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DEVELOPMENT OF NOININVASIVE GENETIC TECHNIQUES TO MONITOR ELUSIVE CARNIVORES; A CASE STUDY OF BOBCATS (*LYNX RUFUS*) IN THE NORTHERN LOWER PENINSULA, MICHIGAN

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ABSTRACT

DEVELOPMENT OF NONINVASIVE GENETIC TECHNIQUES TO MONITOR ELUSIVE CARNIVORES; A CASE STUDY OF BOBCATS (*LYNX RUFUS*) IN THE NORTHERN LOWER PENINSULA, MICHIGAN

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Effective and cost-efficient methods of obtaining noninvasive genetic samples would greatly aid monitoring efforts for elusive or rare carnivores. We compared the efficacy of two methods for obtaining bobcat (*Lynx rufus*) genetic samples in the Northern Lower Peninsula (NLP), Michigan: detector dogs trained to find scat samples, and hair snares. Hair snares generated many more samples than detector dogs; however, only two hair samples yielded bobcat multilocus genotypes, while scat samples yielded 9 genotypes. Our results support the detector dog method over hair snares for monitoring the MI NLP bobcat population, both in terms of overall efficacy (# of genotypes obtained) and cost-efficiency. We also quantified the effect that environmental variables have on the probability of scat sample detection by detector dog teams, by placing scat samples from captive bobcats in known locations. Detection of scat samples by detector dogs was most strongly impacted by the distance of the sample from the handler and wind strength, and to a lesser extent, scat degradation. There was also significant variation in success between dog/handler teams. Additionally, we examined the effect of observable scat characteristics on microsatellite genotyping success in order to optimize cost-efficiency of the scat-collection methodology. There was no significant correlation between amplification success and observable scat characteristics, suggesting caution when culling samples based on human perception of scat degradation.

This dissertation is dedicated in memory of my grandfather, Dr. Fowler White, who inspired all around him to appreciate the magnificent detail of the natural world.

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PREFACE

Solitary, cryptic, or wary carnivores, such as bobcats (*Lynx rufus*), are notoriously difficult to monitor. The success of noninvasive genetic population estimation has been hampered for species with cryptic behavior or low density across large geographic areas by a lack of an efficient sample collection technique. The underlying impetus of this project was the development of a cost effective and reliable noninvasive genetic sample collection method for the bobcat of the Northern Lower Peninsula (NLP), Michigan.

Chapter 1 of this dissertation details the biological need for population estimation of the NLP bobcat population. It then discusses the methodological need for genetic sample collection methods and their potential utility within capture-mark-recapture population estimation. Chapter 1 also includes a literature review of bobcat natural history, and of the field and laboratory methods employed during this project. Chapter 1 ends with a summary of the research questions raised by the biological and methodological needs. Chapter 2 discusses the field trials and experiments conducted to address the research questions outlined in Chapter 1. All activities conducted as part of this thesis fall under the IACUC approval # 07/06-091-00.

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CHAPTER 1
INTRODUCTION TO NONINVASIVE GENETIC TECHNIQUES AND BOBCAT (*LYNX RUFUS*) NATURAL HISTORY

BIOLOGICAL IMPETUS:

Bobcats (*Lynx rufus*) have the largest geographic range of indigenous North American native felids (Anderson et al. 2003). Bobcats were listed in Appendix II of the Convention on International Trade of Endangered Species (CITES) in 1975. Appendix II listing dictates that controlled export of bobcat pelts will "not be detrimental to the survival of that species." (www.CITES.org 2006). In 1983, the bobcat was reclassified to a subsection of Appendix II that allowed for bobcat management to include harvest, but the species was not removed from CITES due to a concern over population viability in some local areas of its range as well as its physical similarity to the endangered Mexican lynx (*Lynx rufus escuinapae*) (Anderson et al. 2003). Within the United States, the U.S. Fish & Wildlife Service (USFWS) is charged with regulating species protected under CITES. The Michigan Department of Natural Resources and the Environment (MIDNRE) must prove to the USFWS that state-regulated harvest is not detrimental to the population. The MIDNRE website states that, "The Michigan Department of Natural Resources is committed to the conservation, protection, management, use and enjoyment of the State's natural resources for current and future generations." (www.michigan.gov/dnr 2006) Biological information including abundance, sex ratio, genetic diversity, fecundity, and survival provide the foundation for management regulations of natural populations (Williams et al. 2002; Anderson et al. 2003). It is our responsibility

as scientists to provide biological information regarding the sustainability of natural resource use to our policy makers.

Furbearing wildlife were extensively harvested throughout the 1800s and into the early 1900s leading to dramatic declines in abundance and distribution (Hubert 1982). Harvests were motivated by fur-trade, sport, and livestock protection (Woolf and Hubert 1998). The state of Michigan maintained a bounty on bobcats until 1965 (Hubert 1982). Today, the species' distribution within Michigan is primarily restricted to the NLP and Upper Peninsula (UP)(Woolf and Hubert 1998). Lake Michigan to the west and Lake Huron to the east, form absolute barriers to the NLP population. It is unknown if the Straits of Mackinac to the north is a complete barrier between the NLP and the UP. Dispersal across the strait is probably extremely limited if it occurs at all. The NLP population is partially restricted to the south by high human density and intensive land-use, making the area less suitable to bobcats (Lovallo and Anderson 1996ab, Nielsen and Woolf 2002a), although recent radio-telemetry efforts within MI have shown bobcat locations in residential areas as well as agricultural areas (Svoboda 2008). Considering the virtual 'island' status of the NLP bobcat population, the need for accurate population data may be more important for the NLP population than for bobcat populations where immigration is more frequent. It should be noted, that the western portion of the NLP is closed to harvest, and may serve as a source of individuals for the harvested region of the population. However, the major road systems in the NLP may pose a partial barrier to movement between the harvested

and unharvested regions due to mortality from vehicle collisions (Svaboda 2006ab).

A nationwide state agency survey in 1996 listed the top research needs reported by bobcat managers as: 1. reliable survey methods, 2. demographic data, 3. distribution and abundance, 4. habitat availability and use, and 5. interactions with other carnivores (Anderson et al. 2003). Demographic data of furbearers are often collected as indices, or proxies, of population trends due to cost-efficiency and repeatability (McDonald and Harris 1999) as well as the difficulty in obtaining population estimations from secretive furbearers. Index methods assume that a change in the index count data is proportional to the change in population size. An additional assumption is that proportions remain constant across time periods, or that the proportional change can be estimated (Williams et al. 2002). For example, harvest counts or sightings are assumed to be proportional to the population size. Index counts such as harvest must be corrected for 'effort' which can cause significant variation in the index count unrelated to the actual population abundance (McDaniel et al. 2000, Woolf et al. 2000). Effort can consist of the number of traps set per night, number of days spent in the field per season, number of harvesters registered per year, etc. Reliable reporting rates across years are essential to these indices.

The sensitivity of index methods is debatable (Slade and Blair 2000, Warrick and Harris 2001, Piggott et al. 2006). For example, a change of 10% in the actual population abundance may be undetectable by index methods due to the large variability in the index count data. Undetected changes may be highly

significant for population persistence. At the time of this study, bobcat abundance in MI was monitored using several population indices (Frawley et al. 2005), primarily relying on harvest-effort data (Frawley and Etter 2008). Harvest rate index models use harvest effort, based on fur-taker registration data and surveys, to calculate the relative change in the abundance (Cooley et al. 2007).

Statistical population abundance estimation is a more robust method for evaluating demographic trends than are indices. Population estimation uses a sampled fraction of the population to statistically infer population abundance. Population estimations can be used to validate index methods by concurrently obtaining a population estimate across a number of years to demonstrate index sensitivity. A robust population estimation technique, coupled with validated index methods will provide the data needed to ensure biologically sustainable and cost-effective management of the NLP bobcat population.

METHODOLOGICAL IMPETUS:

Bobcats are solitary, crepuscular, cautious, and elusive (Rollings 1945, Nielsen and Woolf 2002a). These characteristics make the bobcat an extremely challenging animal to study and are the reasons that the population size is currently monitored using population indices. Methods available for population monitoring include life-table population models and population reconstruction, accounting type models, and mark-recapture population estimation. Life table population models require a large amount of demographic data, often making them infeasible for elusive carnivores (Anderson et al. 2003). Population density

estimates can be developed using home-range sizes and expanded into a population estimate for a specified geographic area (Pruess and Gehring 2007). This method also requires labor-intensive trapping and radio-telemetry activities. The ability to expand local density estimates to a regional population estimate is also questionable (Nielsen and Woolf 2002a).

A suite of statistically robust population estimation methods exist, based on basic 'mark-recapture' techniques. The essence of mark-recapture is the estimation of the sampled fraction using count statistics (Pollock et al. 1990, Nichols 1992). The first step is to capture, mark, and release a sample (M) of the target population (N). Traditional methods of marking include minor mutilation (e.g. ear-punch), attached tag, or surface mark (e.g. paint). Next, a second sample is captured from the population (n) and the number of marked animals in the second sample is counted (m). The number of marked (m) animals out of the total number captured in the re-capture session (n), multiplied by initial number of marked animals (M) provides an estimate of population size (Pollock 1990).

$$M/N \approx m/n \quad \rightarrow \quad \hat{N} = \frac{nM}{m}$$

Traditional marking methods have a number of drawbacks including the possibility of injury to the animal, the mark changing, or the mark being lost entirely (ACUC 1998). Minor mutilations may heal over, endanger the health of the animal, or may be difficult to observe in the future without handling the animal a second time (Cattet et al. 2008). Tags or surface marks may reduce the camouflage of cryptic species or can be lost.

