only at low deer density ($\leq$ 2/km^2) and medium to very high moose densities ($\geq$ 0.35/km^2) in spring (Fig. 1.6). Moose would be preferred across the range of moose and deer densities in summer and winter. With higher beaver density (6/km^2), moose would be preferred across the range of moose and deer densities in spring, summer, and winter.

In the Ceded Territory, at low beaver density (2/km^2) moose would be preferred across the range of moose and deer densities in spring and summer (Fig 1.6). In winter, moose would be avoided only at low deer density ($\leq$ 2/km^2) and very high moose density ($\geq$ 50/km^2). With higher beaver density (6/km^2), moose would be avoided across a greater range of moose densities in winter. In the Voyageurs National Park area, at low

beaver density (2/km^2), in spring moose would be preferred only when moose density was low (≤ 0.05/km^2) across the range of deer densities (Fig 1.6). In summer, moose would be avoided at the range of moose and deer densities. In winter, moose would be preferred when moose density was low to medium (≤ 0.35/km^2) at medium to very high deer densities (≥ 30/km^2) or when moose density was low (≤ 0.05/km^2) and deer density was low (≤ 2/km^2). With higher beaver density (6/km^2), moose would be preferred across greater ranges of prey densities in all seasons.

In Grand Portage, at low beaver density (2/km^2) deer would be preferred only at low deer density (≤ 2/km^2) and medium to high moose densities (≥ 0.20/km^2) in spring and winter and only at low deer density (≤ 2/km^2) and high moose densities (≥ 0.35/km^2) in summer (Fig. 1.7). With higher beaver density (6/km^2), deer would be preferred across greater ranges of moose densities in summer. In the Ceded Territory, deer would be preferred only at low deer density (≤ 2/km^2) and medium to high moose densities (≥ 0.20/km^2) across the range of beaver densities in all seasons. In the Voyageurs National Park area, deer would be preferred only at low deer densities (≤ 2/km^2) across the range of moose and beaver densities in all seasons.

In Grand Portage, beaver would be preferred at medium to high moose densities (≥ 0.20/km^2) across all deer densities in spring and summer and also when moose density was low (≤ 0.05/km^2) and deer density was low (≤ 2/km^2) in spring (Fig. 1.8). In winter, beaver would be preferred only at high deer densities (≥ 30/km^2) and high moose densities (≥ 0.35/km^2). In the Ceded Territory, beaver would be preferred only at high deer densities (≥ 30/km^2) and high moose densities (≥ 0.35/km^2) in spring and would be avoided across the range of prey densities in summer and winter. In the Voyageurs

National Park area, beaver would be preferred across the range of moose and deer densities in spring and summer. In winter, beaver would be preferred only at medium to high moose densities ($\geq 0.20/km^2$) and medium to high deer densities ($\geq 30/km^2$). With higher beaver density ($6/km^2$), beaver would be avoided at a broader range of prey densities in all areas, except in summer and winter in the Ceded Territory and in summer in the Voyageurs National Park area when beaver would be avoided and preferred at the same range of prey densities as with low beaver density.

Number of Prey Consumed in Grand Portage

With the estimated population of 15 wolves in Grand Portage, about 15 adult and sub-adult moose, 66 adult and sub-adult deer, and 79 beavers would be consumed each year based on diet composition from scats. An estimated 7 calves and 78 fawns would be consumed in spring and summer (Table 1.5). The average moose population in Grand Portage in winter 2011 to 2013 was 48 moose with 59% cows (Grand Portage, unpublished data). With a pregnancy rate of 83% (Grand Portage, unpublished data), there were likely at least 24 calves born each year. With 7 calves consumed in spring and summer, wolves would have consumed about 30% of calves born each year. With 15 adult and sub-adult moose, wolves would have consumed about 30% of the adult moose population. One wolf would consume about 4 deer, 1 moose, 5 beavers, 0.5 calves, and 5 fawns each year.

Discussion

Differences in Diet among Locations

The primary prey was deer, moose, and beaver, but relative importance of each species varied by study area. In Grand Portage, moose were the primary prey, deer were

secondary, and beaver were third. In the Ceded Territory, deer were the primary prey, with moose secondary and beaver not important as a prey species. In Voyageurs National Park area, deer was the primary prey, with beaver secondary and moose third. Availability of prey varies across northeastern Minnesota, and wolf diet composition changes across the landscape with prey availability. In past studies of wolf diets based on scat analysis in Minnesota and Canada, the primary prey during winter and summer was deer and moose, with moose the primary prey item in Ontario and deer the primary prey item in northwestern and central Minnesota and Voyageurs National Park area (Fig. 1.9). In summer, beaver was the third most important prey, and there was minor consumption of fawns and calves (Table 1.6). Snowshoe hare and other species composed a minor portion of wolf diets.

Differences in Diet among Years

There was variation in wolf diet among years, some of which was likely caused by differences in snow depth and timing of spring snow melt. In winters with greater snow depth, wolf predation on white-tailed deer is higher (Nelson and Mech 1986). During winter of 2012-2013, consumption of deer was higher and moose was lower in Grand Portage and consumption of deer was higher in Voyageur National Park, when compared to winters of other years. December 2012 through April 2013 had higher than normal average snowfall, and snow depths in March and April 2013 were greater than in 2011 and 2012 (NOAA 2010-2013). Also, during winter of 2011-2012, in Grand Portage consumption of deer was lower, which was likely attributable to less snowfall in December 2011 and January 2012 (NOAA 2010-2013). Furthermore, in spring 2013, consumption of adult deer was higher than in spring of other years in all three study

areas. The extended winter and greater amount of snow depth in April may have caused higher wolf predation of deer, even into spring.

Seasonal Differences in Diet

Moose and deer were the primary prey year-round but importance of prey types varied by season. Consumption of adult moose and deer was highest in winter in Grand Portage. Consumption of adult moose did not vary among seasons in the Ceded Territory or the Voyageurs National Park area. Consumption of adult deer was highest in summer and winter in the Ceded Territory and in winter in the Voyageurs National Park area. Beaver was important as a prey item in spring and summer in Grand Portage and the Voyageurs National Park area. Consumption of calves was higher in spring than in summer in the Ceded Territory. Consumption of fawns was higher in summer than in spring in the Voyageurs National Park area.

Importance of Calves, Fawns, and Beaver

Calves did not comprise a significant portion of wolf diet in Grand Portage or the Voyageurs National Park area but were important prey in spring in the Ceded Territory. Fawns were important prey in spring and summer in Grand Portage and in summer in the Voyageurs National Park area. Beaver comprised a significant portion of wolf diet in spring and summer in Grand Portage and the Voyageurs National Park area. Beaver was frequently found in scats collected in April through December. Four scats collected in February and March in Grand Portage and the Voyageurs National Park area contained beaver, but this occurred only in 2012 when there was an early snow melt.

Even though they did not comprise a major portion of wolf diet by biomass, a significant number of calves may be preyed upon by wolves. If there are high numbers

of wolves in some areas, they may impact the moose population through predation on calves in spring and summer. Wolves can effectively limit moose populations through predation on calves (Testa et al. 2000, Bertram and Vivion 2002). In Grand Portage with a population of 15 wolves, about 7 calves (30%) and 78 fawns would be consumed in spring and summer. If the wolf population were larger, they would potentially be preying on over 50% of calves born each year.

Prey Preference

Moose, including calves, were preferred at most prey density estimates in Grand Portage and the Ceded Territory and were avoided at a broader range of prey density estimates in Voyageurs National Park. Deer were avoided at most prey densities in all three study areas. Deer were usually preferred when deer density was low and moose density was medium to extremely high. With higher beaver density estimates, moose were usually preferred across greater ranges of prey densities, while differences in density estimates for beaver did not usually affect preference or avoidance of deer.

Deer densities can be extremely high, especially along the Lake Superior shore in winter, thus deer can comprise the majority of available prey in the environment. However, even with high availability of deer, moose still compose a significant proportion of wolf diet in winter. In a 3,000 km^2 area of northeastern Minnesota, deer have been absent in winter for at least thirty years due to wolf predation, severe winters, and deer migration behavior (Nelson and Mech 2006). Deer have not recolonized the area even with an increasing population nearby, thus moose have been the primary prey for wolves in this area (Nelson and Mech 2006).

Assumptions

Canids were a minor prey item (≤ 3% by biomass, in 2% of scats) in all three study areas. Many scats contained one or a few canid hairs, which were attributed to grooming. However, when a scat contained more than four canid hairs out of 25 sampled, the data was included in the analysis. These hairs may have also been from grooming, especially if they were from wolves affected by mange. About 60% of wolves captured in Grand Portage from 2007 to 2014 had at least 5% hair loss due to mange (Grand Portage, unpublished data). By including canids as prey in the analysis, other species would be underrepresented in the diet of wolves. However, because the proportion of canids in the wolves' diet was minor, the estimate of diet composition would not be significantly affected.

When interpreting wolf diet through scat analysis, there are assumptions that may be violated that need to be considered. First, the results of this diet study represent consumption of prey, not necessarily predation. We were unable to differentiate between prey that were scavenged versus killed. Adult moose health is compromised in Minnesota (Cornicelli et al. 2012), and wolves may be preying upon moose that are already sick or consuming moose that have died from other causes. Additionally, moose adults may not be protecting the young as well due to poor health. The health of the prey was not considered in this study. Prey in poor condition would likely weigh less and have more hair per digestible material, causing wolves to produce more scats (Weaver 1993), and thus, the prey would be overrepresented in diet.

When estimating percent biomass ingested to determine diet, some assumptions must be considered. We did not address the amount of prey lost to caching or other

scavengers or whether the entire carcass was consumed when estimating biomass consumed by wolves. Wolves prefer to consume soft components of the carcass (Carbyn 1983), and may not eat the entire carcass (Pimlott et al. 1969, Peterson 1977, Carbyn 1983, Potvin et al. 1988, Bobek et al. 1992, DelGiudice 1998), especially with larger prey (Floyd et al. 1978). To address these factors, the proportion of carcass that was not eaten or was lost to scavengers or cached would need to be subtracted from live weight estimates when estimating biomass consumed by wolves (Peterson and Ciucci 2003). We did not address these factors, thus larger animals may have been underestimated in wolf diet.

Wolf pack size and other factors may also affect diet composition results. Food availability per wolf and amount of prey lost to scavengers decreases with larger pack size (Peterson and Ciucci 2003). However, there was no difference in the amount of food obtained per wolf in different sized packs (Schmidt and Mech 1997), and lone wolves and pairs are able to kill moose (Thurber and Peterson 1993). We estimated average wolf diet over northeastern Minnesota. It would have been impossible to determine differences in diet among wolf packs over such a large area with many wolf packs. Additional factors, such as the length of time after consumption before depositing scats, which is 8 to 56 hours (Floyd et al. 1978) and how the scats collected were distributed over time and space relative to specific kills may have affected the random collection necessary to determine average wolf diet.

However, we were able to collect extensive data on wolf diets in northeastern Minnesota from a broad range in space and time. These data can be used in conjunction with predation and prey collaring studies to evaluate the predator/prey interactions in

northeastern Minnesota. Wolves in Minnesota consume mainly deer and moose, but the relative importance of deer or moose will vary in areas with varying prey densities. Beaver contributes to wolf diets in spring and summer, as do moose calves and deer fawns. Calves do not comprise a majority of wolf diet. However, with high wolf numbers in areas of high moose densities, wolves have the potential to impact the moose population through predation on calves. Wolves preferred moose over deer at most prey density estimates in Grand Portage and the Ceded Territory. Beaver density affected preference and avoidance of moose but not deer.

Chapter 2: How Many Hairs is Enough: An Assessment of Hair Selection in Scats When Determining Gray Wolf (*Canis lupus*) Diet

Introduction

Scat analysis methods have been modified to reduce time investment when determining carnivore diet; however, additional analyses are needed to determine whether these modified methods cause increased error. Using scat analysis to identify prey items in a carnivore diet is an effective, non-invasive, and commonly used method (Weaver 1993; Trites and Joy 2005). Scat analysis provides essential information about wolf diets by allowing researchers to collect large sample sizes, determine basic food habits, and analyze summer diets, while leaving the wolves unharmed (Peterson and Ciucci 2003, Ciucci et al. 2004). Undigested remains, including hair and bone, in scats are examined to determine prey occurrence. Prey hairs selected from scats are identified by analyzing macroscopic features and cuticular scale patterns.

Performing analysis of whole scats through hand separation is time-consuming and can lead to inaccuracies in identification of prey occurrence due to observer bias (Spaulding et al. 2000). Therefore, systematic sampling of undigested remains in wolf (*Canis lupus*) scats was developed to effectively reduce time needed for analysis and improve accuracy. The point-frame method is a useful and quick method for selecting hairs randomly from each scat (Ciucci et al. 2004). A trained researcher would require about 8.5 months using hand separation of scat contents to analyze 1,162 wolf scats, while only about 52 days would be required when using the point frame method (Ciucci et al. 2004). When using the point-frame method, a grid with pre-marked locations is placed over each scat, and one hair is selected from each location on the grid and identified.

When analyzing wolf diets, selecting 50 hairs per scat using the point-frame method is recommended (Ciucci et al. 2004), but in more recent studies, researchers have been

selecting fewer hairs for identification, thus reducing time needed for scat analysis. In wolf and coyote (*Canis latrans*) diet studies conducted in Algonquin Park (Canada) and southeastern Ontario, three hairs were selected from each scat for identification (Forbes and Theberge 1996, Sears et al. 2003). Analysis of three hairs accounted for 98.8% of prey items in each scat (Forbes and Theberge 1996).

Selecting fewer hairs per scat for identification can reduce intensive time investment, but additional analyses are needed to evaluate whether selecting fewer hairs will increase error when determining diet composition. We performed a sensitivity analysis on hair selection in wolf scats collected in northeastern Minnesota to test for differences in prey species composition. In this study, we tested effect of sample size by determining how estimated prey composition varied when sampling 3, 6, 12, and 25 hairs from wolf scats.