An alternative to traditional marking involves using genetic information to identify individual animals. Genetic 'marks' cannot be changed or lost within the lifetime of the animal, and can be gathered without handling the animal. Noninvasive samples include genetic material shed by an animal. Sample sources could include hair, scat, urine, egg-shells, or feathers (Waits and Paetkau 2005). Mitochondrial DNA extracted from noninvasive samples can be used to identify species or trace maternal ancestry (Mills et al. 2000b, Waits and Paetkau 2005). Microsatellites or other hypervariable genetic markers may be used to identify individuals, or determine population structure (Mcrae et al. 2005; Waits and Paetkau 2005).

Microsatellite genotypes (genetic information unique to an individual animal) can be easily incorporated into standard population estimation methods. Genotypes may be used in place of traditional ear-tags or other marks within mark-recapture population estimation (Schwartz et al. 1998; Miller et al. 2002; Piggott et al. 2006). Each unique genotype observed in the first sampling period is considered a 'captured and marked' individual. Genotypes, rather than individual animals, are 'recaptured' in a second sampling period. In order for marked animals to be recaptured during the second period, the method of initial sample collection must be noninvasive as opposed to a method that would remove them from the population (e.g. harvest). The second, or final, sampling period does not need to be noninvasive. Therefore, it is possible to use a combination of noninvasive samples and tissue samples to conduct a genotype-based mark-recapture population estimation. Dreher et.al (2007) successfully employed a

noninvasive hair-snare method to collect genetic samples from black bears (*Ursus americanus*) throughout the NLP of MI. Genotypes obtained from hair samples were considered marked individuals. The authors then used the annual harvest to collect tissue samples. From the ratio of marked genotypes found in the harvest, they were able to estimate black bear population abundance for the NLP study area.

The switch from handling animals to using noninvasive genetic information constrains the ability to collect certain ecological data. Schwartz et.al. (1998) points out that data pertaining to age structure as well as health and reproductive status are not available when the animal is not captured. However, the development of hormonal analysis from noninvasive samples holds promise for collection of these data (Foran et al. 1997; Wasser et al. 1997).

Two major factors may hamper efforts to use noninvasive genetic mark-recapture methods of estimating population abundance. First, researchers may encounter difficulties in obtaining a sufficient sample size. Secondly, there is potential for high levels of genotyping errors associated with degraded samples (Schwartz et al. 1998). Additionally, the cost of laboratory analysis may be high relative to standard methods. Current methods used to obtain noninvasive genetic samples are numerous, and yet few are effective for many animals. Hair-snares are a common method of obtaining noninvasive genetic samples from carnivores. For hair snares to be successful, the hair station must first attract an animal to a site and then induce the individual to leave a sample (e.g. , hair with root follicle). Visual attractants may be used, including pie tins, bird wings, or bird feeders.

Stations may also be baited with meat or scent lures. Scent lures used for felids have included bobcat urine or beaver (*Castor canadensis*) castor (McDaniel et al. 2000), and even Calvin Klein's Obsession perfume (Mickleburgh and Fisher 2003). Once attracted to a site, hair must be pulled out by the root by a physical device such as a hook or sticky substance. The root contains the hair follicle cell, which is the source of genetic material.

Hair snares have proven to be successful for certain carnivores, including black bear and coyote (*Canis latrans*) (Woelfl and Woelfl 1997, Gehring and Swihart 2002, Dreher et al. 2007) but are unreliable for felids such as cougars (*Puma concolor*), and bobcats (Long et al. 2007a). A study area often needs to be saturated with hair-snares in order to obtain sufficient samples even for basic presence/absence studies (McDaniel et al. 2000). For example, the U.S. Forest Service implemented the national Lynx Survey in 1999 to determine the distribution of lynx (*Lynx canadensis*) on federal land across 12 states. Even though the number of samples per area needed to establish 'presence' was minimal, several hundred permanent and temporary federal and state employees were needed to check the 13,000 hair snares to obtain the required number of samples (Mills 2000b). The need for more robust biological data of the NLP bobcat population combined with doubt surrounding the effectiveness of hair snares due to bobcat natural history led to the overall goal of this study: to assess noninvasive genetic techniques for monitoring populations of elusive carnivores.

BOBCAT NATURAL HISTORY:

Bobcats are representative of elusive carnivores for which managers lack a robust method for noninvasive genetic sample collection. Bobcats have many natural history characteristics that make them particularly enigmatic to researchers. Breeding time, prey items, and home-range size vary considerably across the species' range, and are discussed in more detail below. Habitat preferences also vary widely, making it difficult to conduct habitat-specific searches without *a-priori* knowledge of local habitat preferences. There is considerable variation in suitable habitat depending on geographic region. For example, studies from Massachusetts and Maine have documented a higher use of hardwood associations relative to available habitat (McCord 1974, Litvaitis et al. 1986, Litvaitis et al. 1987). Chamberlain et al. (2003) found that bobcats preferentially used young pine plantations more often than other habitat types in Mississippi. Bobcats were even found to preferentially use old-field areas in Tennessee. These habitat preferences most likely reflect prey abundance in specific habitat types (Kitchings and Story 1984). In Michigan, Pruess and Gehring (2007) found a preferential use of lowland habitats, specifically lowland coniferous forests, compared to available habitat. Lowland coniferous forests provide good cover habitat for stalking prey as well good thermal cover.

In Indiana, where fir and pine associations dominate, there was preferential use of specific under-story vegetation. Bobcats preferred Douglas fir (*Pseudotsuga menziesii*)-mountain mahogany (*Cercocarpus sp.*) but avoided Douglas fir-wheatgrass (*Triticum aestivum*)(Koehler and Hornocker 1989). A

number of studies have found that microhabitat conditions such as stem density (number of woody stems per unit area) and vertical cover (height and density of overhanging vegetation) may play a more crucial role to habitat preference than general stand type (Rollings 1945, Anderson et al. 1990, Kolowski and Woolf 2002). However, the preference for high density of vegetative cover may be more complicated than simple preference (Kolowski and Woolf 2002). For example, in Maine, bobcats avoided sparse understory (<12,000 stem cover units/ha) and preferred dense stands (>36,000 stem cover units/ha), but the habitat preference was not a linear function of stand density beyond a threshold level of cover (Litvaitis et al. 1986).

Other habitat factors that have been suggested to be important to bobcats include: shelter from severe weather, availability of rest shelters, freedom from human disturbance, reduced snow depth, and cover from predators (Rollings 1945; Koehler and Hornocker 1989). Rock-outcropping was found to be important to bobcats in some studies (McCord 1974, Koehler and Hornocker 1989) and not in others (Kolowski and Woolf 2002). In Wisconsin, paved roads were avoided by bobcats, but there was a high density of unpaved roads within home ranges (Lovallo et al. 1996ab). McCord (1974) found that bobcats spent a good deal of their travel time on secondary roads in MA. In Idaho, bobcats were found preferentially on steep slopes (Koehler and Hornocker 1989) while in Maine they preferred flat areas, avoiding slopes greater than 5° (Litvaitis et al. 1986). Kolowski and Woolf (2002) attempted to quantify bobcat microhabitat preference in IL. Twenty-two microhabitat variables were recorded at 121

locations. Microhabitat variables were tested within a linear model framework to predict bobcat presence. None of the resulting models had greater than 70% correct classification when compared to telemetry locations.

Suitability for den sites may also affect bobcat movement. Requirements for den sites are that they are dry, well hidden, and yet accessible to the adult female (Rollings 1945). A variety of den site characteristics have been recorded including under windfalls, hollow standing snags, hollow logs, cliffs, depressions at the base of stumps, piles of brush, rocky terrain, and abandoned woodchuck holes (Rollings 1945, Kitchings and Story 1984). Breeding seasons vary by region, further complicating interpretations of variables associated with habitats occupied. In WY, bobcats are polyestrous, breeding from Feb to June with kittens born May15-June 15 (Crowe 1975). Gestation is approximately 70 days. Litters average 2.8 kittens (Crowe 1975). Overall, the amassed data demonstrate that the bobcat is a habitat generalist, making predictive models of habitat preference extremely difficult to develop.

Prey density is most likely the overriding characteristic that dictates habitat use by bobcat (Rollings 1945, McCord 1974, Kitchings and Story 1984, Koehler and Hornocker 1989, Chamberlain et al. 2003). Lagomorphs are the predominant prey item throughout much of the bobcat's range, including Michigan (Dearborn 1932, Rollings 1945, Progulske 1955, Bailey 1974, Fritts and Sealander 1978, Kitchings and Story 1984). Lagomorph prey species include cottontail rabbits (*Lepus sylvaticus*) in AK, TN, VI, and ID, snowshoe hare (*Lepus americanus*) in MN, ME, and jackrabbits (*Lepus timidus*) in ID. In

Michigan, Dearborn (1932) determined bobcat diet to be composed of 89.6% hare. However, there is a wide range of potential prey items for bobcats. Many state-wide studies have found a significant proportion of bobcat diet composed of white-tailed deer (*Odocoileus virginianus*) between 5.5% occurrence in VI to 35% in MN (Rollings 1945, Progulske 1955). Dearborn (1932) found that white-tailed deer constituted 3.5% of the bobcat diet. It is inconclusive whether the white-tailed deer found in bobcat diet is prey or carrion. There is often a seasonal difference in the proportion of deer in the diet, the highest proportion occurring just after hunting season (Progulske 1955). Other prey items comprising a significant proportion of diet include muskrat (*Ondatra zibethicus*) (2.6%), mice (2.6%), birds (1.2%), squirrel (0.7%), porcupine (*Erethizon dorsatum*) (0.1%), and skunk (*Mephitis mephitis*)(0.1%) (Dearborn 1932). Additional species that contributed to diet included: woodchuck (*Marmota monax*), opossum (*Didelphis virginiana*), shrews, voles, rats, blue jays (*Cyanocitta cristata*), pocket gophers, raccoons (*Procyon lotor*), chipmunks, and chicken (*Gallus gallus domesticus*). Trace occurrences of Canada lynx (*Lynx canadensis*), cow (*Bos grimigenius*), goat (*Capra aegagrus hircus*), red fox (*Vulpes vulpes*), rattlesnakes, rat-snakes, and moose (*Alces alces*) have also been found (Rollings 1945, Progulske 1955, Bailey 1974, McCord 1974, Crowe 1975; Fritts and Sealander 1978, Kitchings and Story 1984, Litvaitis et al. 1986, Koehler and Hornocker 1989, Anderson 1990, Chamberlain et al. 2003). Information on bobcat prey was used to tailor both our study site design and hair-snare attractants.