Study Area

The study area encompasses the northeastern region of Minnesota (Fig. 1.1), including federal, tribal, state, county, and private land. There were three collection areas, including the Grand Portage Indian Reservation, Voyageur's National Park Area, and 1854 Ceded Territory (Fig. 1.1). Cook, Lake, St. Louis, and part of Koochiching counties are included in the study area. Physical descriptions of the vegetation and landscape and estimates of wolf and prey densities have been described in Chapter One.

Methods

Scat Collection and Laboratory Processing

Scats were collected along roads and snowmobile trails from April 2011 to March 2014. Wolf scats were identified by shape and diameter. Scats used for analysis were ≥ 24 mm in diameter (Thompson 1952). Scats were stored in plastic bags and frozen until

laboratory analysis. Full scats were processed following a Standard Operating Procedure (Appendix 3), and data was entered into a standardized data sheet.

Hair Identification

After washing and drying, scats were spread out on a plate. Prey types were identified from hairs. The point-frame method was used to select the hairs that would be identified in each scat (Chamrad and Box 1964, Ciucci et al. 2004). A grid the same size as the plate was pre-marked with 25 points and placed over each scat. One hair was pulled from each point and identified.

Hairs were classified into nine different prey categories, including moose (*Alces alces*), moose calf, white-tailed deer (*Odocoileus virginianus*), deer fawn, beaver (*Castor canadensis*), snowshoe hare (*Lepus americanus*), small mammals, black bear (*Ursus americanus*), and canid, which could include wolf or coyote. I determined presence of young ungulates in scats during May through August and classified all ungulates after August to species level only. Birds, vegetation, insects, and trash were identified as present or absent. I could not differentiate between coyotes and wolves, so these hairs were classified as canid. Single or few canid hairs were found in many of the scats with few scats containing all or mostly canid hairs. The canid hairs were most likely from grooming (James 1983, Muller 2006), but when a scat contained more than four canid hairs, the hairs were included in analysis.

I examined all hairs with a dissecting microscope to compare color, shape, diameter, and length. I used a compound microscope to analyze medulla and scale patterns (Chapter One, Appendix 2). Hair scale patterns were extracted by taking negative impressions of hairs with Duco Cement® (Carrlee and Horelick 2010). Scale

patterns were identified using collected hair samples from the region and a reference manual (Adorjan and Kolenosky 1969). I took a blind test using 100 known hair samples to determine accuracy of identification (Ciucci et al. 1996), which was 95%.

Percent Accuracy when Selecting 3, 6, and 12 Hairs

If wolf scats contain one prey item, identifying one hair from each scat would result in accurate determination of prey species composition. However, selecting one or few hairs in scats that contain multiple prey items may result in missing additional prey items per scat, thus producing error when determining prey species composition. I estimated percent accuracy in determination of prey species composition from wolf scats when selecting 3, 6, and 12 hairs compared to selecting 25 hairs per scat. We analyzed percent accuracy using two methods: 1) overall percent deviation from true prey occurrence and 2) frequency that a prey item was not identified in scats.

Scats used in analysis contained two mammal prey types, and scats used in analysis had 25 hairs selected and identified using the point frame method. For every scat, each of the 25 selected and identified hairs were associated with a random number in excel. The 25 hairs were randomly sampled to select 3, 6, and 12 hairs, replicated ten times.

Percent Accuracy Based on Percent Deviation

I calculated the percent occurrence of each prey type per scat when 3, 6, and 12 hairs were selected for each of the 10 replicates. The absolute value of the deviation in percent occurrence from when 25 hairs were selected per scat was calculated. I averaged the percent deviation for all prey types in 125 scats and then averaged the 10 replicates.

The percent deviation was compared when 3, 6, and 12 hairs were selected, and 95% confidence intervals were calculated using $\alpha = 0.05$.

Percent Accuracy Based on Presence

I summed the frequency that a prey item was not present in a scat when 3, 6, and 12 hairs were selected, compared to when 25 hairs were selected, averaged over 10 replicates. The frequency that all prey types were present was multiplied by the percent of scats that contained multiple prey items. The percent accuracy was added to the percent of scats that contained only one prey item to estimate the accuracy in all scats.

Results

There was only one prey item in 83% of the 1,000 scats analyzed. Of the remaining scats, 125 scats were used to randomly select 3, 6, and 12 hairs. The species composition of these 125 scats was 39% adult deer and 26% beaver, with fawns, moose, calves, snowshoe hares, black bears, small mammals, and canids composing the remaining composition at 3-7% per prey item (Fig. 2.1). Adult moose represent a minor portion of the prey composition in scats because adult moose hairs were not typically found in scats with multiple prey items. Scats collected in spring were more likely to contain multiple prey items, and scats collected in summer contained the second highest number of scats with multiple prey items. When 25 hairs were selected per scat, the proportion of hairs by prey type was most commonly 1 hair of one prey type and 24 hairs of another prey type, with 2 and 23 hairs the second most common proportion (Fig. 2.2).

Percent Accuracy Based on Percent Deviation

As expected, the greatest percent deviation from true prey occurrence was when 3 hairs were selected per scat, with 6 hairs having significantly less difference in deviation

than 3 hairs, and 12 hairs having significantly less difference in deviation from selecting 3 and 6 hairs per scat (Fig. 2.3). Selecting 3, 6, and 12 hairs produced 16%, 10%, and 6% deviations from true prey occurrence when 25 hairs were selected, respectively. When the proportion of hairs per prey type was uneven (24:1 hairs per prey type), the deviation in occurrence from true results was less than when the proportion of hairs was close to equal (13:12 hairs per prey type) (Fig. 2.4). Percent accuracy based on percent deviation in prey occurrence when 3, 6, and 12 hairs were selected in scats that contained two prey items was 86%, 90%, and 94%, respectively (Table 2.1). However, because 83% of scats contained only one prey item, accuracy in determining accurate prey occurrence will be improved when analyzing all scats, not only scats with multiple prey items. Accounting for 100% accuracy in determining prey occurrence in scats with one prey item, the estimated accuracy in all scats was 97%, 98%, and 99% when 3, 6, and 12 hairs were selected, respectively (Table 2.1)

Percent Accuracy Based on Presence

Percent accuracy of identifying presence of all prey items in scats that contained two prey items when 3, 6, and 12 hairs were selected was 42%, 64%, and 83%, respectively (Table 2.2). Accounting for 100% accuracy in correctly identifying all prey items in 83% of scats containing one prey item, the estimated accuracy when 3, 6, and 12 hairs were selected in all scats was 90%, 94%, and 97%, respectively (Table 2.2)

Discussion

Selecting fewer hairs per scat resulted in reduced accuracy in determining prey species occurrence in wolf scats. When estimating accuracy based on percent deviation from true results when 3, 6, and 12 hairs were selected in all scats, the accuracy was

greater than 95%. However, when selecting fewer hairs per scat, prey items were occasionally missed in scats. Accuracy in correctly identifying presence of all prey items in scats was less than 95% when selecting 3 or 6 hairs from scats. This reduced accuracy caused prey items that were not identified in scats to be underrepresented in wolf diet, which may result in greater error when determining prey species composition.

When comparing hand separation of scats to the point-frame method, both methods produced reliable results, but the point-frame method required less time and improved objectivity (Ciucci et al. 2004). When using the hand separation technique to analyze scats, researchers may have differing interpretations of complete and thorough inspection, however, using a grid in the point-frame method increases objectivity among researchers by allowing for random selection of hairs in scats (Spaulding et al. 2000, Ciucci et al. 2004). The researchers sampled 50 hairs per scat using the point-frame method but recommended potentially sampling 100 hairs per scat for wolves with a more diversified diet and concluded that even by doubling the number of hairs to analyze, sampling time will still be reduced (Ciucci et al. 2004).

Selecting 3 or 6 hairs per scat for identification will reduce time invested in scat analysis but will cause error greater than 5% when determining prey occurrence in wolf scats. Selecting 12 hairs per scat will require less time for scat analysis than selecting 25 hairs, while producing similar accuracy. When selecting 12 hairs per scat, the accuracy of identifying all prey items in scats and the accuracy based on percent deviations in prey occurrence were both greater than 95%. Therefore, I recommend selecting 12 hairs per scat when using the point-frame method to determine prey occurrence in wolf diets.

The results in this study were based on scats collected in northeastern Minnesota, where 83% of scats contained one prey item and prey was assigned to nine prey categories. When analyzing wolf diets that have a higher percentage of scats with multiple prey items, more hairs may need to be selected per scat to accurately determine diet composition. Additionally, more hairs may need to be selected per scat when determining diet composition of wolves that have a more diversified diet.

Table 1.1. Estimated live weights of animals used for biomass equation. Adult deer and moose weights were estimated combining both sexes (Forbes and Theberge 1996).

Prey	Prey Live Weight (kg)
White-tailed Deer	75
Fawn (May - June)	4
Fawn (July - Aug)	14
Moose	444
Calf (May-June)	20
Calf (July-Aug)	57
Beaver	20
Snowshoe Hare	1.5
Bear	100
Small Mammal	0.25
Canids	32

Table 1.2. Number of scats collected by location, year, and season. A total of 1,000 scats were collected in all three study areas.

		Spring	Summer	Winter
Ceded Territory	2012	28	12	38
	2013	71	10	84
	Total	99	22	122
Grand Portage	2011	47	55	112
	2012	44	17	128
	2013	71	26	24
	Total	162	98	264
Voyageurs National Park area	2012	43	20	26
	2013	60	8	76
	Total	103	28	102

Table 1.3. Proportion of prey availability in the environment. Prey availability was estimated based on expected densities of deer, moose, and beaver across northeastern Minnesota. Densities were multiplied by prey weight to determine biomass available.

Density (prey/km^2)			Biomass Available (kg/km^2)			Percent Available		
Deer	Moose	Beaver	Deer	Moose	Beaver	Deer	Moose	Beaver
2	0.05	2	150	22	40	71	10	19
2	0.2	2	150	89	40	54	32	14
2	0.35	2	150	155	40	43	45	12
2	0.5	2	150	222	40	36	54	10
30	0.05	2	2250	22	40	97	1	2
30	0.2	2	2250	89	40	95	4	2
30	0.35	2	2250	155	40	92	6	2
30	0.5	2	2250	222	40	90	9	2
55	0.05	2	4125	22	40	99	1	1
55	0.2	2	4125	89	40	97	2	1
55	0.35	2	4125	155	40	95	4	1
55	0.5	2	4125	222	40	94	5	1
80	0.05	2	6000	22	40	99	0	1
80	0.2	2	6000	89	40	98	1	1
80	0.35	2	6000	155	40	97	3	1
80	0.5	2	6000	222	40	96	4	1
2	0.05	6	150	22	120	51	8	41
2	0.2	6	150	89	120	42	25	33
2	0.35	6	150	155	120	35	37	28
2	0.5	6	150	222	120	30	45	24
30	0.05	6	2250	22	120	94	1	5
30	0.2	6	2250	89	120	92	4	5
30	0.35	6	2250	155	120	89	6	5
30	0.5	6	2250	222	120	87	9	5
55	0.05	6	4125	22	120	97	1	3
55	0.2	6	4125	89	120	95	2	3
55	0.35	6	4125	155	120	94	4	3
55	0.5	6	4125	222	120	92	5	3
80	0.05	6	6000	22	120	98	0	2
80	0.2	6	6000	89	120	97	1	2
80	0.35	6	6000	155	120	96	2	2
80	0.5	6	6000	222	120	95	4	2

Table 1.4. Wolf diet composition in Grand Portage (2011-2013), the Ceded Territory (2012-2013), and Voyageurs National Park area (2012-2013). Frequency of occurrence was converted to biomass using the equation Y = 0.439 + 0.008 * X (Weaver 1993). N represents number of scats collected.

	Spring		**Summer**		**Winter**	
	% Freq.	% Biomass	% Freq.	% Biomass	% Freq.	% Biomass
Grand Portage	**N=162**		**N=98**		**N=264**	
Deer	32	35	31	28	58	38
Fawn	23	11	19	9		
Moose	7	30	11	41	22	54
Calf	8	5	3	2		
Beaver	25	16	25	13	17	6
Hare	1		1			
Bear	1	1	2	2	1	1
Small Mammal	1		5	2	1	
Canid	2	1	3	2	2	1
Ceded Territory	**N=99**		**N=22**		**N=122**	
Deer	38	39	71	55	72	60
Fawn	12	5	4	2	1	
Moose	8	30	13	37	10	32
Calf	21	12				
Beaver	8	4			5	2
Hare	4	2	4	1	6	2
Bear	4	5	4	4	1	1
Small Mammal	4	2	4	1	5	2
Canid	1	1			2	1
VNP Area	**N=103**		**N=28**		**N=102**	
Deer	52	61	31	44	81	78
Fawn	7	4	26	19		
Moose	2	11			4	13
Calf	2	1	3	3		
Beaver	33	22	37	30	12	7
Hare	2	1			2	1
Small Mammal	2	1				
Canid			3	3	2	1

Table 1.5. Number of prey consumed by wolves per year in Grand Portage. The number of prey consumed is calculated using the biomass proportion of prey in wolf diet, estimated daily food requirement of a wolf, and an estimated population of 15 wolves in Grand Portage.

	# Prey Consumed			
	Parturition	Summer	Winter	**Total**
Deer	9	13	44	**66**
Fawns	55	23		**78**
Moose	1	3	11	**15**
Calves	5	2		**7**
Beavers	20	29	30	**79**
Hares	3	9	8	**20**
Bears	0	1	1	**2**
Small Mammals	39	368	89	**496**
Canids	1	3	3	**7**

Table 1.6. Wolf diet compositions in Ontario, Canada and Minnesota based on scat analysis. Diet is represented as percent biomass, which was converted from frequency of occurrence using a regression equation (Floyd et al. 1978, Weaver 1993).