Approximate adult weights for bobcats range from 9.0 kg (female) to 12.3 kg (male) (Crowe 1975, Litvaitis et al. 1986). Average body length was recorded as, female= 786 mm, male = 869 mm (Anderson et al. 2003). Crowe (1975) estimated the average lifespan of female bobcat to be 12 years, and found that individuals remained sexually active until death. Vehicle collision was the primary source of mortality within unharvested populations. During a study in TN, two out of six radio-collared cats were killed by automobiles (Kitchings and Story 1984). In IL, 10 of 19 bobcats were killed by automobiles and 2 were killed by trains (Nielsen and Woolf 2002b). Other sources of mortality include natural causes such as old age, starvation, disease and predation, as well as incidental trapping injury. Annual survival rates of harvested and unharvested populations in Idaho were 0.61 and 0.87, respectively (Knick 1990). Within the NLP, major sources of mortality include the annual harvest, trap-related injury, and vehicle collisions (Svoboda 2006a,b).

Home range size varies across the geographical range of bobcats, between sexes, seasons and individuals. Smallest estimated home range for a female was estimated to be 8.64 ± 0.51 km^2 (Chamberlain et al. 2003). The largest estimated home range for a male was estimated to be 112.2 ± 22.4 km^2 (Litvaitis et al. 1987) (Table 1.1). Some studies have found a significant difference between seasons (Koehler and Hornocker 1989; Chamberlain et al. 2003) and others have not (Nielsen and Woolf 2001)(Table 1.2). Bobcat social organization is characterized by higher levels of female-male overlap than same-sex overlap, and virtually exclusive female core areas (Bailey 1974; Nielsen and Woolf 2001; Pruess and

Gehring 2007). This spatial organization is rather unusual for mammalian species where it is more common for higher overlap among females rather than males involved in mate competition. This may be a result of greater female competition for kitten-rearing resources than for male mate competition. Population density has been estimated as low as 0.05 per km^2 to as high as 1.53 per km^2 (Table 1.3).

Table 1.1: Average Estimated Adult Bobcat Home Range Size (km^2) by Sex. (± = Standard Error) and Method of Estimation by State. (MCP = Minimum Convex Polygon)

State[a]	Estimate Female km^2	Estimate Male km^2	Method of Estimation
TN	25.9	76.77	Principal Component Method 95%
ME coast	30.2 ± 10.0	71.1 ± 54.3	MCP
ME inland	27.5	112.2 ± 50.6	MCP
MS	8.63 ± 0.95	17.25 ± 2.11	Adaptive kernel 95%
IL	9.1 ± 1.2	19.4 ± 2.2	Fixed kernel 95%
MN softwood	49 (Range: 14-85)	46 (Range: 35-59)	MCP
MN mixed	32 (Range: 6-67)	61 (Range: 14-156)	MCP
MI	11.9 ± 5.7	36.6 ± 20.5	MCP
MI	14.4 ± 8.7	51.8 ± 33.1	Adaptive kernel 95%

[a][MS (Chamberlain et al. 2003)]; [MN (Fuller et al. 1985)]; [(Kitchings and Story 1984) TN]; [ME (Litvaitis et al. 1986)]; [IL (Nielsen and Woolf 2001)]; [MI (Pruess 2005)]

Table 1.2: Average Bobcat Home Range Size by Season (ID)[b]

Summer	Winter
22.7 ± 16.5km^2	88.1 ± 60.3 km^2

[b](Koehler and Hornocker, 1989)

Table 1.3: Bobcat Population Density Estimates by State.

State[c]	Population Density Estimation (bobcat/km^2)
ID	0.43
IL	0.27 - 0.34
CO (harvested)	0.05 - 0.10
CO (un-harvested)	0.77 - 1.53

[c][ID (Koehler and Hornocker 1989); [IL (Nielsen and Woolf, 2002a)]; [CO (Anderson and Lovallo, 2003)]

It is unclear how social boundaries are maintained. Evidence of aggression during transition periods (after a home range is vacated by death or migration and before re-settled) has been documented in Mississippi (Benson et al. 2004). However, literature suggests that borders are maintained by passive means such as scent marking (feces, urine, anal gland), sign (scrapes), and avoidance (Bailey 1974, Anderson 1988, Nielsen and Woolf 2001). In an unusual case, four individuals of different sexes occupied a single rock-pile refuge for a period of two weeks with no aggression (Bailey 1974).

Chamberlain and Leopold (2005) conducted a study of interspecific competition among bobcats, coyotes, and grey fox (*Urocyon cinereoargenteus*) in Mississippi. Although all three species had extensively overlapping home ranges, foxes actively avoided establishing core areas within areas of high bobcat or coyote density. They found considerable overlap of bobcat home ranges within coyote core areas.

GENETIC SAMPLE COLLECTION TECHNIQUES:

The field of molecular ecology draws upon the disciplines of genetics, evolutionary biology, landscape ecology, population ecology, conservation biology, and animal behavior. As the field of molecular ecology emerged, genetic information was predominantly obtained from tissue or blood samples. More recently, noninvasive genetic samples, such as hair and scat have allowed researchers to obtain information on wildlife populations will little to no impact on individual animals (Waits and Paetkau 2005). Noninvasive samples contain much smaller quantities of DNA than do tissue or blood. Hair samples contain DNA in the hair follicle at the base of the hair shaft. Scat samples contain DNA from the sloughed cells of the intestine. The advent of Polymerase Chain Reaction (PCR) allowed for DNA amplification from very small quantities of template material (Creel et al. 2003, Eggert et al. 2003). In addition to the small quantity of DNA found in noninvasive samples, DNA is often degraded or commingled with PCR inhibitors that reduce PCR efficiency (Wasser et al. 1997). The technology to overcome these challenges has only recently been developed, making noninvasive genetic analysis possible for many species (Waits and Paetkau 2005). However, the low quantity and quality of DNA remains a significant source of failed DNA amplification from noninvasive samples. Therefore, it may be necessary to collect a far larger number of noninvasive samples than tissue or blood samples in order to reach project goals. However, it is may be easier to collect a larger number of noninvasive samples than tissue samples, resulting in a more representative sample of the population.

Collecting noninvasive samples is relatively simple for those species that use roads or trails as movement corridors, as humans may easily monitor for sample deposition by wildlife (Ruell and Crooks 2007). Other species, such as black bear, are easily induced to deposit hair samples (Dreher 2004). However, for many wildlife species, the largest challenge to the development of noninvasive genetic studies lays in sample collection methods. Species that have wary or timid behavior, low population densities, or cryptic movement or hunting patterns are particularly challenging. Species that lack specific habitat preferences are even more difficult due to the uncertainty surrounding sample locations.

Bobcats have behavioral characteristics, population densities, movement patterns, and habitat preferences that pose a challenge to population monitoring. Therefore, the bobcat represents a prime candidate species for trials of innovative noninvasive genetic sample collection techniques. This project evaluates three sample collection techniques: 1. hair snares, 2. detector dogs, and 3. trapper mail-collection system.

Hair Snares: Hair-snares require that an animal is first attracted to a site, and then induced to deposit a hair sample. The snare must pull the hair out by the root so that the follicle cell is attached. Hair snare stations may consist of barbed wire around a tree, carpet nails through a piece of carpet tied to a tree, or Velcro pieces with staples punched outward. More elaborate hair snares may consist of glue pads on spring-loaded trip-wired arms or frayed break-away neck snares. Success rates for these techniques vary significantly by species and by

environment. Hair snares that act as scratch-posts are less likely to affect animal behavior (less likely to elicit a trap-shy response), less likely to injure the animal, and less likely to mechanically fail. Hair snares that are on trip wires or have break-away mechanisms are only capable of obtaining a single sample. For example, once a neck snare is broken, the snare cannot be used again. Single-sample traps do, however, eliminate the problem of multiple animals depositing samples on a single snare. Multiple visitations are problematic in areas with high population density. Considering the relatively low densities of bobcat populations, it may be more important to consider the expense of replacing snare elements than with multiple visitations when designing a bobcat monitoring program. Hair snares are ideally placed in areas of high animal traffic. In areas where bobcats are not harvested, they often use trails or road systems (Ruell and Crooks 2007). However, in areas of bobcat harvest, including our study area, bobcats may avoid road systems where human presence is more pronounced.

Baits used to attract animals to hair snare sites may include white-tailed deer (Kitchings and Story 1984) and/or other animal meat (Nielsen and Woolf 2001). However, bait is not a necessary component of hair snares and may be a strong attractant for non-target animals. Scent lures may be detectable at longer geographic ranges and produce stronger olfactory cues than meat baits. Many scent lures are designed to target groups of wildlife, such as felids, or canines specifically. The efficacy of many lures, both natural and commercial, were tested for lynx by McDaniel et.al. 2000. Beaver (*Castor canadensis*) castoreum and catnip oil were significantly more effective than were commercial lures.

Bobcat urine has also been proposed by local MI trappers as well as Kitchings and Story (1984) as an effective lure for bobcats. Bobcats are considered wary overall, but may tend to be curious about unusual (especially shiny) objects in the environment (Rollings 1945). Many scientific studies and fur-trappers have taken advantage of this behavior by adding visual attractants to their hair snares or traps (McDaniel et al. 2000, Nielsen and Woolf 2001). Bird wings and pie tins are two popular visual attractants.

Detector Dogs: The locations and environmental characteristics surrounding wildlife defecation are central questions when developing a scat sample collection technique. While defecation is an integral part of the daily routine of every animal, literature on bobcat defecation rates and locations in the wild is extremely scarce. It is difficult to interpret the significance of scat locations encountered by humans. Scat locations may not reflect a particular preference by the animal, but rather those sites that are easily visible to humans, and therefore recorded preferentially. Dearborn (1932) noted that feces were usually found on high ground and less than 100ft from the edge of swamps on bare soil. Rollings (1945) stated that bobcats usually defecated off game trails and covered their droppings. Rollings also noted that bobcats would often deliberately leave the worn trail to deposit scat on slightly elevated spots such as snow drifts, or on top of logs. However, in snow covered terrain, the warmth of the feces usually causes the scat to settle into the snow and may lead the observer to believe the scat was covered by the animal. The behavior of covering scats

does not seem to be uniform, nor does inconspicuous vs. conspicuous placement. Bailey (1974) writes, "Although adult bobcats sometimes covered their feces, at other times they left exposed, conspicuous feces along their routes of travel and near caves and rockpiles." Of the 60 scats found by Bailey (1974) 60% were covered and 40% exposed. This ratio is very similar to Erickson (1944) where 52% of 42 scats were covered and 48% exposed.

There is uncertainty regarding the use of latrines by bobcats. Latrines are defined as conspicuous groups of feces and urine possibly deposited by multiple individuals. Bailey (1974) found that latrines could contain over 50 feces in an area 1 meter in diameter. Bailey found 42% of feces were near known den sites and 58% near frequently traveled areas such as ridgelines. Bailey stated that most of the recently-deposited feces at the latrines were made by females with kittens, but it is unknown how they determined which individuals were visiting the site. Many of the latrines were established late summer and early autumn, corresponding to the time kittens begin traveling with their mothers. Bailey found no regularity of the rate at which these latrines were used. One location was used once every three months, while another was left alone from Sept until the following spring. Interestingly, two bobcats defecated at the latrine only after feces were experimentally removed by researchers.

Previous carnivore studies using scat samples as genetic material have relied on opportunistic collection along trails (Kohn et al. 1999, Ernest et al. 2000, Ruell and Crooks 2007) or have watched animals to know where feces are deposited (Creel et al. 2003). Neither of these methods is feasible for bobcats in

the NLP. NLP bobcats most likely, use thick cover, are crepuscular, solitary, defecate off of trails, and may cover scats. Latrines exist in low density, may not be used often, and then, only by a subset of the population (biased toward females) (Bailey 1974).

Dogs trained to detect scat of specific species offer an alternative for searching for scat samples. Training techniques for scat-detector dogs were adapted directly from canine narcotics techniques (Wasser et.al. 2004). Dogs are chosen for high play-drive and appropriate size for field conditions. They are trained to find scat from specific species, and are rewarded with play time with a ball. Many of the dogs employed as wildlife detector dogs are rescued from shelters because their energy level and ball-obsession make them unfit as domestic pets.

While several wildlife monitoring studies have employed detector dogs, only a few have quantitatively evaluated which environmental factors influence scat detection by dogs (but see Long et al. 2007b). Determining the relative influence of climactic factors, vegetation, scat degradation, and differences between dog/handler teams is critical to the future application of detector dogs to wildlife research. However, environmental considerations are critical concerns to researchers when choosing a noninvasive genetic sample collection method. Is the vegetation too dense for dogs to maneuver? Will the high winds of this region affect scat detection? Will the dogs find scat even if it was deposited a long time prior to sampling? This study addressed questions such as these related to the efficiency of detector dogs.

Trapper Mail Collection: During the legally regulated coyote trapping season, fur-trappers inadvertently catch bobcats while trapping for coyote (Frawley et al. 2005, Michigan Trapper Association, Pers comm). Bobcats are released if found alive in a trap. If killed in the trap, the trapper may obtain an incidental take permit, but must surrender the bobcat to the MIDNRE. In 2004, 2,180 furtakers in the NLP obtained a trapping license. In this year, there was also an officially regulated bobcat trapping season in the NLP for 11 days only (December 10-20) with a 1 bag limit per person. Approximately 15% (326) of the registered furtakers set for bobcats and 151 bobcats were trapped, and nearly 50% (68) of these animals were released alive (Frawley et al. 2005). Obtaining genetic samples from released animals has the potential to dramatically increase both sample size and geographic coverage possible by a single research team. Prior to requesting the assistance of MI trappers, fur-takers were asked for input on the best method for retrieving hair samples from incidentally caught bobcats during a MIDNRE furbearer stakeholder meeting in late 2005. During this meeting, fur-takers were presented with the idea of assisting with bobcat research by submitting samples through the mail. The response was extremely positive.

Fur-takers must register all harvested bobcats with MIDNRE. We were provided the list of registered furtakers for the 2006 season by the MIDNRE. The Pigeon River Study site is encompassed by Cheboygan, Otsego, Presque Isle, and Montmorency counties. The extent of these counties includes a substantial amount of area beyond the study site. We assumed that the majority of furbearer trapping activities would be conducted within the county of residence. Collection

packets were mailed to all 500 households with registered furtakers throughout the four counties. Coyote trapping season began October 15, 2006 and the collection packets were distributed the following week. Collection Packets included an informational flier and three sample collection envelopes (Figure 1.1). The informational flier detailed the purpose of the project, how the addition of trapper samples would assist with the project goal, and how to handle the hair so that it remained viable until its arrival at the lab. Emphasis was placed on returning samples from only live and released bobcats. Sample collection envelopes included spaces for date of capture and location of capture.

Unfortunately, only one envelope was returned with a hair sample, and no DNA was retrieved from that sample. An additional 3 packets were returned with notes indicating that their trapping activities precluded the possibly of incidental bobcat capture. Failure of the Trapper Mail Collection was most likely due to insufficient communication between the research team and the public prior to packet distribution. The lack of communication was due to complicated political conditions surrounding the management of the MI bobcat population at the time of this study. Improved communication and publicity could dramatically improve the success of this method, especially considering the positive response of furtakers during early planning discussions. A trapper mail collection should not be discounted as a sample collection method since it was relatively inexpensive and has great potential to supplement CMR sample size if participation was bolstered.

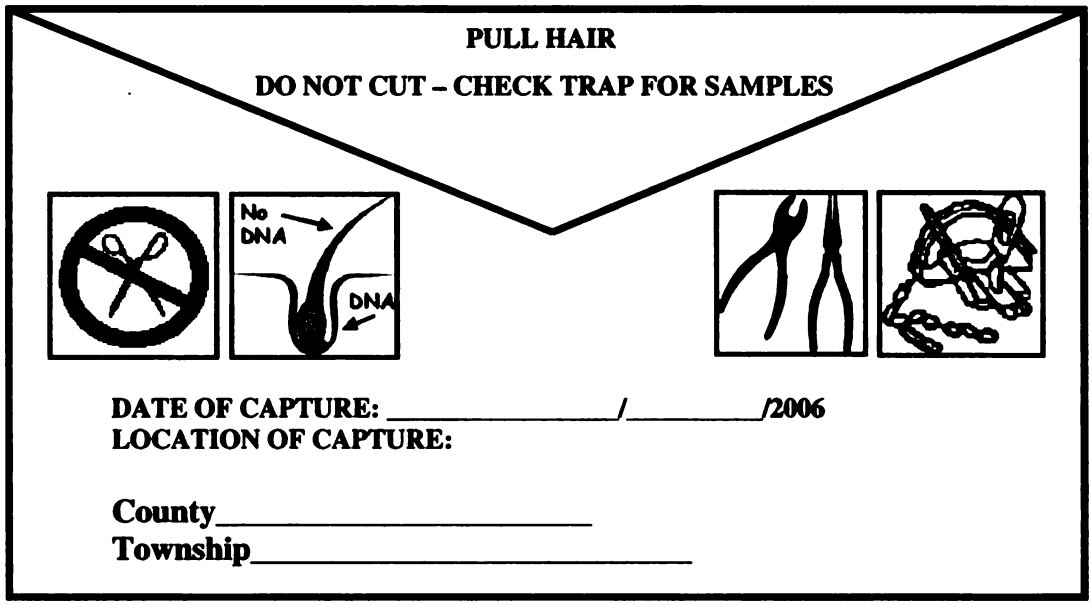

Figure 1.1: Example of Sample Collection Envelope used in the Trapper Mail-Collection System. Reverse side was the pre-printed MSU address and paid postage.

LABORATORY TECHNIQUES:

Microsatellites are nuclear, co-dominantly inherited genetic markers comprised of short-tandem repeats of base pairs (e.g.: CACACACACA). Microsatellites are useful for identifying individual animals, estimating relatedness, determining population genetic structure, and determining gene flow (Kohn and Wayne 1997, Schwartz et al. 1998, Scribner and Pearce 2000). Nauta and Weissing (1996) caution that microsatellites have a limited utility and should be specifically used for small populations (<5000 individuals) and short time-spans (<2000 generations). For each microsatellite locus there are a certain number of possible alleles. As the number of possible alleles per locus (polymorphism) increases, there is a lower probability that one individual animal will have the same allele as another. A genotype consists of the specific combination of alleles across several loci. The probability of identity (PID) (the

probability that two individuals share the same genotype by chance) is obtained by multiplying the probability of sharing an allele by chance across loci (assuming loci are independent). Therefore, PID decreases with increased polymorphism per locus and with additional loci. Conversely, PID may be increased by certain demographic patterns that cause individuals to share alleles at a higher probability than expected. For example, severe demographic fluctuations may lead to reduced genetic variation within a population (genetic bottleneck) due to higher level of relatedness of subsequent generations (Schwartz et al. 1998). Bottlenecks may result in misleading PID due to a lower level variation at individual loci than expected based on number of alleles (Taberlet and Luikart 1999).