Summer			*% biomass*						
Source	*Year*	*Location*	*moose*	*calf*	*deer*	*fawn*	*beaver*	*hare*	*other**
Forbes & Theberge 1996	1987-92	Ontario	75	7	4	1	13	0	0
	1987-92	Ontario	54	4	24	4	15	0	0
	1987-92	Ontario	57	7	21	4	11	0	0
Fritts & Mech 1981	1972-76	NW MN	34		57		1	1	7
Gogan et al. 2004	1988-89	VNP Area	5		67	8	16	1	3
Fuller 1989	1980-83	N central MN			77	13	7	2	
This study	2011-13	GP	35	4	31	10	14	0	4
	2012-13	NE MN	33	6	47	4	2	2	6
	2012-14	VNP Area	5	2	53	12	26	0	2
		Average	**37**	**5**	**42**	**7**	**12**	**1**	**3**
Winter									
Forbes & Theberge 1996	1987-92	Ontario	87		4		9	2	0
	1987-92	Ontario	65		23		14	1	0
	1987-92	Ontario	72		14		8	0	0
Fritts & Mech 1981	1972-76	MN	21		75		0	1	4
Gogan et al. 2004	1988-89	VNP Area	7		90		3	0	0
Fuller 1989	1980-83	N central MN			92		5	3	
This study	2011-13	GP	54		38		6	0	2
	2012-13	NE MN	32		60		2	2	3
	2012-14	VNP Area	13		78		7	1	1
		Average	**44**		**53**		**6**	**1**	**1**

*includes wolf, livestock, black bear, small mammals, vegetation, fish, birds, garbage

Table 2.1. Percent accuracy in determining prey occurrence when selecting 3, 6 and 12 hairs per scat based on deviation from true prey occurrence. Percent deviation from true prey occurrence when 25 hairs were selected per scat was summed across all prey types and scats, averaged over 10 replicates. Sample size was 125 scats that contained two prey items. Percent accuracy in all scats was determined by accounting for 100% percent accuracy in scats with only one prey item.

Hairs Selected per Scat	% Deviation in Scats with 2 Prey Items	% of Scats with >1 Prey Item	% Accuracy in All Scats
3	16	17	97
6	10	17	98
12	6	17	99

Table 2.2. Percent accuracy based on presence of all prey items in scats when selecting 3, 6, and 12 hairs per scat. The frequency that a prey item was missed in 125 scats containing two prey items was summed, averaged over 10 replicates. Percent accuracy in all scats was determined by accounting for 100% accuracy in scats with only prey item.

Hairs Selected per Scat	% All Prey Items Present in Scats with 2 Prey Items	% of Scats with >1 Prey Item	% Accuracy in All Scats
3	42	17	90
6	64	17	94
12	83	17	97

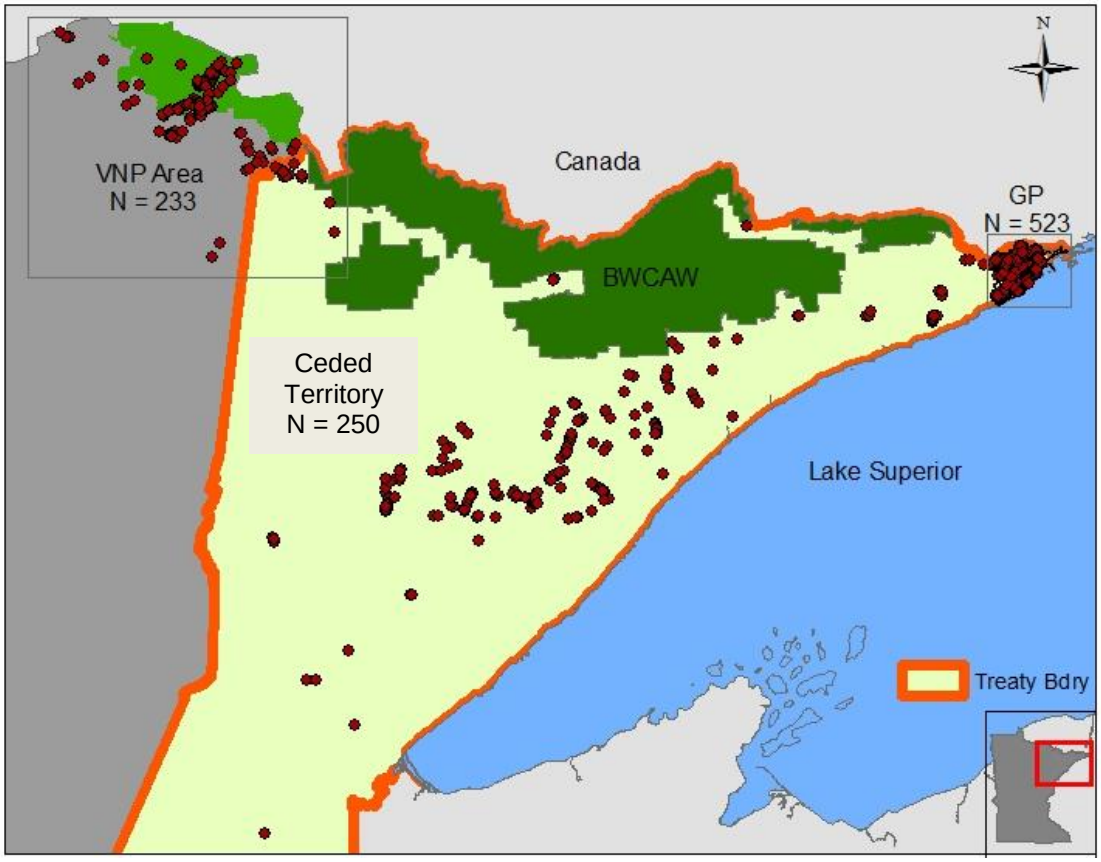

Fig. 1.1. Map of wolf scat collection locations in northeastern Minnesota. There were three collection areas, Grand Portage (GP), 1854 Ceded Territory (Ceded Territory), and Voyageurs National Park area (VNP Area). All scats collected outside Grand Portage and Voyageurs National Park area were designated to the Ceded Territory study area. Dots represent wolf scat collection sites from 2011 to 2013.

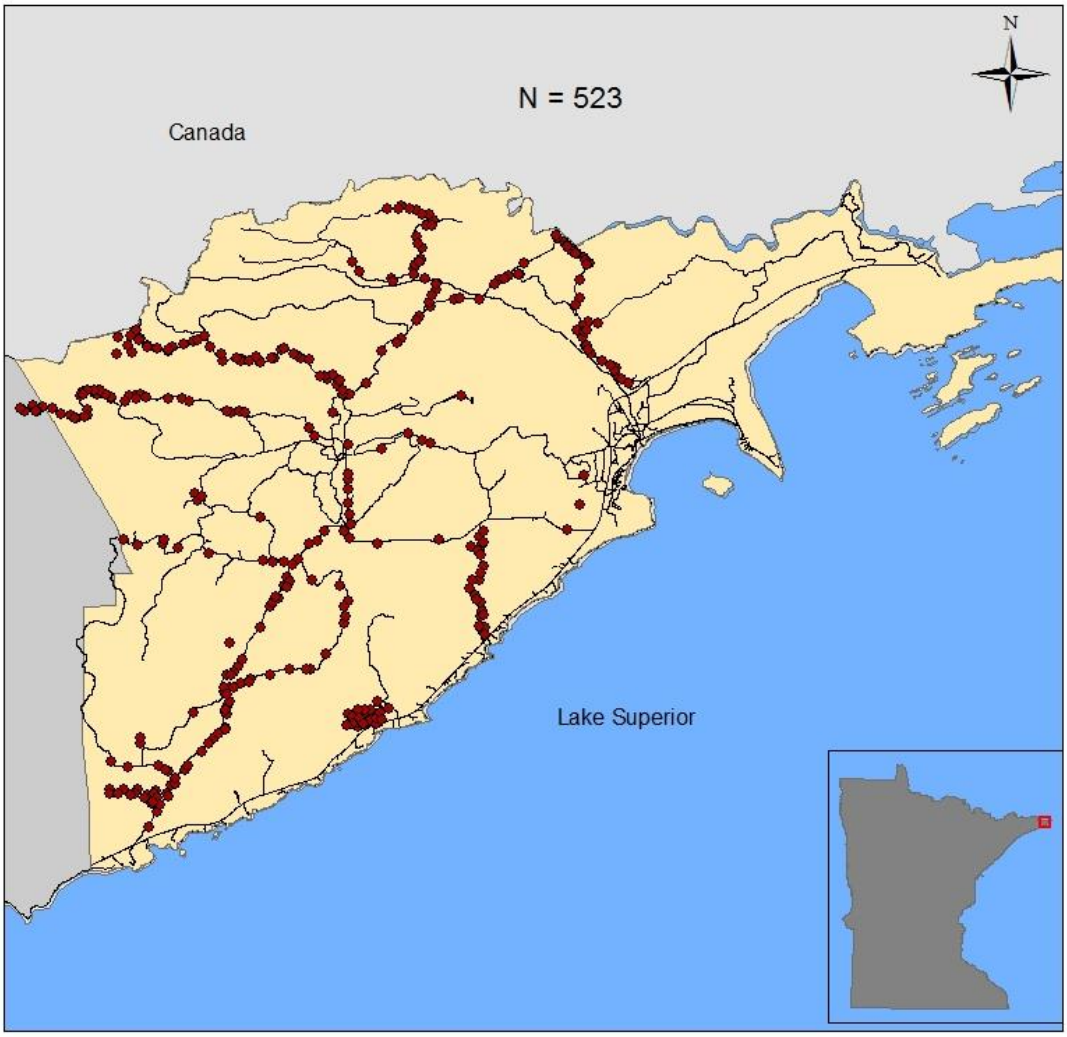

Fig. 1.2. Map of wolf scat collection sites in the Grand Portage Reservation in northeastern Minnesota. Dots represent collection sites from April 2011 to June 2012.

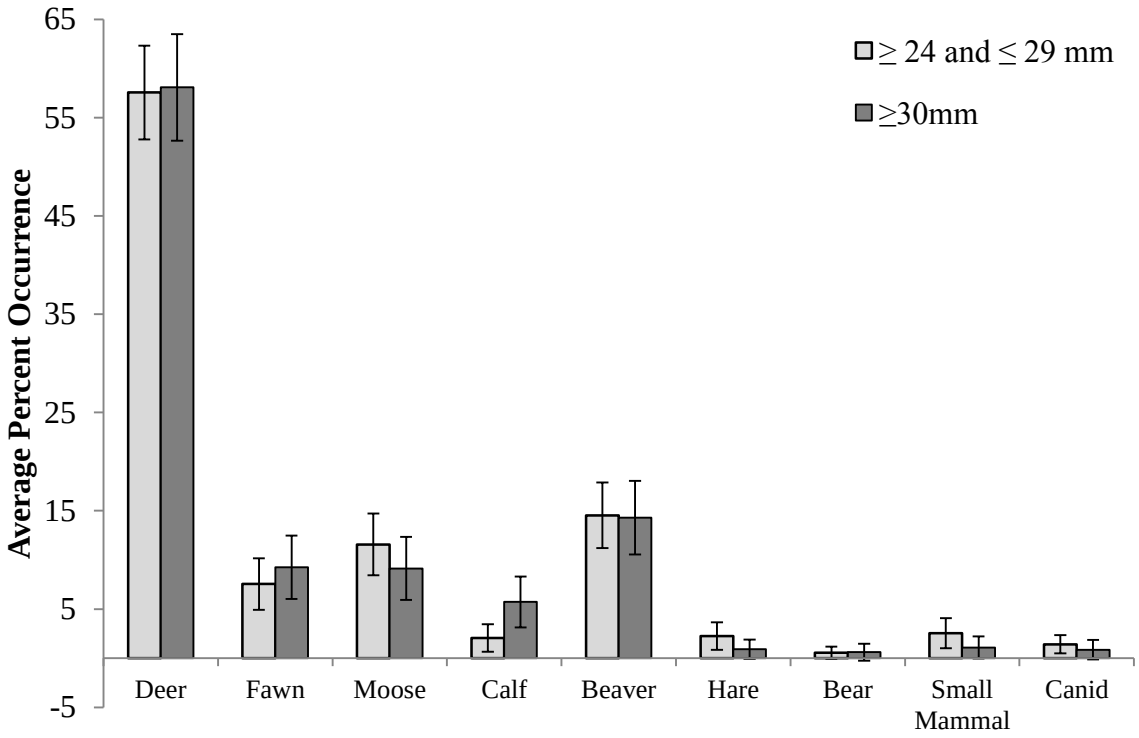

Fig. 1.3. Comparison of prey occurrence in scats with diameters ≤ 29 mm and scats with diameters ≥ 30 mm. Sample size was 689 scats, 386 scats with diameters ≤ 29 mm and 303 scats with diameters ≥ 30 mm. Error bars represent 95% confidence intervals.