Genotyping (assigning a genotype to an individual) has several possible sources of error. Before the sample arrives at the lab, contamination of samples, mislabeling of containers, or mishandling of data may lead to errors. Within the laboratory, errors may be caused by allelic dropout (failed amplification of a single allele within a heterozygote resulting in false homozygotes) (Taberlet et al. 1996), null alleles (when the primers do not recognize a mutation because it occurred in the flanking region), false alleles (erroneous alleles generated by slippage early in the amplification process) (Taberlet et al. 1997), and human error (Miller et al. 2002). The probability of error is multiplied with the addition of each locus. Therefore, the ideal number of loci is a balance between desired probability of identity and potential increase in error rate. Factors to consider when choosing microsatellite loci include: 1. level of polymorphism of the loci

(fewer loci are needed if each locus has many alleles), 2. size of microsatellite (larger sequences have higher probability of failed amplification), and 3. quality of the DNA (noninvasive samples tend to be degraded and are prone to allelic dropout) (Taberlet et al. 1996). Extraction of DNA from both hair and scat samples should be conducted as expediently as possible following sample collection in order to optimize DNA quality and minimize mishandling of samples while in storage.

Genotypes resulting from noninvasive sample are particularly prone to errors (Broquet and Petit 2004, Broquet et al. 2007). Means of investigating error rates of false alleles and allelic dropout are numerous. A method proposed by Taberlet et.al. (1996, 1997) includes repeated extraction and a "multiple tubes approach". "Multiple tubes" refers to multiple PCR for each DNA extraction. Taberlet et.al. (1997) ran 1-5 extractions per single-hair sample for Pyrenean brown bears (*Ursus arctos*) and then ran PCR with the multiple-tubes approach. A "matching approach" proposed by Creel, et.al. (2003) accepts genotypes if they are observed more than once. Requiring an exact match between genotypes may cause bias in the population estimation if there are genotyping errors. Rarefaction technique is another attempt to incorporate error rates into population estimation (Frantz and Roper 2006). The rarefaction method plots the cumulative number of unique genotypes as the number of sampled individuals increases, forming an asymptotic curve. The point at which the plot asymptotes is the population estimate. The plots are simulated multiple times (>1000x) and the frequency of

the asymptotic estimates are plotted, the peak of the curve being the presumed population estimate.

Most genotyping errors can be resolved with repeated PCR of the same samples. However, in the case where two PCRs yield different genotypes due to error, when they are actually from the same individual, populations size may be overestimated (Frantz et al. 2003, Paetkau 2003). The opposite effect, called the "shadow effect" is caused when different samples are thought to be same individual due to genotyping errors when they are actually from different individuals. The "shadow effect" is evident by heterogeneity of individual capture probabilities and can be accounted for within model software such as CAPTURE (Mills et al. 2000a).

Miller et.al. (2002) describes a maximum likelihood approach that is less costly than using multiple tubes, and less stringent than 'matching' technique. The maximum likelihood approach focuses additional PCRs on genotypes that differ at only a few loci, or genotypes that are most likely scored inaccurately, such as those with many homozygotes. Genotypes are considered the same individual if they mismatch by a certain number of alleles. Many studies have employed maximum likelihood approach with minor alterations (Frantz et al. 2003, Paetkau 2003).

STUDY OBJECTIVES:

This chapter has summarized the impetus for this project and introduced the field and laboratory methods. Three study questions arose from the biological and methodological needs outlined above:

Question 1: How do the noninvasive sample methods compare (e.g., number of samples obtained, number of samples per unit effort, etc.) when employed in the field? To address this question, hair snares and detector dogs were employed to collect bobcat noninvasive samples in the NLP, Michigan.

Question 2: Once scat has been collected, is it possible to effectively cull "poor-quality" samples using observable characteristics of the scat? The scat samples collected during field trials were used to address this question. The ability to cull scat samples based on characteristics observable by human senses could decrease the costs of using scat as genetic material.

Question 3: Are there environmental variables that will affect the ability of detector dogs to locate scat samples? The development of an efficient method for collecting samples must include optimization in the field as well as the laboratory. Captive bobcat scat samples placed in known locations were used to address this final question. Chapter 2 details the methods, results, and conclusions from our investigation of these three questions.

CHAPTER 2
RELATIVE EFFICACY OF GENETIC SAMPLE COLLECITON METHODS FOR BOBCAT (*LYNX RUFUS*) IN THE NORTHERN LOWER PENINSULA, MI & QUANTITATIVE ASSESSMENT OF ENVIRONMENTAL FACTORS AFFECTING SCAT DETECTION BY DETECTOR DOG TEAMS.

INTRODUCTION:

Traditional methods for monitoring wildlife, such as collaring or capture-mark-recapture (CMR), become logistically difficult and expensive when wildlife species are rare, have large geographic ranges, preferentially use areas of dense vegetation, or have particularly wary behavior. The development of molecular techniques within the field of wildlife ecology has allowed scientific investigation of many elusive wildlife species. Noninvasive samples have become a valued source of genetic and physiological information (Waits and Paetkau 2005), especially when the species is difficult to observe directly. Genetic data gathered from noninvasive sources have been successfully incorporated into CMR population estimation techniques for the wide ranging black bear (*Ursus americanus*) (Dreher et al. 2007), presence-absence survey for the endangered San Joaquin kit fox (*Vulpes macrotis mutica*) (Smith et al. 2005), and population genetic analysis for the wary cougar (*Puma concolor*) (Ernest et al. 2000).

The bobcat population of the Northern Lower Peninsula, MI is an excellent system for testing the efficiency of novel noninvasive genetic sample collection for several reasons. First, the bobcat (*Lynx rufus*) possesses many of the enigmatic characteristics typical of felid carnivores. For example, they are crepuscular, use dense cover for ambush hunting (Schaller 1996, Preuss and

Gehring 2007), maintain large homeranges (between 11.9-51.8 km^2 in MI; Pruess and Gehring 2007), and have cryptic behavior (Rollings 1945, Nielsen and Woolf 2002a). Secondly, there is substantial political and scientific impetus for improving monitoring capabilities for the MI bobcat populations. The NLP bobcat population is subject to annual harvest and the sustainability of the harvest is monitored by the MI Department of Natural Resources and the Environment (MIDNRE) using indices including population reconstruction and harvest effort data (Earle and Tuovila 2003, Cooley et al. 2007, Frawley and Etter 2008). A more quantitative population monitoring technique would involve genotype-based mark-recapture population estimation using noninvasive genetic samples and harvest samples. The underlying impetus of this project was to develop an effective and cost efficient noninvasive genetic sample collection method for NLP bobcat population.

Two commonly used sources of noninvasive genetic samples are hair and scat (Waits and Paetkau 2005). Hair samples must include the root, for the hair follicle cells are the source of DNA. A wide variety of "hair snare" devices have been employed to collect hair samples. Squeeze tubes with glue traps were used in Michigan's Upper Peninsula to collect hair samples from fisher (*Martes pennanti*) and American marten (*Martes americana*) (Williams et al. 2009). Barbed wire snares were also successfully employed in Michigan to collect hair samples from black bear (Dreher et al. 2007). This study used a typical hair-snare configuration of a carpet patch with tacks tied to a tree, as well as several alternative configurations. One drawback to hair snares is the necessity of luring

the animals to the device and inducing them to leave a sample, which may bias capture probabilities between individuals or sexes due to differing levels of trepidation to visit the snare. Additionally, if multiple hair samples from the same individual are deposited between checks, it is impossible to determine whether they were from one or multiple visitations.

An alternative method to hair snares is the collection of scat samples. The majority of carnivore studies using scat samples have relied on opportunistic collection along trails (Kohn et al. 1999, Ernest et al. 2000, Ruell and Crooks 2007) or have watched animals defecate (Creel et al. 2003). Collection of scat samples solely along trails or roads can bias sample collection if certain individuals or sexes use linear features more than others (Vynne 2010). Furthermore, neither direct observation nor trail collection are possible for species, such as the bobcat, that use thick cover, are crepuscular, solitary, cryptic, may avoid trails, or cover scats (Rollings 1945, Nielsen and Woolf 2002a). A solution is to employ scat detector dogs (Wasser et al. 2004) to find samples on or off-trail. Detector dogs are working domestic dogs (*Canis lupus familiaris*) trained to detect scat of specific species. Training techniques for scat-detector dogs were adapted from canine narcotics techniques. Dogs are rewarded for each target-species scat they find with play-time with a ball. Detector dogs search based on olfactory cues, but are capable of learning to visually cue their search to specific areas where the species of interest is likely to defecate. Dog handlers must be aware of the mannerisms of their dog to facilitate the search, and must also be aware of wind direction and topography. The guidance of the handler and

the learned search patterns of the detector dogs may help increase scat detection, but may also introduce bias if the search is preferentially directed towards features in the landscape used by certain individuals or sexes. An additional caveat to the scat detection method is that the location of scat samples may not perfectly reflect movement patterns of an animal if defecation occurs in specific locations, such as territorial boundaries. A benefit to scat collection is that each scat sample can be assumed to represent a single deposition event, and therefore multiple scats in an area represent multiple visitations to that site. Within the context of CMR population estimation, each separate scat represents an independent sample.

Only a handful of studies have evaluated relative merits of scat sample collection using detector dogs compared to other methods such as hair snares (Wasser et al. 2004; Smith et al. 2005; Long et al. 2007; Furtado et al. 2008). There has also been little comparative cost/effort data between alternative genetic sample collection methods, which is vital information for any entity planning a genetically-based wildlife monitoring program (Long et al. 2007a). Therefore, our first research objective was to determine the efficacy (number of bobcat genotypes obtained) and cost- efficiency of hair snares versus scat detector dogs for obtaining genetic samples from bobcats. We also investigated whether our survey effort was sufficient to yield a CMR population estimation by obtaining genetic samples from bobcat harvest season immediately following the field trials.

The success of our alternative noninvasive genetic sample collection methods ultimately depends on species-specific natural history and behavioral characteristics. Previous studies have shown that bobcat preferentially use linear

features such as low-use roads or riverbeds as movement corridors (McChord 1974, Harrison 2006, Ruell and Crooks 2007). However, there is a strong possibility that bobcats within our study area avoid roads due to the associated hunting pressure. Both sexes are more territorial toward same-sex individuals than toward opposite sex individuals. Females generally occupy smaller home-ranges, and female home-ranges may partially overlap or lay completely within the home-range of a male (Pruess 2005). The average adult male home-range size for a bobcat varies widely throughout its range. In Michigan, the estimated mean home-range size for adult female bobcats is 11.9 ± 5.7 km^2 and adult male is 36.6 ± 20.5 km^2 using the minimum convex polygon method (Pruess 2005).

Successful genetic sample collection methods for bobcat in the NLP MI will need to be able to cover a large geographic extent given the home-range size of bobcats. Additionally, it would be beneficial for a method to detect individuals at a fine spatial grain (in other words, to detect two individuals in close proximity to each other) due to bobcat territorial behavior leading to partial overlap of individual home-ranges. Finally, a successful collection method should be flexible enough to accommodate both road-use and road-avoidance movement behavior. Both hair snares and detector dogs may be employed in a manner to accommodate these three criteria for success. Detector dogs are reported to be extremely target-specific (Smith et al 2005) and so the number of bobcat genotypes obtained from scat samples may be greater than for hair snares which are not as target specific a method. However, hair snares have been successfully employed to collect lynx hair samples when the study area is saturated with snares

(McDaniel et al. 2000). Therefore, we predicted that hair snares and detector dogs would generate approximately the same number of bobcat genotypes over the duration of the study season.

The success rates for obtaining multilocus genotypes from noninvasively collected samples has been shown to be highly variable (Mills et al. 2000; Waits and Paetkau 2005). The presence of bacteria, moisture, and PCR inhibitors degrade DNA within scat samples and prevent successful DNA amplification. Given the variation in quality of scat samples detected by dogs in previous studies (See Wasser et.al. 2004, Vynne 2010), researchers would benefit from the development of criteria for culling "poor quality" samples based on observable scat characteristics prior to laboratory analysis. The 'age' and odor of scat samples has previously been suggested as predictors of DNA quality (Wasser et al. 2004). Scat diameter may also predict genotyping success since the loci chosen for this project are designed to amplify felid DNA only, and non-target samples may be larger or smaller than bobcat scat. Since detector dogs are trained to be species-specific, the strength of their "alert" may indicate their confidence that the sample comes from the target species. Long et al. (2007b) also assessed "confidence", but in their case, they assessed whether the handlers could correctly identify scat samples to species. In our case, we were interested in whether the alert of the dog could predict both the target-specificity and quality of the sample (both are necessary for successfully genotyping). Therefore, our

second study objective was to examine the effect of observable scat characteristics on microsatellite genotyping success.

Few qualitative studies have been conducted that demonstrate the extent to which environmental factors impact the rate of scat sample detection by dog/handler teams (but see Long et al. 2007; Cablk et al. 2008). Distance of the sample, wind speed and direction, and vegetation characteristics are likely factors that impact scat detection. Specifically, understory vegetation has been considered in previous studies as a potentially important factor affecting species detection via scat samples (Long et al 2007b). The impenetrability of local understory vegetation could potentially impede detector dogs and/or handlers. Additionally, dense understory vegetation may disrupt air-current between the sample and the dog, preventing odor detection. Microhabitat, defined as the physical characteristics of the vegetation immediately surrounding scat samples, may also impact scat detection. For example, dogs may be more likely to detect a sample at the base of a tree, because they are visually drawn to the tree as an object in space, rather than if the sample was in the middle of an open field with no "focal-point". Characteristics of the scat samples (age or odor), as well as wildlife diet, or behavior of the dog/handler teams may also influence scat detection. Behavior of the dog/handler teams, both as the sample period progresses and between the teams themselves, may affect scat detection. Climactic factors, including temperature, wind-speed and direction, or precipitation can affect the availability of scent to the dog and hence scat

detection. The third study goal was to quantitatively evaluative the effect of environmental variables on scat detection by dog/handler teams.

The approaches used to address our three study objectives were as follows: Objective 1: To compare the efficacy and cost- efficiency of collecting bobcat noninvasive genetic samples from hair snares versus scat detector dogs, we used field trials of both methods across the Pigeon River study area in the NLP MI. We predicted that the Catch per Unit Effort (CPUE) would be higher for the detector dog method than for hair snares due to the target-specificity of the dog's search. However, due to the greater expense of the detector dog method, we predicted that the Cost-per-Genotype would be lower for the hair snare method. Objective 2: We used scat samples collected during the field trials to quantify the effect of observable scat characteristics on microsatellite genotyping success. We predicted that increased odor, older age, and extreme scat sizes will negatively affect genotyping success. We also predicted that the strength of the dogs' alert at the sample will be positively correlated with genotyping success. Objective 3: We conducted experimental transects with captive bobcat scat to quantitatively evaluate of the effect of environmental variables on scat detection by detector dog teams. We predicted that stronger wind would be the primary factor affecting scat detection and that dense understory vegetation and distance to sample would reduce scat detection. We also predicted that the scat content (diet), scat age, and precipitation would have lesser impact on scat detection. This prediction was made based on the purported robustness of the detector dog search to sample quality and weather. We also expected that the categories of microhabitat that

contained a focal point for detector dog search will have an increased probability of detection due to the potential for dog/handler teams to visually direct the search. Based on findings by Long et al. (2007b) we expect to see significant variation in dog/handler team scat detection rates.

STUDY AREA:

The NLP is a mosaic of vegetation types under both private and public ownership including portions of Pigeon River and Mackinaw State Forests. Stand types include lowland conifer dominated by cedar and fir, pine plantations, upland deciduous, lowland mixed conifer/deciduous and upland mixed. The Pigeon River Study Site (Figure 2.1) was chosen for its accessibility and historically high bobcat population density based on analyses of many years of harvest data (D. Etter, MIDNRE, *pers. comm.*). Boundaries between public and private land primarily consisted of barbed wire fence, and were assumed not to pose barriers to bobcat movements. Several private land-owners were contacted for permission to establish sample locations within their property bounds. Bobcat core home-ranges may be primarily confined to lowland areas (Press and Gehring 2007), but movement, and therefore sample capture is possible across a wide range of habitat types. Therefore, the entire study area was treated as a contiguous area of potential bobcat habitat covering approximately 1,320km^2. The study area falls completely within the MI counties of Cheboygan, Otsego, Presque Isle, and Montmorency.

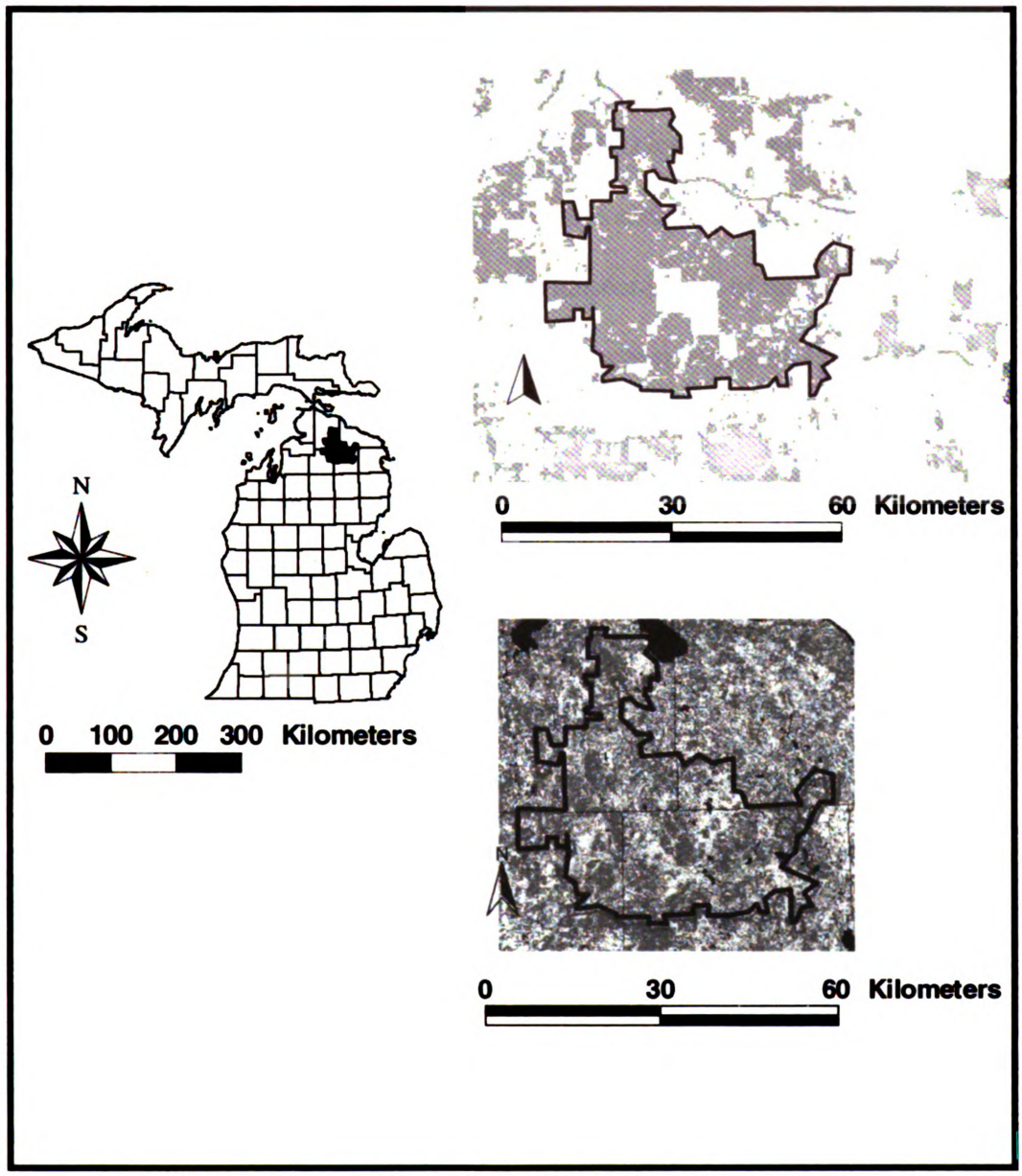

Figure 2.1: (*Left*): Pigeon River Study Site (shaded black) in the Northern Lower Peninsula, MI. 1,320 km^2 (*Top Right*): Public land shaded. (*Bottom Right*): Bobcat Habitat Quality Prime (dark grey), Marginal (light grey), Poor (white), Open Water (black).