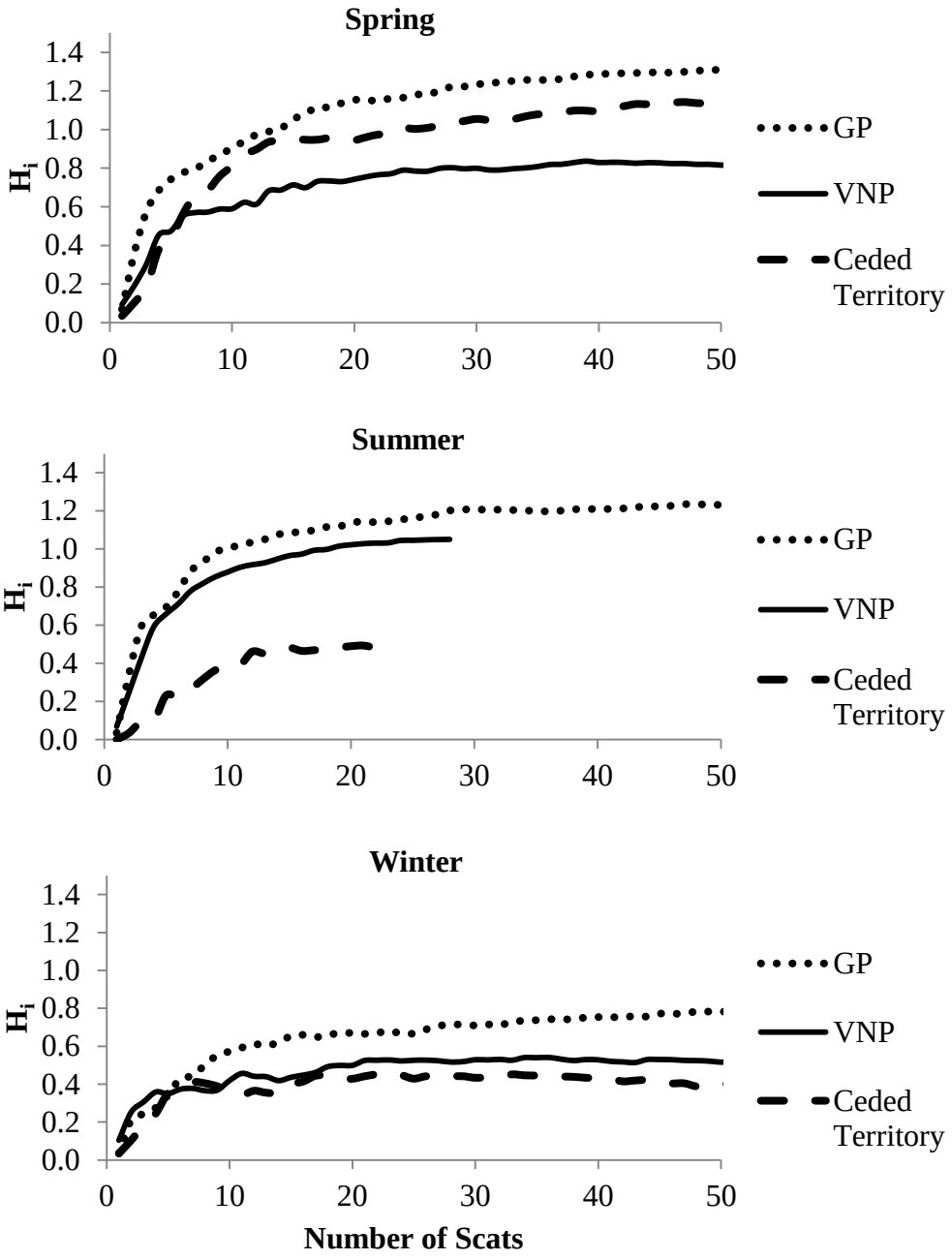

Fig. 1.4. Dietary diversity of wolves calculated using Brillouin Index, Hi, to measure adequacy of sample size analysis.

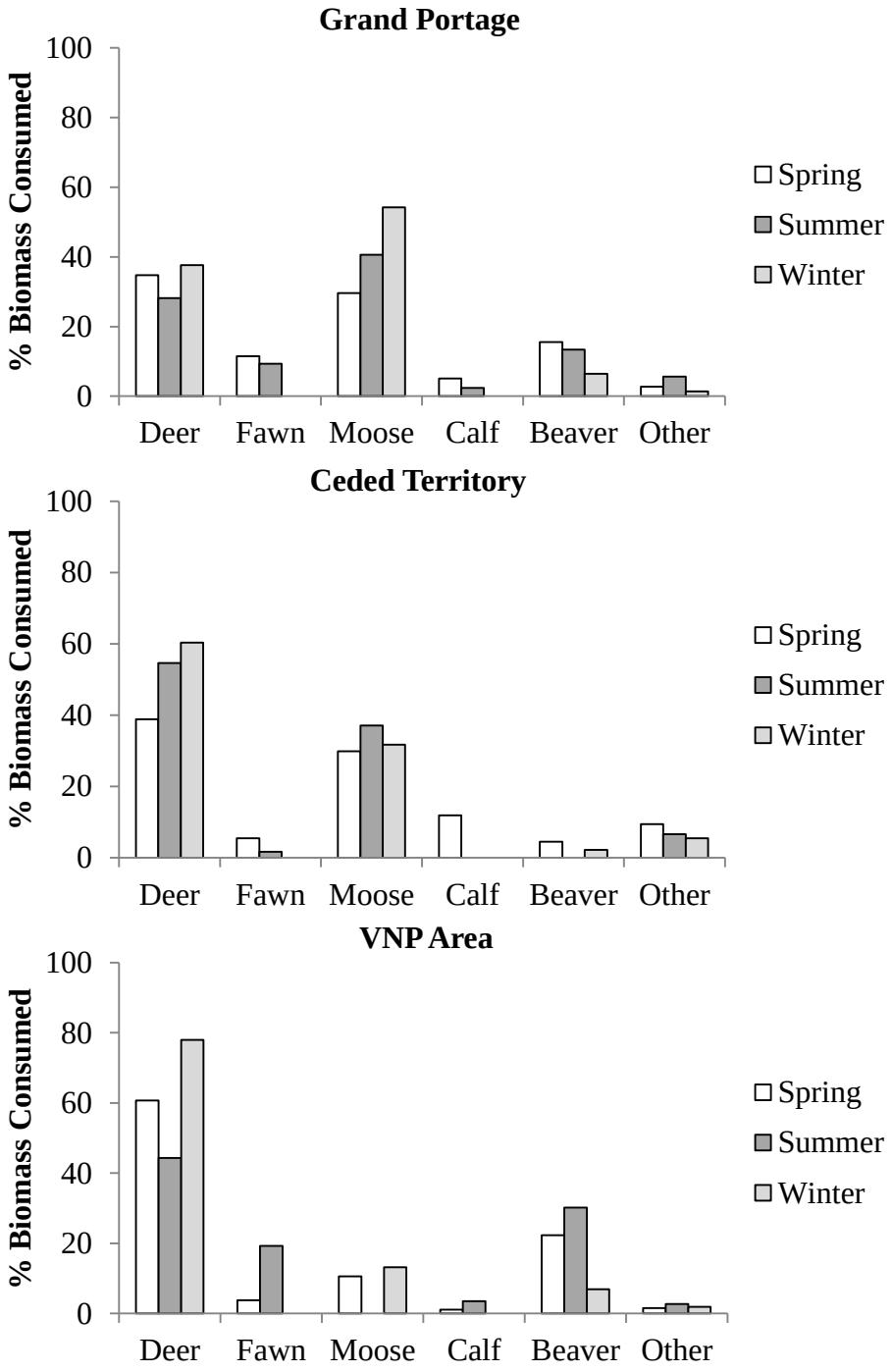

Fig. 1.5. Percent biomass of prey consumed by wolves in Grand Portage (2011-2013), 1854 Ceded Territory (2012-2013), and Voyageurs National Park area (2012-2013). Bars represent % biomass consumed using a regression equation (Weaver 1993).

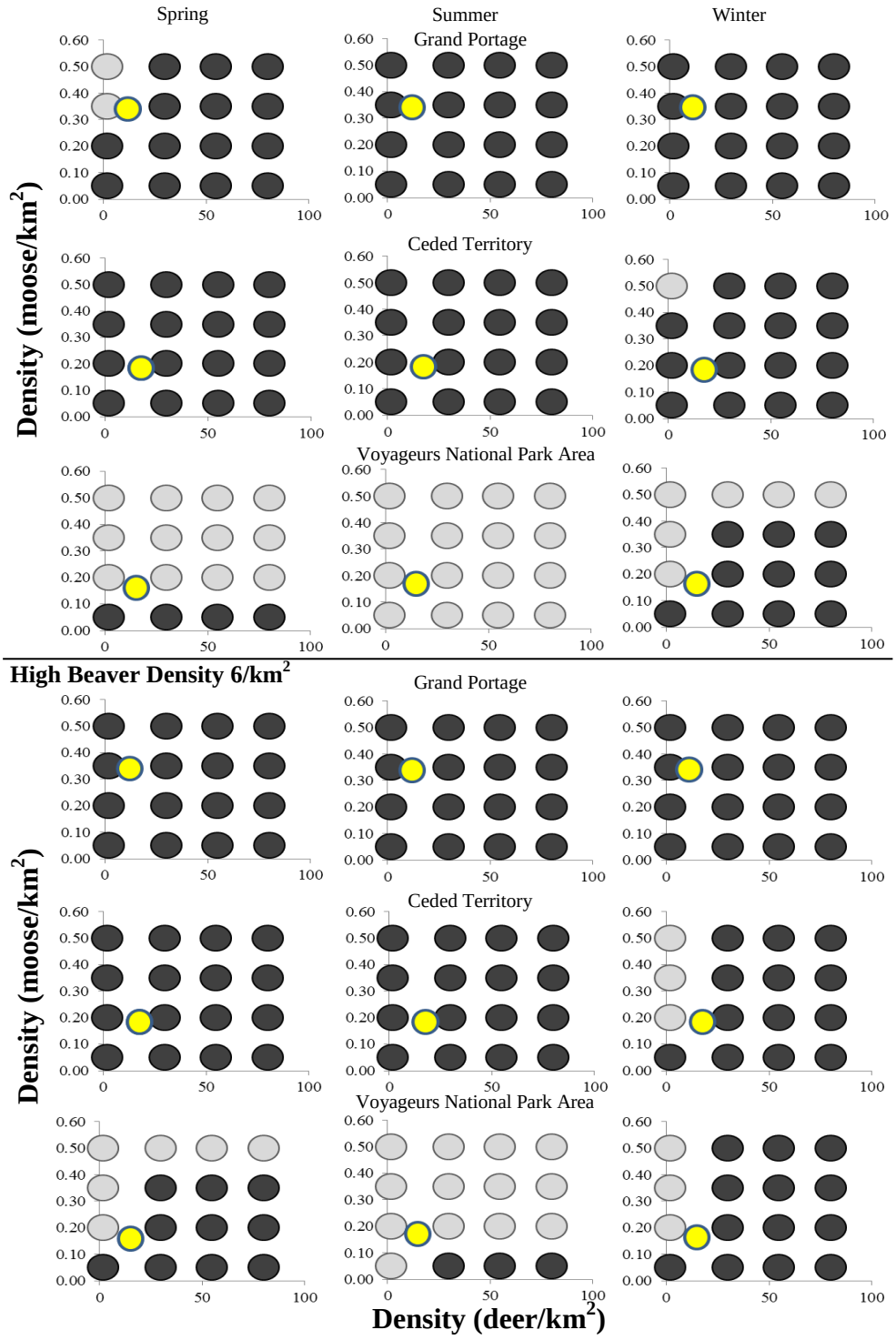

Fig. 1.6. Preference and avoidance of moose by season and study area. Black dots represent preference for moose and gray dots represent avoidance. Yellow dot is the approximate density of moose and deer. Estimated beaver density is 2/km2 and 6/km2.

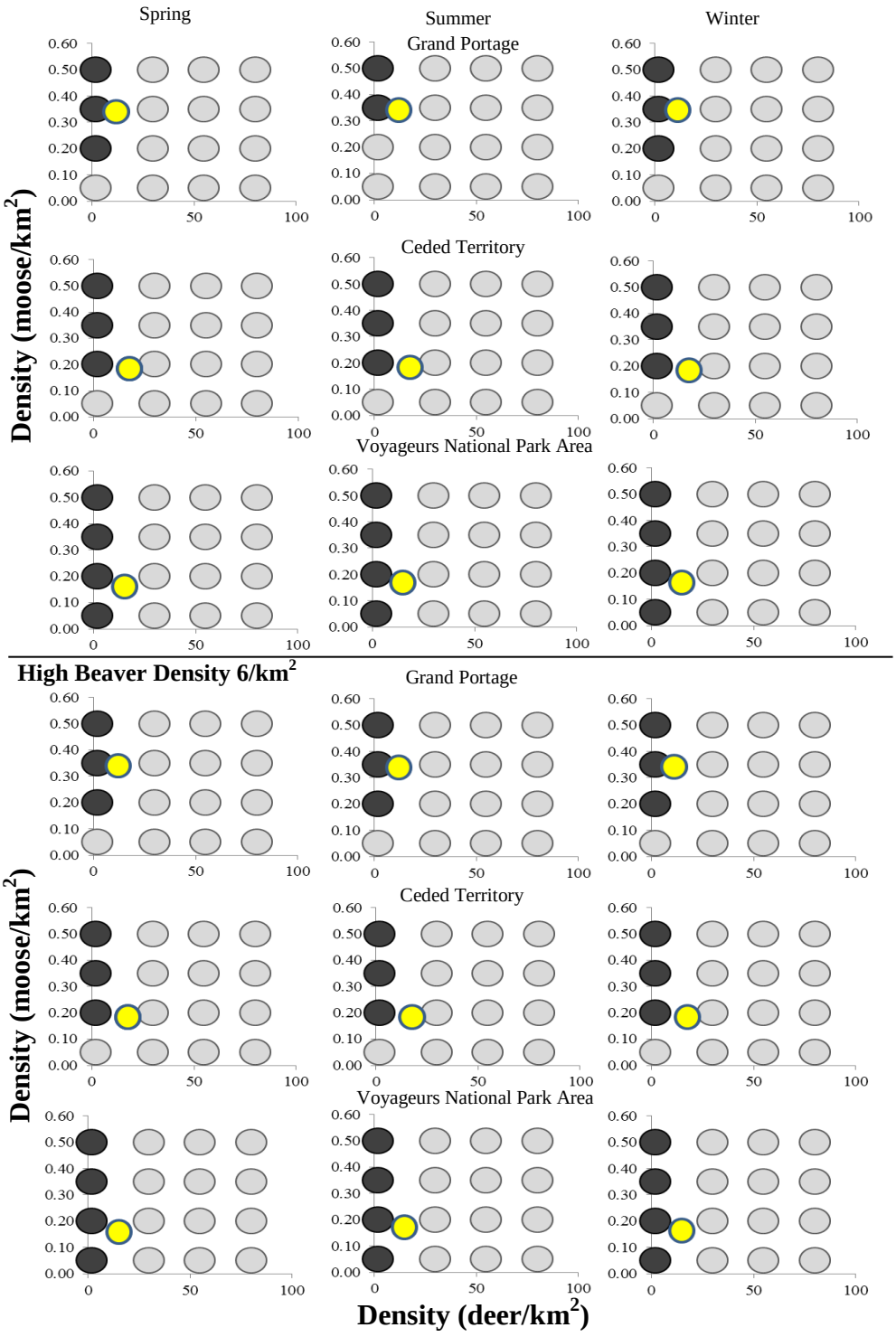

Fig. 1.7. Preference and avoidance of deer by season and study area. Black dots represent preference for deer and gray dots represent avoidance. Yellow dot is the approximate density of moose and deer. Estimated beaver density is 2/km^2 and 6/km^2.