METHODS:

Objective 1. Compare the efficacy of detector dogs versus hair snares.

Field Methods Hair Snares: A total of 100 hair snares were deployed along twenty transects (5 stations each). Within each transect, the hair-snares were separated by at least 1 km, for a total transect length of approximately 5 km. Hair snare transects were established in a grid system with each transect approximately 5 km apart so that even a small bobcat home-range would overlap with at least one hair snare (See Figure 2.5). The DNA found in the hair follicle is highly susceptible to degradation from heat or moisture. Therefore, snares should be checked often to ensure the highest possible quality of DNA could be obtained from hair samples. To facilitate weekly checks across the entire study site, hair-snare transects were established along roads. Hair snare transects along roads also take advantage of possible use of roads by bobcats as travel corridors. However, to accommodate possible road-avoidance behavior due to hunting pressure within the study region, 3 of the 5 snares per transect were set 0.25 km away from the road. The first, third, and fifth station of each transect were set off the road, for a total of 60 "off-road" hair snares. At 0.25 km from roads, the sounds of vehicles were significantly decreased or were not detectable by investigators. The scent lure placed in the canopy was designed to attract animals that may be traveling further off-trail. Therefore, we assume that locating stations at 0.25 km from the road was adequate to accommodate any road-avoidance behavior. The second and fourth stations along each transect were set within the tree-line adjacent to the road, for a total of 40 "near-road" hair snares.

All 60 off-road snares consisted of carpet pieces with tacks punched through from the back (tacks were lightly hammered to create a hook at the end). Three types of snares (carpet pieces = 15, sections barb-wire = 15, and pieces of Velcro® = 10) were used at the near-road stations, and they were tied to a wooden stake in the ground. The near-road stations also had sand track plates around the stake to monitor wildlife visitation (Figure 2.2). Snares were tied 0.5 m from the ground to facilitate cranial rubbing behavior (McDaniel et al. 2000). In addition to the hair snare device, stations included the two visual attractants (feathers and pie-tin), a long distance commercial scent lure suspended in the canopy, bobcat urine <5 ft from the snare, and catnip oil on the snare itself. Transects were deployed in August 2006 and re-baited with scent lures weekly through mid-November 2006. Hair snares were deployed during the fall season so that potential genetic 'marks' from the samples would be obtained just prior to the hunting season (Jan 1 – March 1, 2006) to minimize any violation of the demographic population closure assumption.

Figure 2.2: Examples of Hair Snare Stations: (*Left*) Carpet patch with tacks tied to tree (L) with feathers as a visual attractant. (*Right*): Barbed wire tied to wooden stake including feathers and cotton balls soaked in catnip oil surrounded by sand for track counts. All stations also included long-distance lure in canopy, and a pie tin hanging from a nearby branch.

Field Methods Scat Detector Dogs: Data layers from the Michigan Geographic Data Library (www.mcgi.state.mi.us/mgdl/) were analyzed using ArcView 3.0 GIS. The numerous land-use classifications of the "Lower Peninsula Land Cover 2001 IFMAP" data layer were reclassified into four classes of bobcat habitat: 1.prime, 2.marginal, 3. poor, and 4.open water (Figure 2.1) based on knowledge of NLP bobcat habitat preference (Preuss and Gehring 2007) and similar reclassifications of land-use to bobcat habitat (Nielsen and Woolf 2001). Forested and scrub land-use were classified as prime. Open fields were classified as marginal as bobcats may use these areas for ambush hunting but

likely do not frequently use these areas (Pruess and Gehring 2007). Poor habitat included active agricultural or developed land.

A stratified random design based on bobcat habitat was chosen to optimize the limited time with detector dogs, while still sampling all possible habitat classifications. More sampling effort was placed in prime habitat where we were more likely to encounter bobcat scat. However, to account for uncertainty regarding habitat use by bobcats, some sampling locations were placed within marginal or poor habitat classifications. Animal Movement Extension in ArcView 3.0 was used to generate random points within 20 m of roads, and within each level of habitat suitability (prime, marginal, and poor). Transect start points within prime (n=19), marginal (n= 13), and poor (n= 3) habitat were chosen randomly from the generated points. Thirty-five triangular transect routes from start points were laid out to avoid limitations such as river crossings (See Figure 2.7). Transects were 9 km long (3 km each leg) to maximize distance searched, and yet ensure that the entire transect could be completed within one day even when traveling through difficult terrain. Triangular routes were chosen to return the dog/handler team to the field vehicle at the end of the search, and to maximize the area searched off trail. Triangular transects were used rather than wandering or circular transects to give the handlers concrete geographic locations (the off-road 'points' of the triangles) to reach, and thereby standardize their search patterns. Two detector dog teams (handler and dog) completed the 35 transects between December 1 and Dec 19, 2006. There was heavy snow fall immediately preceding the detector dog field trials, and the first week of field

trials were in deep snow. The snow melted rapidly, and the rest of the field trial was conducted in clear weather or rain. Team J.Sayers/Bruiser searched 2 poor, 6 marginal, and 10 prime transects, while J.White/CJ searched 1 poor, 7 marginal, and 9 prime transects. Scat samples were also opportunistically collected while completing other field activities such as checking hair-snares and searching for captive-scats, as the dogs could not be dissuaded from finding scat while in the field for other activities. Scat samples were collected by inverting a plastic bag, with one scat per bag to avoid con-specific contamination as well as human contamination. Relative confidence in the 'alert' from the dog was recorded for each scat detected (None = passed without hesitation, Low = Checked out but did not sit, Medium = sit response but not strong, High = strong sit). These confidence levels were assigned based on the range of behavior observed during training with known bobcat and non-bobcat scat samples. Photo-documentation of the surrounding vegetation was also taken at each scat (4 photos in cardinal-directions).

Laboratory Methods: Hair samples were stored dry at –20°C and extracted within 3 days of field collection. Up to five hairs were used per sample (greater than five hairs tends to clog the filtration system). All scat samples were frozen (-20°C) until transport to Michigan State University campus for DNA extraction. Known bobcat tissue samples were obtained from the MIDNRE from all individuals harvested within the four counties encompassing the study area. Tissue samples were frozen (–20°C) until DNA extraction.

DNA was extracted from hair and tissue samples using DNeasy® Tissue Kit and from scat samples using QIAmp DNA Stool Mini Kits (protocol for large stool samples) (*Qiagen* Inc., Valencia, CA). Following the multiple-tubes approach to minimize genotyping error, each scat sample was extracted twice (given sufficient material) and DNA from each extraction was genotyped using two independent PCR (Taberlet et al. 1996).

Low quality DNA from noninvasive samples is particularly prone to low amplification success and genotyping errors which can lead to incorrect identification of individuals (Taberlet and Luikart 1999, Paetkau 2003). The first step to addressing these problems is to appropriately choose molecular markers to minimize genotyping error. Fewer loci are needed to obtain a sufficient probability of identity (PID) as the polymorphism of individual loci increases. However, with each additional locus needed, you increase the chance of genotypic error (Waits et al. 2001). Therefore, past studies (e.g. Paetkau 2004) have recommended that noninvasive genetic studies should use the fewest loci necessary to yield an expected PID that is sufficient to identify individuals. Shorter fragment lengths have a lower probability of genotyping error associated with the PCR process (Frantzen et al. 1998, Roon et al. 2003). Three microsatellite markers developed for domestic cat (*Felis catus*) were chosen for this study based on high level of polymorphism and short fragment length; FCA026, FCA043, and FCA090 (Menotti-Raymond and O'Brien 1995, Menotti-Raymond et al. 1999) (Table 2.1). Four additional microsatellite loci were used to distinguish between potential matching genotypes (6HDZ056, 6HDZ057,

6HDZ610, 6HDZ700) (Williamson et al. 2002) (Table 2.1). Two nuclear sex identification markers, SRY/ZFX (Aasen and Medrano 1990, Sinclair et al. 1990, Pomp et al. 1995) were used to determine the presence of DNA of sufficient quality for microsatellite amplification (Pilgrim et al. 2005), as they consistently amplified DNA from local carnivores in prior studies in Michigan (Williams 2006).