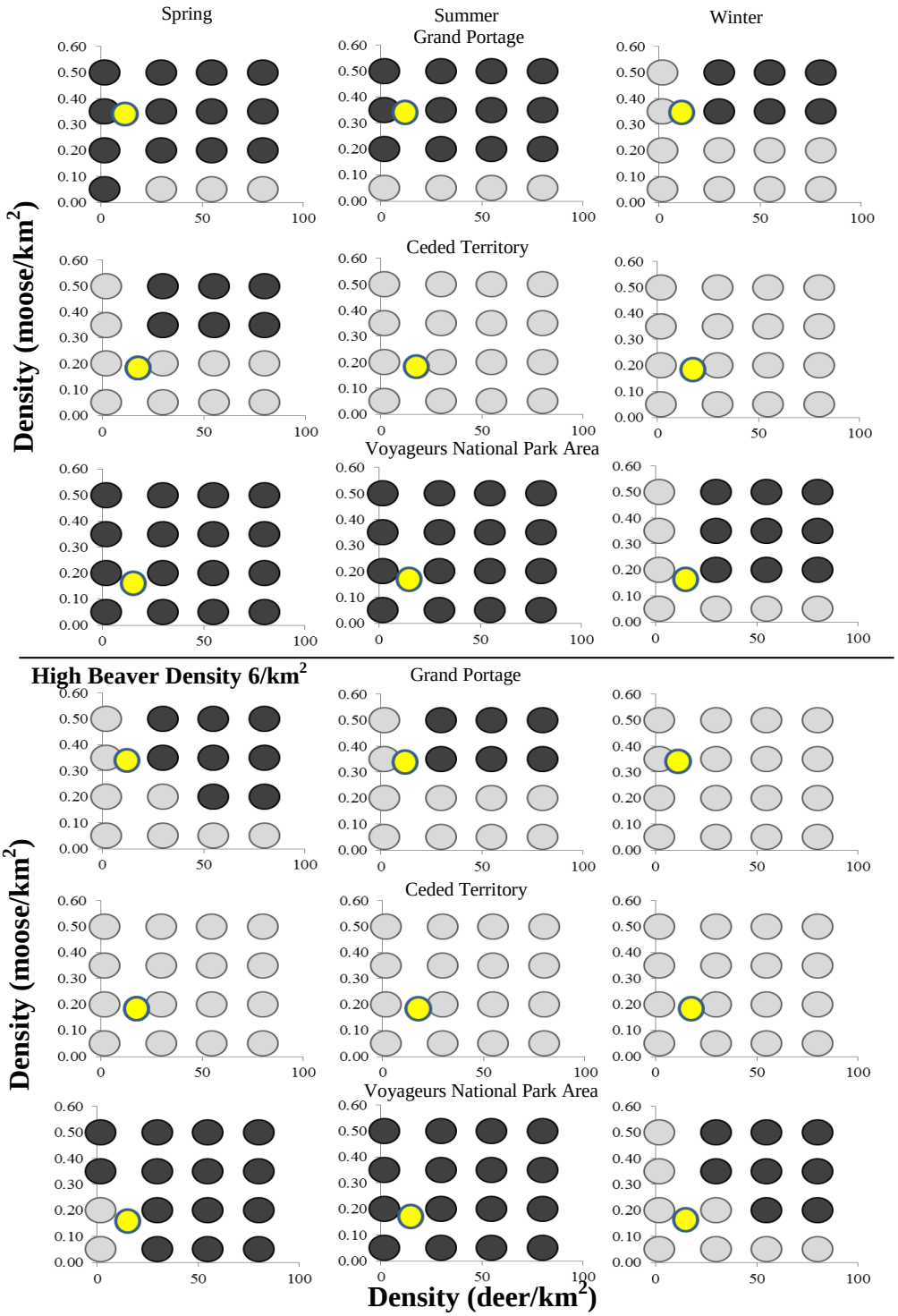

Fig. 1.8. Preference and avoidance of beaver by season and study area. Black dots represent preference for beaver and gray dots represent avoidance. Yellow dot is the approximate density of moose and deer. Estimated beaver density is 2/km^2 and 6/km^2.

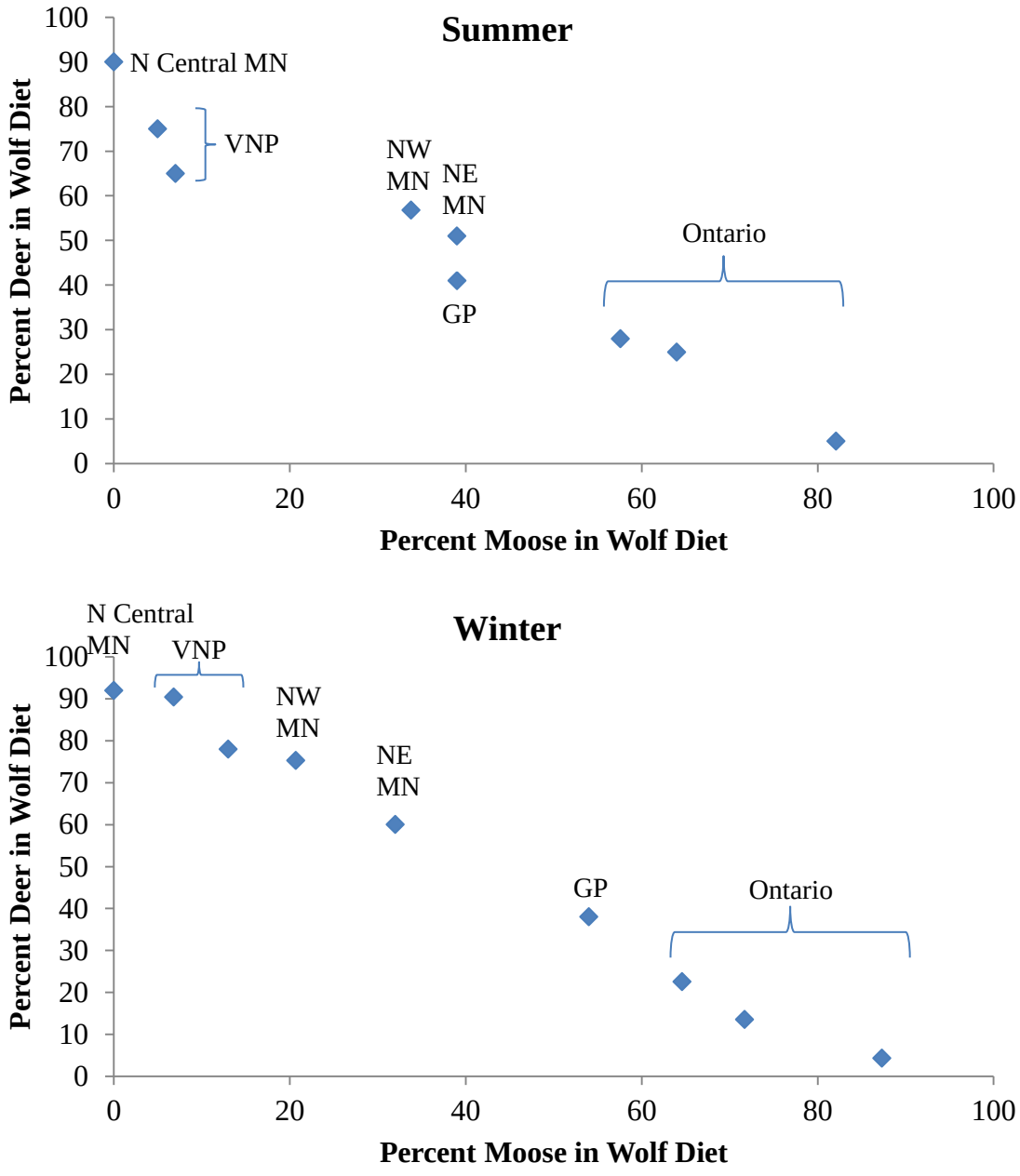

Fig. 1.9. Comparison of deer and moose in wolf diets in Ontario, Canada and Minnesota based on scat analysis. Diet is represented by percent biomass, which was converted from frequency of occurrence using a regression equation (Floyd et al. 1978, Fritts and Mech 1981, Fuller 1989, Weaver 1993, Forbes and Theberge 1996, Gogan et al. 2004, Ibrahim et al. 2015).

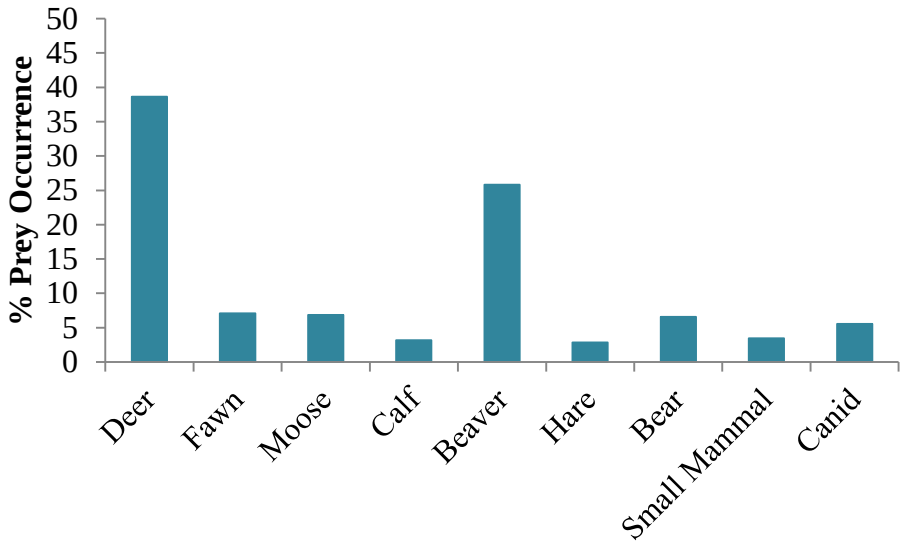

Fig. 2.1. Species composition in scats used for hair selection analysis. Bars represent average percent occurrence in 125 scats that contained two prey items.

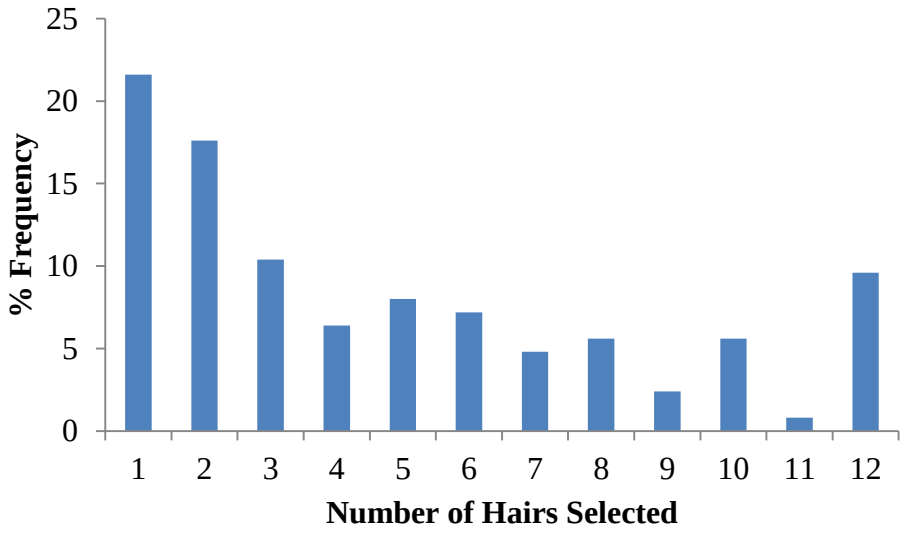

Fig. 2.2. Proportion of hairs by prey type when 25 hairs were selected per scat. Bars represent occurrence of hairs by prey type in 125 scats containing two prey items. Frequency of 24-13 hairs was identical to 1-12 hairs.

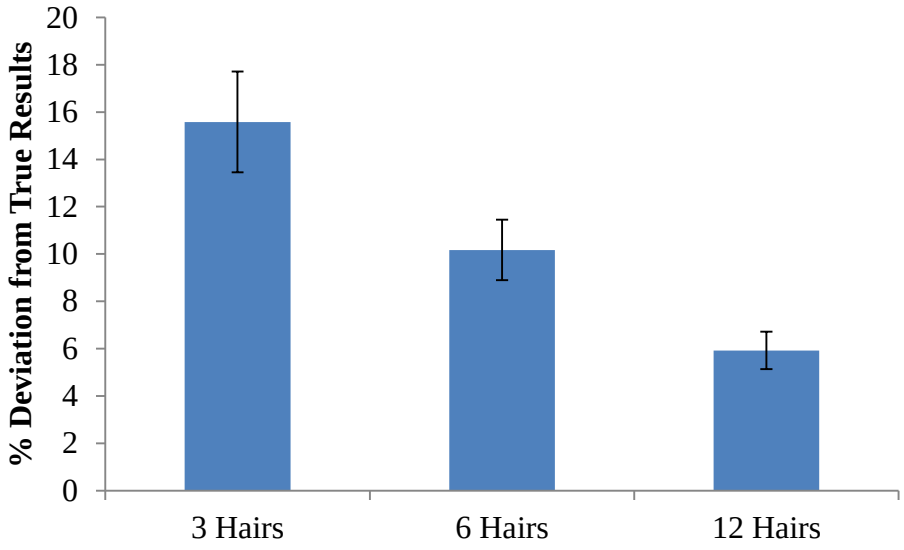

Fig. 2.3. Deviation from true prey occurrence when selecting 3, 6, and 12 hairs per scat. Bars represent percent deviation from prey occurrence when 25 hairs were selected per scat. Sample size was 125 scats that contained two prey items. Error bars are 95% confidence intervals.

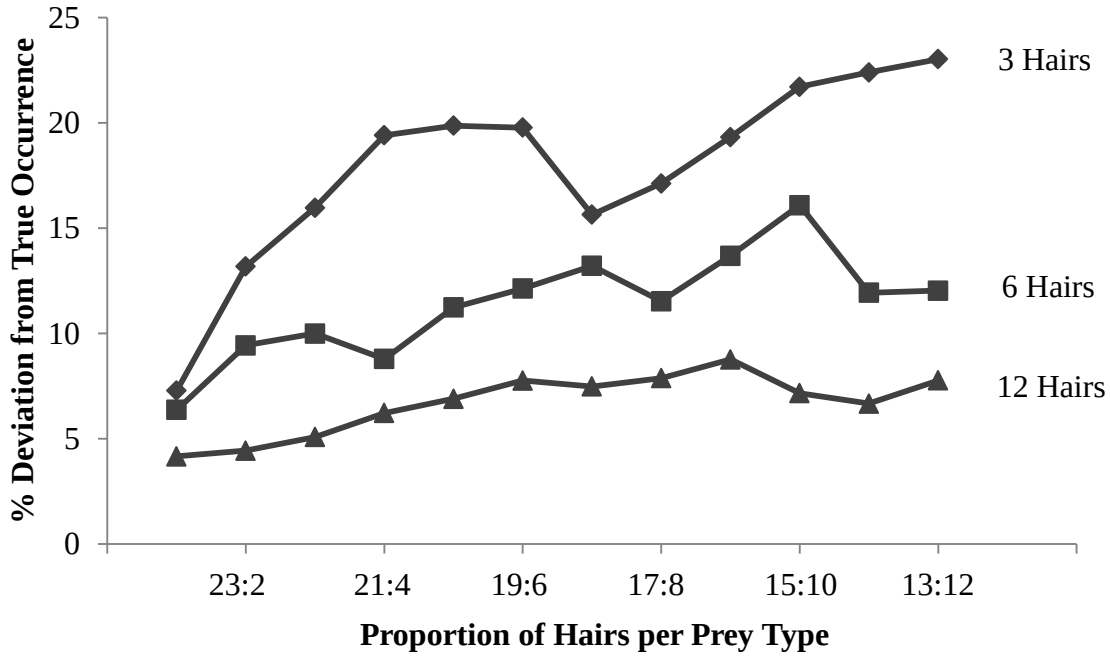

Fig. 2.4. Deviation from true prey occurrence by proportion of hairs per prey type when 3, 6, and 12 hairs were selected per scat. Points represent percent deviation from occurrence when 25 hairs were selected per scat. Sample size was 125 scats with two prey items per scat. Deviation for 12-1 hairs was identical to 13-24 hairs.

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Appendix 1: Wolf Scat Collection Data Sheet

Date _____ Name of Collector _____

UTM x and y (preferred) or location description (e.g., Google earth to get an approximate lat/long or directions from a map):

_____

_____

Check the following:

_____ Fresh because _____moist

_____strong smell

_____tracks present

_____scat is on top of new snowfall

_____ Old because _____crumbly

_____white

If you are following routes regularly or if there was a recent storm, mark dates if known:

_____Date last time road was traveled and scat picked up

_____Date of last snowplow

Appendix 2: A Manual for Identification of Prey Species in Gray Wolf (C*anis lupus*) Scats in Northeastern Minnesota

Introduction

Prey items are identified in carnivore scats by analyzing macroscopic features of hair and cuticular scale and medulla patterns, but learning to identify species and age class through hair analysis can be a time-consuming process. Using manuals with recommended techniques will allow a researcher to become more quickly trained in hair identification. We developed this manual with recommendations for identifying prey hairs from gray wolf (*Canis lupus*) scats collected in northeastern Minnesota. Using scat analysis to identify prey items in a carnivore diet is an effective, non-invasive, and commonly used method (Weaver 1993; Trites and Joy 2005). This manual can be used in different regions in conjunction with other manuals and by collecting prey hair samples from the region.

Gray wolves prey primarily on ungulates but may consume animals that range in size from 1 to 1,000 kg (Mech and Boitanti 2003). Prey hairs that we identified in wolf scats included moose (*Alces alces*), moose calf, white-tailed deer (*Odocoileus virginianus*), deer fawn, beaver (*Castor canadensis*), snowshoe hare (*Lepus americanus*), small mammals, black bear (*Ursus americanus*), and canid, which could include wolf or coyote (*Canis latrans*). After young ungulates molt in early fall, the hairs are no longer distinguishable from those of adults. Thus, adult and young ungulate hairs can only be differentiated from birth until late summer (Pimlott et al. 1969, Voigt et al. 1976, Fritts and Mech 1981, Peterson et al. 1984, Gauthier and Theberge 2010). We did not differentiate among small mammals or between wolves and coyotes. In this manual, we provide guidelines for identification of prey items through macroscopic and microscopic analysis of prey hairs in gray wolf scats.

Methods

Health Hazards

Wolf scats can contain harmful parasites. The disease you are most likely to catch from handling wolf scat is echinococcosis, or cystic hydatid disease. Humans can get this disease by ingesting or inhaling eggs of the tapeworm. Wear gloves at all times when handling scat, scat bags, and any items used when handling un-sanitized scats. Each scat should be treated like it is contaminated. Wash hands after collecting or processing scat, particularly before eating. Wash hands thoroughly with soap and water if you touch wolf scat with bare skin. When analyzing hairs from sanitized scats, use tweezers to handle hairs and wash hands thoroughly after analysis.

Laboratory Procedure

We collected wolf scats on roads and trails and identified them by shape and diameter, with scats used for analysis $\geq$ 24 mm in diameter (Thompson 1952). Scats were stored frozen in plastic bags until laboratory analysis. Scats were transferred to nylon stockings and boiled for >30 minutes under a fume hood to kill parasites (Patterson et al. 1998, Chavez and Gese 2005, Klare et al. 2011). Scats were washed in a dishwasher to remove digestible material until only bone and hair remained in the stocking. The scats were rewashed and wrung out by hand to remove remaining digestible material if needed. The undigested remains were air-dried in a fume hood for 24 hours and then weighed.

After washing and drying, scats were spread out on a plate. Prey species were identified from hairs. The point-frame method was used to select the hairs that would be identified in each scat (Chamrad and Box 1964, Ciucci et al. 2004). A grid the same size of the plate was pre-marked with 25 points and placed over each scat. One hair was randomly selected and pulled from each point and identified.

Hairs were classified into nine different prey categories, including moose, moose calf, white-tailed deer, deer fawn, beaver, snowshoe hare, small mammals, black bear, and canid, which could include wolf or coyote. I determined presence of young ungulates in scats during May through August and classified all ungulates after August to

species level only. Birds, vegetation, insects, and trash were identified as present or absent.

Hair Identification

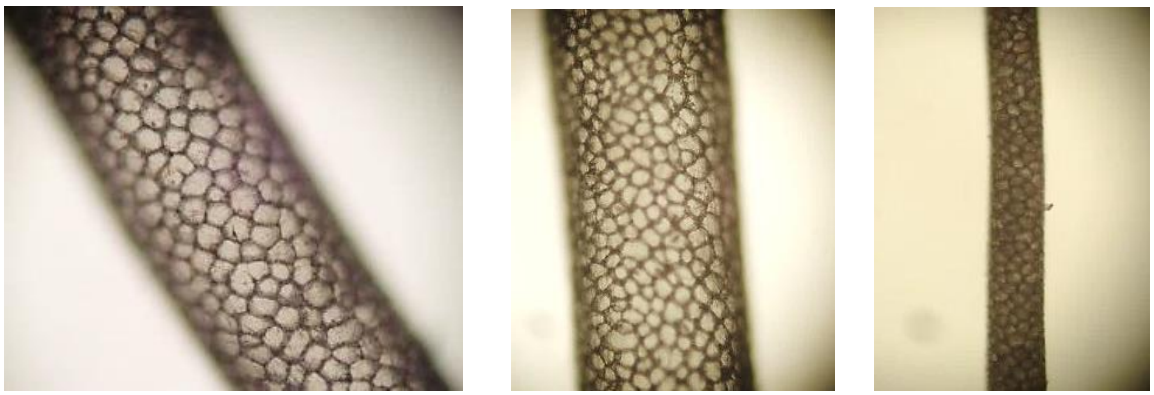
I examined all hairs with a dissecting microscope to compare color, shape, diameter, and length. I used a compound microscope to analyze the medulla, the innermost part of the hair, which can be observed using a compound microscope with the contrast positioned to the brightest light. The hairs of ungulates and snowshoe hares have distinctive medulla patterns, which allowed for initial classification of the hair as ungulate, snowshoe hair, or other. I identified all beaver hairs and most hairs of other species macroscopically. However, identification of hairs occasionally required additional analysis by examining scale patterns, especially when differentiating between moose and deer and identifying to age class.

Hair scale patterns were extracted by taking negative impressions of hairs with Duco Cement® (Carrlee and Horelick 2010). A thin layer of Duco Cement® was spread on a microscope slide, and a hair was placed in the cement. After three minutes, the hair was pulled out and taped to the slide. Scale patterns were identified using collected hair samples from the region and a reference manual (Adorjan and Kolenosky 1969). Scale patterns vary along different sections of the hair shaft, with the shaft divided into three sections: base, medial, and distal (Adorjan and Kolenosky 1969). The base and distal sections were typically the most useful in identification. Before performing hair identification in scats, I took a blind test using 100 known hair samples with each expected prey species present, including calves and fawns (Ciucci et al. 1996). Accuracy in identification was 95%.

Hair Descriptions

Notes on Ungulate Hair

Ungulate hairs are hollow like a straw, which is easily viewed using a dissecting scope. The hairs also have a distinctive medulla pattern (Fig. 1). These features allow for easy and quick differentiation from hairs of all other species. The hairs of adult moose, calves, adult deer, and fawns can then be differentiated from each other by color, diameter, length, shape and often, by scale analysis.



Adult Deer | Adult Moose | Moose Calf

Fig. 1. Medulla patterns of adult white-tailed deer, adult moose, and moose calf hairs at 200x magnification using a compound microscope. There is no discernable difference in the medulla patterns among ungulates, but there is a difference from other non-ungulate prey types.

Beaver – *Castor canadensis*

Beaver hairs were easily identified macroscopically due to the distinctive hair shape (Fig. 2) and large amounts of gray, cottony underfur (Fig. 3). Because of these features, scale analysis was never necessary to identify beaver hair (Fig. 3).

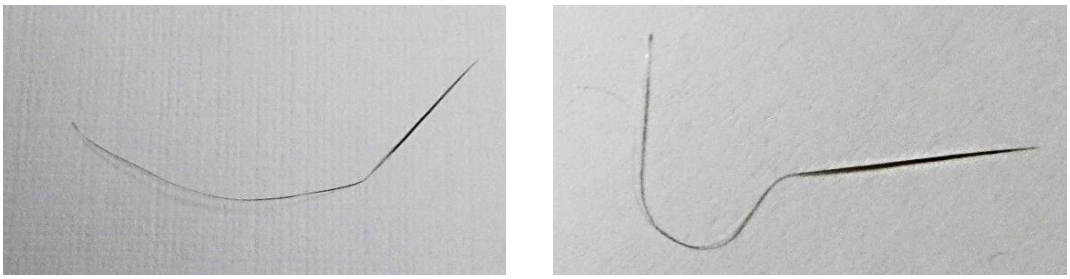
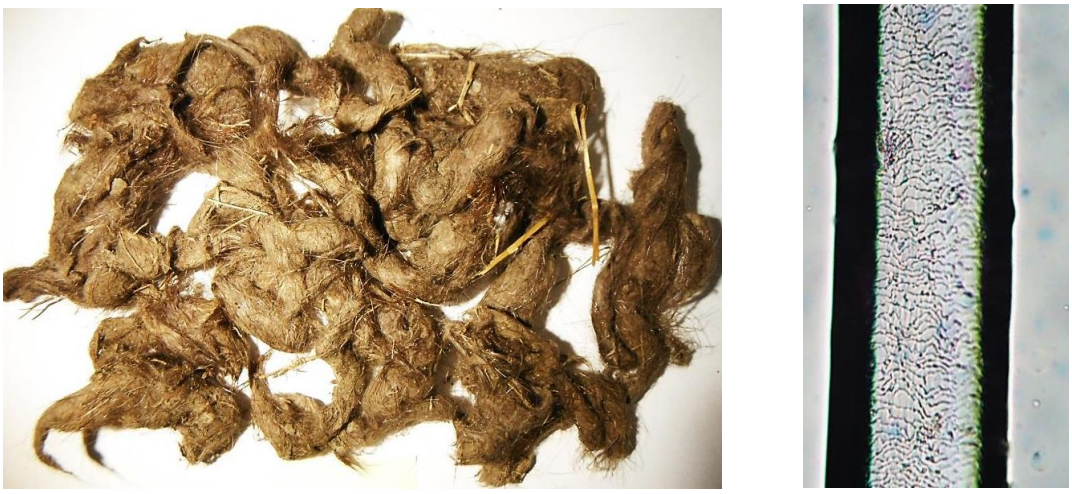


Fig. 2. Macroscopic view of beaver hairs. Beaver hairs typically have a distincitve spear shape with the base of the hair thinner (left image) and the distal end thicker (right image).



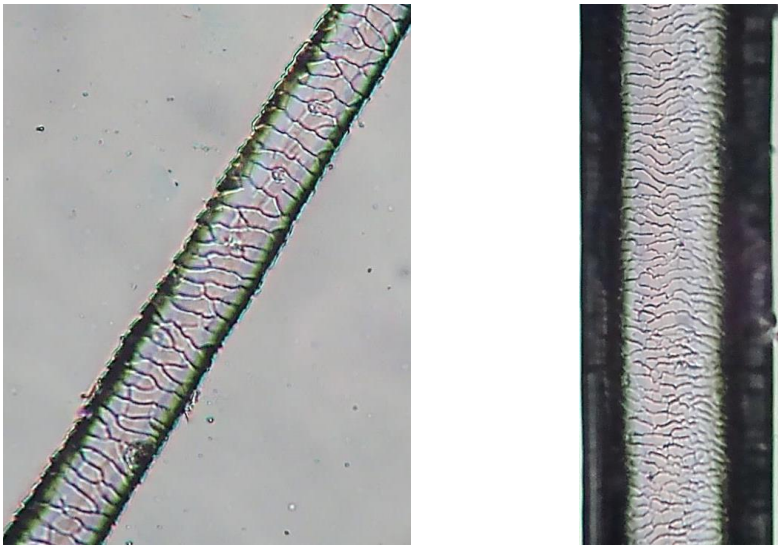
Distal

Fig. 32. Washed scat containing beaver hair and scale pattern of beaver hairs, extracted using Duco Cement©. The downy underfur makes the scat appear fluffy and soft.

Black Bear – *Ursus americanus*

Hairs of black bears are typically black but can be a dark brown. The hairs are long and are often wavy at the distal end of the hair shaft (Fig. 4). Scale patterns of black bears (Fig. 5) and wolves can be very similar. Wolf hairs usually have a distinctive scale pattern in the medial region (Fig. 7) that allow for differentiation, but this pattern is not always present, making differentiation through scale analysis difficult.

Fig. 3. Washed scat containing black bear hairs. Bear hairs are typically black but can be a dark brown. The hairs are very long and are often wavy at the distal end of the shaft.



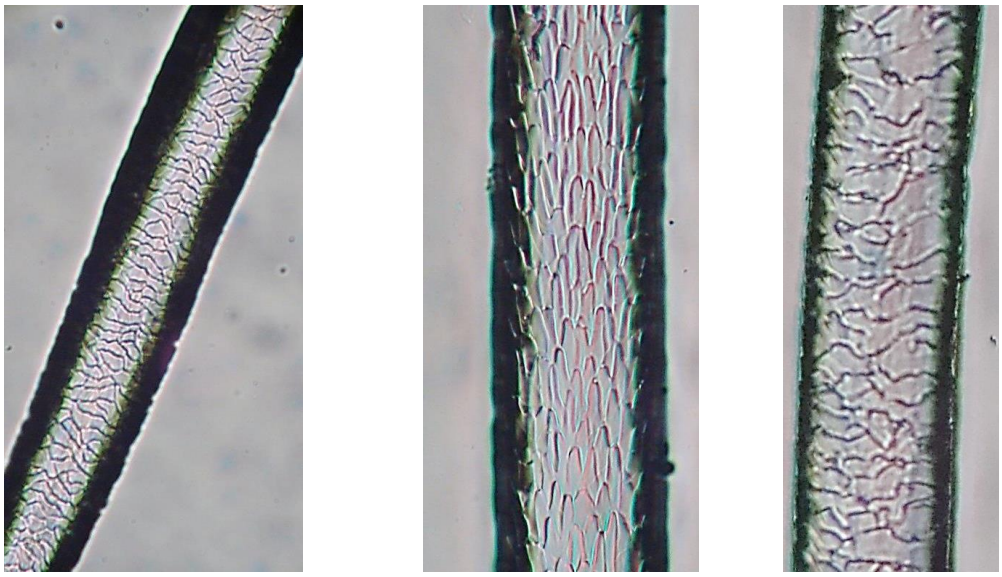
Base **Distal**

Fig. 4. Scale patterns of black bear hairs, extracted using Duco Cement©.

Gray Wolf – *Canis lupus*

Many scats contained a few wolf hairs that were likely from grooming. However, some scats contained a large proportion of wolf hair, and I was unable to determine whether the hairs were from grooming or consumption. Wolf hairs can be a variety of colors, including black, blonde, brown, or banded (Fig. 6). Wolf hairs usually have a distinctive scale pattern in the medial region of the shaft (Fig. 7). Without this pattern, wolf hair is not always distinguishable from black bear hair through scale analysis.

Fig. 5. Washed scat containing wolf hairs. Color can be black, blonde, brown, or banded.



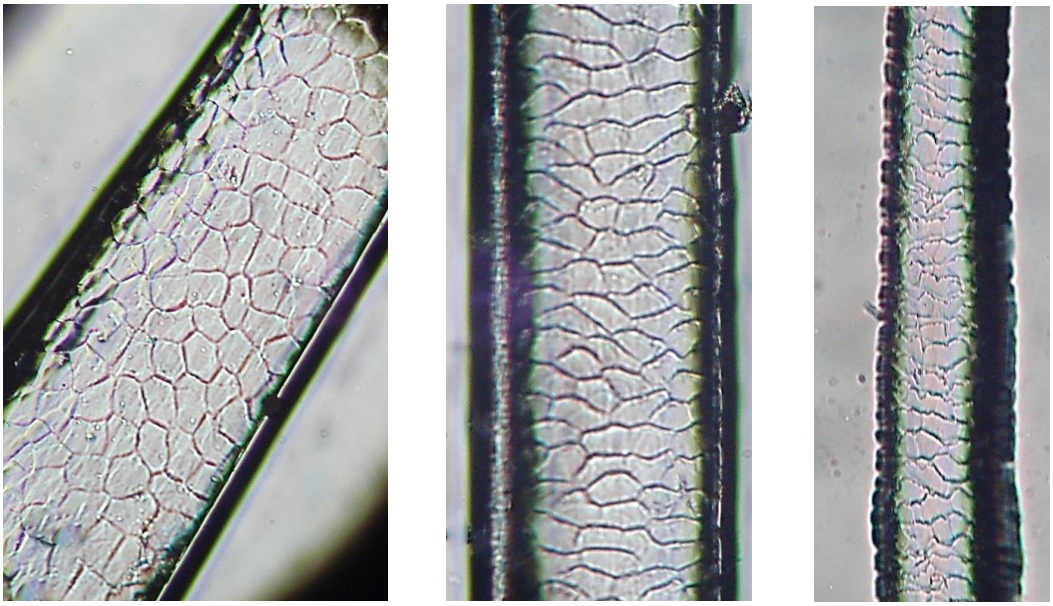
Base **Medial** **Distal**

Fig. 6. Scale patterns of gray wolf hairs, extracted using Duco Cement©.

Moose Adult – *Alces americanus*

Moose hairs are typically dark brown, very thick in diameter, and hollow (Fig. 8). Moose hairs can have a banded color and be lighter, which can be very similar to adult deer hair. These hairs can be differentiated using scale analysis (Fig. 9).

Fig. 8. Washed scat containing adult moose hairs. Moose hairs are hollow and are usually dark brown and thick in diameter.



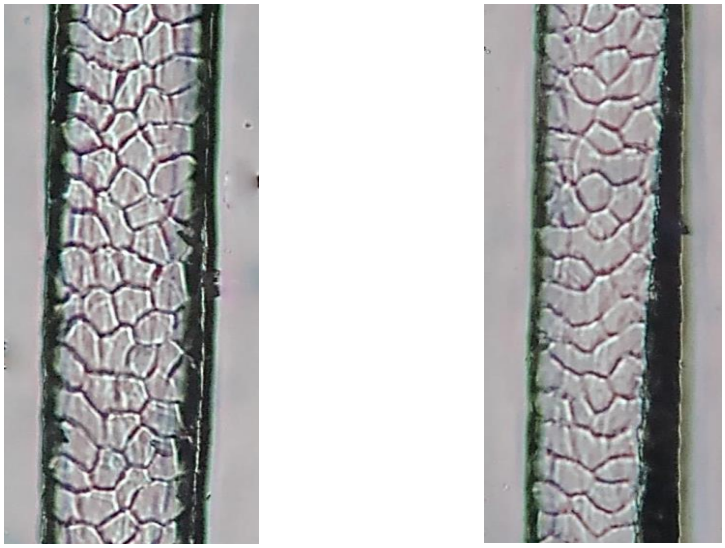
Base **Distal** **Distal**

Fig. 9. Scale patterns of adult moose hairs, extracted using Duco Cement©. The distal portion of the hair can vary in pattern but will always have smaller scales than the base.

Moose Calf – *Alces americanus*

Moose calf hairs are dark brown, downy, and are usually wavy at the distal end of the shaft (Fig. 10). Calf hairs are much thinner in diameter than hairs of adult moose and deer. Calf hairs can be differentiated from deer fawn hairs because the hairs are darker brown and wavy, but occasionally scale analysis is necessary (Fig. 11).

Fig. 10. Washed scat containing moose calf hairs. Moose calf hairs are dark brown, downy, have a thin diameter, and are wavy at the distal end of the shaft.



Base **Distal**

Fig. 11. Scale patterns of moose calf hairs, extracted using Duco Cement©. Scale patterns are similar along the entire shaft, which differs from adult moose and deer and fawns.

Small Mammals

Scats containing small mammal fur have small clumps of underfur with few short, thin guard hairs and many small bones and teeth (Fig. 12). Hairs of small mammals can be identified to the species level using scale analysis (Adorjan and Kolenosky 1969), however, it was very difficult to extract scale patterns of smaller hairs using cement. Hairs of small mammals in scats can be differentiated from other species and grouped into one category by macroscopic analysis because the scats look very different due to the small clumps of underfur and presence of small bones and teeth. The hairs of small mammals are easily distinguishable from snowshoe hare because the hairs of snowshoe hare have easily identifiable medulla and scale patterns. If small mammals need to be distinguished from each other, then the cement method may need to be modified or another scale extraction method may be necessary.

Fig. 12. Washed scat containing small mammal hairs. The scats contain mostly small clumps of underfur and many small bones and teeth.

Snowshoe Hare – *Lepus americanus*

Snowshoe hare hairs have a unique medulla pattern (Fig. 13), which usually allows for species confirmation solely by medulla analysis. If the medulla pattern is not visible, then scale analysis is necessary. Scats containing snowshoe hare often contain teeth, and mostly underfur is visible (Fig. 13). The scats looks similar to scats containing small mammal fur, but the hairs can be differentiated through medulla analysis or by examining scale patterns (Fig. 14).

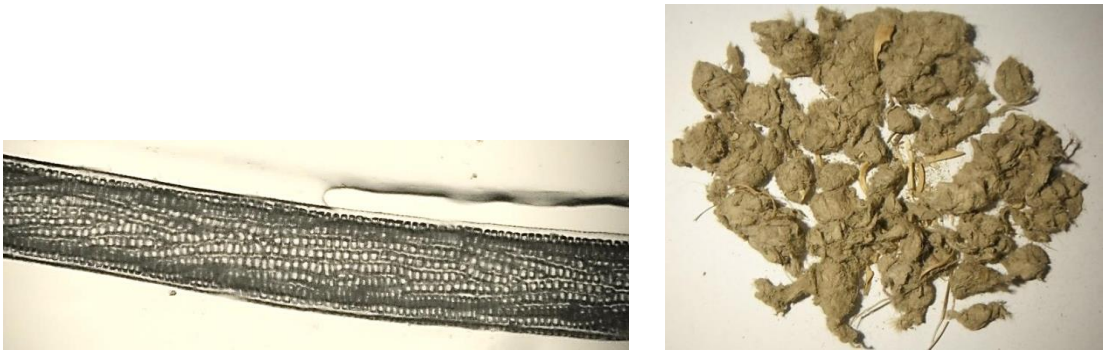
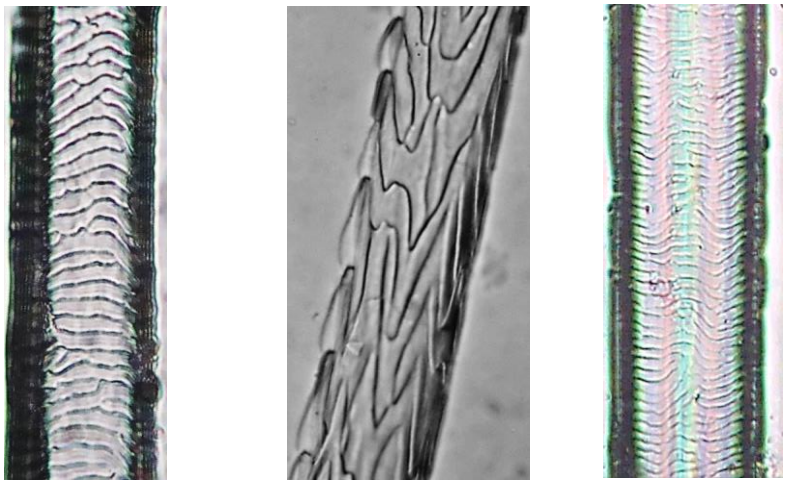


Fig. 13. Medulla pattern and washed scat containing snowshoe hare hairs. The medulla pattern looks like a spiraled ladder and is visible at 200x magnification using a compound microscope. The scats often contain teeth and mostly small clumps of underfur.



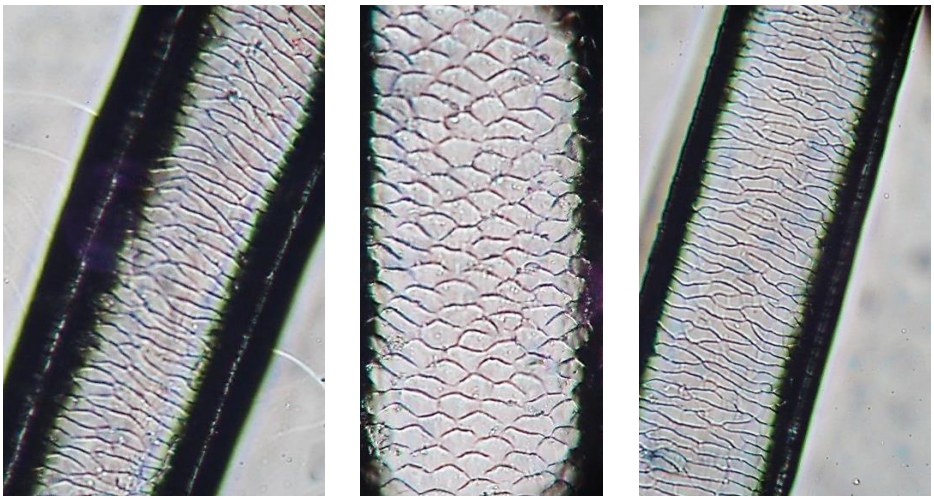
Base **Medial** **Distal**

Fig. 14. Scale patterns of snowshoe hare hairs (400x magnification), extracted using Duco Cement©. Higher magnification was necessary because of small hair diameter.

White-Tailed Deer Adult – *Odocoileus virginianus*

Color and diameter can be used to differentiate between hairs of adult deer and moose, with adult deer hairs typically thinner in diameter and lighter in color than adult moose hairs (Fig. 15). However, both adult moose and deer hairs can have a banded pattern and appear light in color, requiring scale analysis to differentiate between species (Fig. 16).

Fig. 15. Washed scat containing adult deer hairs. Adult deer hairs are hollow and are usually light colored.



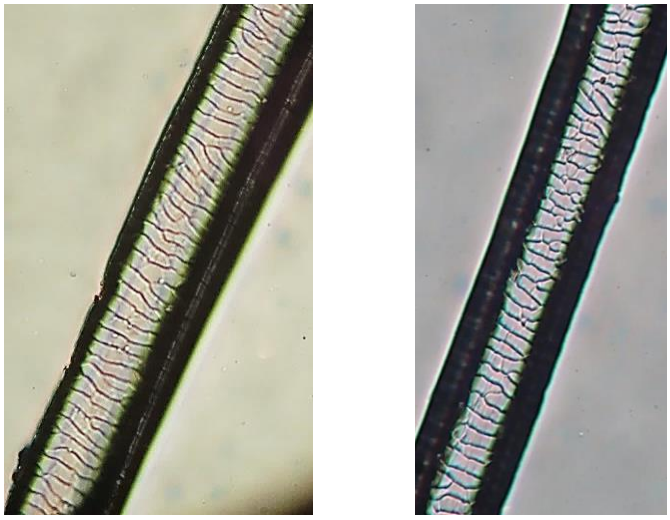
Summer – Base **Winter – Base** **Winter – Distal**

Fig. 16. Scale patterns of adult deer hairs, extracted using Duco Cement©.

White-Tailed Deer Fawn – *Odocoileus virginianus*

Fawn hairs are light red in color, have a thin diameter, and are very downy (Fig. 17). Adult deer hairs are also red in summer and can have a thin diameter, thus these features cannot be used to differentiate between adult deer and fawn hairs. I was not always able to differentiate between adult deer and fawn hairs using scale analysis (Fig. 18), so I differentiated between them by the downy quality of fawn hair. Fawn hair is downy and distinguishable from adult hair from birth through late August.

Fig. 17. Washed scat containing deer fawn hairs. Hairs are fawn colored and downy.



Base **Distal**

Fig. 18: Scale patterns of fawn deer hairs, extracted using Duco Cement©.

Appendix 3: Standard Operating Procedure (SOP) for Handling Wolf Scat

1. Describe health hazards for working with wolf scat

- Wolf scat can contain harmful parasites
- Wear gloves at all times when handling scat, scat bags, and items used in hood
- Treat every scat like it is contaminated
- Wash hands after collecting or processing scat, particularly before eating
- Wash hands thoroughly with soap and water if you touch wolf scat with bare skin
- The disease you are most likely to catch from handling wolf scat is echinococcosis, or cystic hydatid disease. Humans can get this disease by ingesting eggs of the tapeworm. Below are links to various websites that provide information about the parasite:

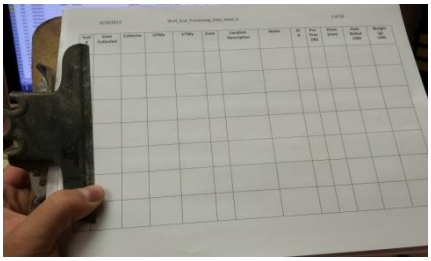
  Washington Dept. of Fish and Game
  Alberta Fish and Wildlife (Sustainable Resource Development)
  Michigan Dept. of Natural Resources
  Newfoundland Natural Resources
  Wyoming Game and Fish

2. Preprocessing Procedure

- Bring the following down from lab to shop

  - Scat caliper (brought down in labeled Ziploc bag)

  - White unsanitized wolf scat bucket

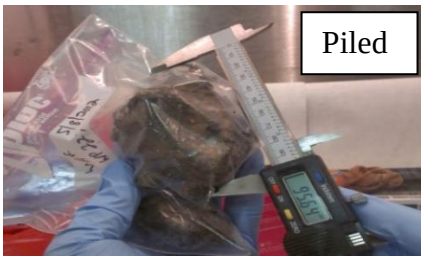


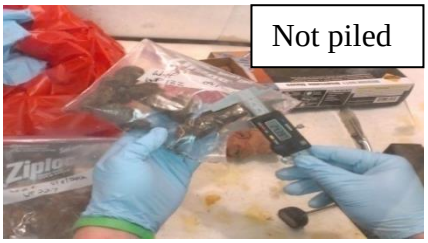
- Clipboard with data sheets

  - Temporary ID tags
  - Latex gloves (in pockets or bucket, not to be worn in hallways)
- Find bag of scat in shop freezer without ID tags
- Designate a recorder (records information) and observer (scat handler)
- Observer responsibilities
  - Read all information given with each scat
  - Measure scat diameter
  - Assign temporary ID tag to each scat
- Recorder responsibility
  - Record all information given by observer onto datasheets
  - Assign permanent ID # to each scat
  - Record observer and recorder initials onto datasheet

- Read all information given with each scat aloud
- Record information on datasheet, also assigning permanent ID# to each scat

- Use calipers to measure widest diameter of scat - while in plastic bag. Record.





- If piled, indicate and measure the widest diameter possible

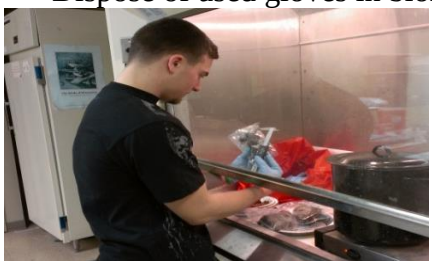
- Assign temporary ID# (stamped brass tag or penny) to each scat

- Drop ID tag/penny into scat bag. Used to identify during processing
- Place preprocessed scats into storage bag with other preprocessed scats
- Invert and remove gloves in shop
- Transfer used gloves to Lab 475 for disposal in biohazard bag
    - Gloves used in wolf scat project should not be thrown in regular trash
- Transfer data from datasheet to excel file
    - See Daily Requirements for directory and saving instructions
- Bring equipment back to lab (caliper inside labeled bag when transporting)

3. Fume hood procedure:

- Never take items used to handle scat out of hood unless directed to in this SOP
- Items taken out need to be labeled (biohazard bag/calipers bag/etc.)
- Do not allow unsanitized items to come into contact with other surfaces
- Work with mask on (recommended)
- Wear latex gloves at all times when handling scat and items in hood
- Use a new pair of latex gloves for each scat to eliminate contamination

- Dispose of used gloves in biohazard bag



- Keep hood cover lowered – should not be raised above chest height

- Keep unsanitized scat (bags and nylons) on 'Unsat Wolf Scat' tin

4. Describe wolf scat sterilization protocol

Small hood in Room 475

- Fill pot with ~3-5 inches of water from sink

- Plug in hot plate. Turn left burner all the way ‘on.’ Bring water to a boil

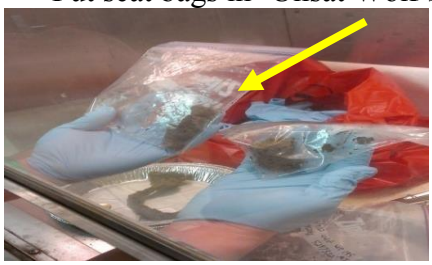
Downstairs in shop

- Bring latex gloves and white ‘Unsanitized scat’ bucket from Lab 475 to shop

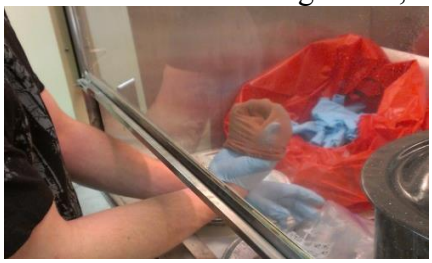
- Put preprocessed scats(ones with temporary Id Tag) in bucket to bring upstairs for sanitizing
- Follow same protocol (as in preprocessing) for glove removal, transport, disposal
- Carry bucket upstairs to Room 475

Small hood in Room 475

- Put scat bags in ‘Unsat Wolf Scat’ tin. Scat should not touch other surfaces

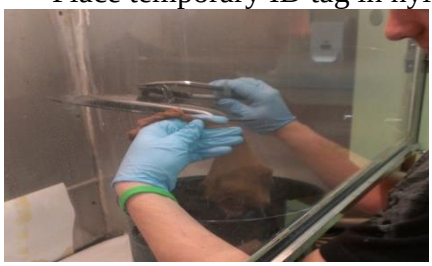


- Record temp ID# and current date on scratch paper provided inside hood
- Break off 1-2” of scat to leave in original bag
- Label outside of bag “done,” with red marker provided inside hood



- Put gloved hand in nylon, grab scat (except for 1-2” piece), invert nylon over scat

- Place temporary ID tag in nylon with scat. Knot top of nylon to close



- Place in boiling water and boil for 45-60 minutes
- Compress air out of “done” bags before sealing closed
- Return “done” bags to shop freezer. Put with other “done” scats
- While scats are boiling, transfer information from scratch paper to datasheets
- After boiling has finished turn off hot plate and unplug

- Using tongs, remove nylons from pot and place in clean tin/plate

- Do not set clean tin/plate down inside hood
- Let scat cool by running under cold water in sink
- While still in nylons, carefully break up scat clumps (may contain sharp bones)
- Put nylons in orange “Sanitized scat” bucket
- Bring bucket and dishwasher contact sign downstairs to Room 141
- Enter all washing data from datasheet to excel file before end of shift

Downstairs in Room 141

- Place nylons between wood frame, lock door, and start dishwasher
- To place in frame:

  - Remove wings, washers, and screen

  - Remove top pieces of wood

  - Manipulate scat to middle of nylon and put nylon ends over wood frame

- Replace wood, screen, washer, and wings – pinching nylon ends in place

- Settings of dishwasher:

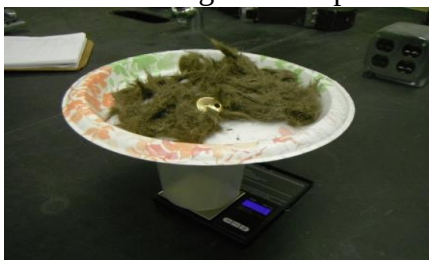
- Heavy Duty
- No drying option selected (all lights off)
- Press Start/Resume
- Cycle takes approximately 150 minutes (2.5 hrs)

- Tape Contact sign (from Room 475) to dishwasher door while in use
- Note: if wood is drying in open dishwasher, OK to set on counter and use
- After washed, return scat to Room 475 in orange "Sanitized scat" bucket
- Remove Contact sign from dishwasher and return it to Room 475

Room 475

- In large hood, cut nylon and empty contents into a clean tin/plate to dry

- Dispose nylon in biohazard bag
- Let sample dry for at least 1 full day
- Record weight of sample



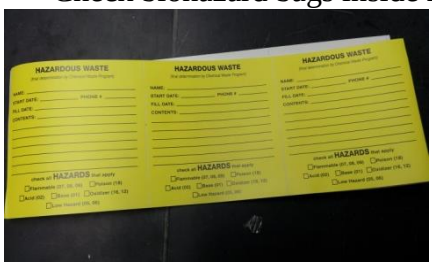
- To record weight:
    - Tare scale (with plastic container)
    - Weigh scat
    - Using temp ID#, find scat on datasheet
    - Record weight and your initials on datasheet
    - Indicate what is included in weight (ex. ID tag, tin or paper plate, etc.)

- Label manila envelope with permanent ID# and empty sample into envelope
- Do not put temporary ID tag in with sample. Remove and wash for re-use

Daily requirements

- Transfer all recorded data from datasheet to excel daily
- This includes:
    - Your initials and current date for everything you did that day
    - Preprocessing information
    - Sanitizing information
    - Massing information
- Directory: Z:\Wolf , Named: 'Wolf_Scat_Processing_Data_X.xls'
- After entering, SaveAs and label with consecutive letter ("X" above, SaveAs "Y")

- Check remaining supplies. If low, indicate on "Scat notes" in 475 and email Ron
- Check biohazard bags inside hood. If full, fill out biohazard label



- To fill out:
    - Write in fill date, your name, and Ron's office number (218)720-4372
    - In contents, write "contains material used in wolf scat processing"
    - For bag in large hood: remove, tie shut, and attach label
    - For bag in small hood: tie shut, remove, and place in 2nd biohazard bag, tie shut and place label on outside of 2nd bag
- Bring bag downstairs
    - If not in, email him about drop off
    - If office is closed, return bag to Lab 475 and bring down the following day

5. Specify decontamination procedures after handling of wolf scat

- Wash counter, sink, drain, and faucet daily
- To clean:
    - Wear latex gloves when handling cleaner
    - Spray directly onto surface, let sit for 5 minutes
    - Wipe with paper towel
    - Spray again and let sit for 2 minutes
    - Rinse thoroughly with hot water and dry with paper towel
- Wash hands thoroughly with soap and hot water after finished

Employee Signature

This is to certify that the employees named below have been trained on this SOP, have read and know the location of this SOP and understands the hazards and safe work practices as detailed in this SOP.

Name	Signature	Date

Supervisor Signature: _____

Date: _____