Allele size ranges for FCA loci are documented in the literature (Menotti-Raymond and O'Brien 1995; Menotti-Raymond et al. 1999; Ernest et al. 2000). However, it was unknown if the FCA microsatellite loci would amplify from non-felid species. Tissue samples from sympatric carnivore species were analyzed using FCA loci to ensure our ability to exclude non-target carnivore samples from bobcat. Non-target species included badger (*Taxidea taxus*), coyote (*Canis latrans*), grey fox (*Urocyon cinereoargenteus*), red fox (*Vulpes vulpes*), river otter (*Lontra canadensis*), and grey wolf (*Canis lupis*). Additionally, the 6HDZ loci allele ranges were documented for bobcat and cougar (Williamson 2002), but were unknown for domestic cat. Hair samples from 8 domestic cats were analyzed using the four 6HDZ loci to provide further distinction between bobcat and non-target samples. (Table 2.2)

Each 10μL polymerase chain reaction (PCR) contained: 5μL DNA template, 1μL PCR buffer, 200 pM dNTP, 2 pmol forward and reverse fluorescently labeled primers, nuclease-free bovine serum albumin (BSA), $MgCl_2$, and 0.5μ *Taq* DNA polymerase (New England Bio Labs, Beverly, MA). PCR buffer contained 1mM Tris-HCl at pH 8.5, 1.5 mM $MgCl_2$, 50 mM KCl,

10μg/mL BSA, and 0.0025% Tween-20). The amounts of BSA and $MgCl_2$ in the reactions were locus specific (BSA= 1.7 μL FCA026, FCA090, and SRY/ZFX, 1.9μL FCA043) (MgCl2 =(0.4μL FCA026, FCA090, or 0.2μL FCA043) (Appendix I). PCR was performed using Strategene® RoboCyler®. PCR thermal profile included an initial denaturation at 94°C for 2 min, followed by 40 cycles of 94°C for 30 sec, 1 minute at a loci-specific annealing temp (Table 2.1), 72°C for 1 min, followed by a single 5 min extension cycle at 72°C. The amplified DNA fragments are separated on a 6% polyacrylamide gel using a LiCor®IR^2 4200 Global Edition DNA Sequencer (NEN™, LI-COR, Inc., Lincoln NE). Fragments are viewed using Saga Generation 2™ software (LI-COR, Inc., Lincoln, NE). All scores were confirmed by independent calls from two trained laboratory personnel.

The "matching approach" to multilocus genotype assignment only accepts a genotype if that set of allele scores is observed more than once (Creel et al. 2003). Requiring an exact match between genotypes may cause bias in the population estimation if there are genotyping errors. A modification on the matching approach focuses additional PCR on genotypes that differ at only a few loci, or genotypes that are most likely scored inaccurately, such as those with many homozygotes (Miller et al. 2002). This study accepted allele scores if they were heterozygous and matched across a minimum of two PCR amplifications. Homozygotes were accepted if they amplified and were scored consistently a minimum of three times. Problematic loci (mismatching or failed amplification) were amplified a maximum of 5 times. If no conclusive genotype resulted from 5

PCR, then the locus was not given a score. It is ill-advised to exhaustively PCR samples when there are obvious problems with DNA quantity or quality, due excessive costs and an increased probability of assigning an incorrect genotype. Program GENECAP (Wilberg and Dreher 2005) was used to search for potential matches between hair, scat, and tissue genotypes.

Table 2.1: Nuclear Microsatellite Primers. Primer Sequences, Annealing Temp (Ta), Genbank Accession No. and allele size range (bp) specific to bobcat (*Lynx rufus*).

Locus Name	Primer Sequence (5' to 3')	T_a (^oC)	Genbank accession No.	Size (bp) (*Lynx rufus*)
6HDZ056	F: ACT AGG TCT GTA ACC ACG CCC R: CAG TCA AAC AAC TGC CCT TTC	52	AF296743	172-176
6HDZ057	F: CTA CCT TTC TTT CAC CTT CTT TTT G R: TCG TGC GTT AGA GGA ATT GG	52	AY045524	92-100
6HDZ610	F: ATC AGG AGT TCT ATC ACC AAC CC R: CAC ATG ATT AGG GAG TTG AGA AGT C	56	AF296744	168-172
6HDZ700	F: TCC TCC TTC CAG GAT GCC A R: AGG ATG GGG GAA AAT CTC TC	52	AF296747	141-143
Fca026	F: GGA GCC CTT AGA GTC ATG CA R: TGT ACA CGC ACC AAA AAC AA	53	AF130482	130-136
Fca043	F: GAG CCA CCC TAG CAC ATA TAC C R: AGA CGG GAT TGC ATG AAA AG	52	AF130487	116-124
Fca090	F: ATC AAA AGT CTT GAA GAG CAT GG R: TGT TAG CTC ATG TTC ATG TGT CC	53	AF130516	103-125

Table 2.2: Microsatellite Allele Ranges of Non-Target Species. Sample sizes: For the FCA loci, n = 4 for badger, coyote, grey fox, river otter, and grey wolf and n=3 for red fox. For the 6HDZ loci, domestic cat n = 8. All other allele ranges were taken from literature (6HDZ: (Williamson 2002), FCA: (Menotti-Raymond and O'Brien 1995; Menotti-Raymond et al. 1999; Ernest et al. 2000) X= no amplification across all samples, NA = data not available.

	Allele Size (bp)								
Locus:	**Bobcat**	**Cougar**	**Domestic Cat**	**Badger**	**Coyote**	**Grey Fox**	**Red Fox**	**River Otter**	**Grey Wolf**
FCA026	**130-136**	140-152	136-154	116-162	130	132-168	132-164	X	122-168
FCA043	**116-124**	124-136	116-128	98-132	132-134	X	102-154	98-140	102-120
FCA090	**103-125**	105-119	90-120	X	94-102	X	101	79	101
6HDZ056	**160/176**	182-196	X	NA	NA	NA	NA	NA	NA
6HDZ057	**92-100**	X	80	NA	NA	NA	NA	NA	NA
6HDZ610	**168-172**	164-172	166-174	NA	NA	NA	NA	NA	NA
6HDZ700	**134-150**	133-141	132-150	NA	NA	NA	NA	NA	NA

Cost Analysis: Costs of DNA extraction, amplification, and visualization vary between laboratories, and rapid advances in lab protocols will cause costs to fluctuate from year to year. The cost of laboratory analysis of hair samples vs. scat samples will also vary between laboratories depending on DNA extraction equipment, protocols employed, and personnel expertise. Once DNA has been extracted, the cost for analyzing samples should be approximately the same, whether the DNA came from hair or scat, but will still vary between laboratories. Additionally, the cost of laboratory analysis will vary widely depending on the study goals and type of analysis required. For example, the cost of determining species is significantly lower than the cost of providing a multi-locus microsatellite genotype. The purpose of this study was to determine relative efficiency of two non-invasive sample collection methods. For reasons listed above, the cost analysis was confined to field expenses only and did not include laboratory expenses.

To compare the collection methods for this project, the costs of hair snares and detector dogs were broken down into "Initial investment" and "Operational Costs". Initial Investment includes the fixed costs of initial capital investments (training/materials/etc.), while Operational Costs are those incurred over units of time through the duration of the project. This will allow future projects to consider how their relative costs may change given the duration of their project compared to this one.

Catch per Unit Effort (CPUE) and "Cost-per-Genotype" may be a more accurate way to judge cost-efficiency due to the different nature of these two

methods. Catch was defined as the number of samples yielding a bobcat multilocus microsatellite genotype. Sampling "effort" was defined as the number of person-days spent conducting the survey. CPUE is the number of bobcat genotypes divided by the number of person days employed collecting samples. The cost was defined as the total cost of field expenses for the duration of the search. This cost did not include any laboratory expenses associated with genotyping. The Cost-per-Genotype was defined as the field expenses divided by the number of bobcat genotypes obtained.

Objective 2. Quantify the effect of observable scat characteristics on microsatellite genotyping success.

Scat characteristics were recorded prior to DNA extraction from the 84 scat samples collected during field trials. The primers used in this study were designed specifically for the felid genome. Therefore, based on the primer design, and our data regarding carnivore non-target amplification at these loci, it is unlikely that a non-felid scat sample will amplify at all loci. If we assume that scat size differs between different carnivore species, the diameter of scat may predict whether the scat is from a bobcat, and therefore, whether it will yield a bobcat genotype or not based on our felid-specific loci. In other words, if bobcat scat has a specific diameter range, and that range differs from non-target species, scats within the bobcat size range should have a higher rate of genotyping success. Diameters of the scats were binned into 1-centimeter categories (0-0.9 cm, 1-1.9 cm, 2-2.9 cm, 3-3.9 cm, 4-4.9 cm, 5-5.9 cm, and 6-6.9 cm). Previous

studies have found a positive correlation between odor of the scat and genotyping success (Wasser et al. 2004, Vynne 2010). Odor was scored 0 – 5 (none, light, med, strong, or very strong), similar to categories used by Wasser et al 2004. The 'age' (level of degradation) of a scat can be qualitatively assessed by observing the overall appearance and moisture level of a cross section of the scat (Long et al 2007ab). External moisture or dry/wet weight can be misleading as precipitation may influence both of these measures regardless of scat age. Scat age was classified as "old", "medium", or "fresh". These classes more general than previous studies that assigned age to specific ages (e.g. 1-2 days, 3 days – 1 wk) yet specific enough to apply to future projects that may use 'age' to cull samples. Both age and odor were scored for all scat samples by one individual to maintain a consistent assessment of relative condition.

When detector dogs located a sample in a latrine, the handler collected all scats from that latrine, regardless of age, presence of mold, or whether the dog seemed interested in the scats. Therefore, latrine scats potentially have a higher probability of being from a non-target, or severely degraded than scats individually located by trained detector dogs. While the diameter and 'age' of scat may also be related to non-target or DNA degradation (respectively), it would be informative to know whether to attempt laboratory analysis from multiple scats from a latrine, or simply collect the one scat indicated by the detector dog.

Detector dogs are trained to find scat samples from the target species only. Therefore, we were interested to see if 'confidence' of the dog's alert was correlated with successful genotyping. Confidence in the dog alert was scored by

each handler as "high", "medium", or "low". We also noted the date of extraction to assess whether there were any issues with laboratory protocols on certain days that might have affected genotyping success. In summary, the variables recorded for each scat sample were: 1. diameter, 2. odor, 3.qualitative age, 4. whether the scat was found in a latrine (a conspicuous collection of multiple scats) or alone, 5. date of extraction, and 6. confidence in dog alert (Table 2.3).

The effect of scat characteristics on successful genotyping was analyzed using a general linear model (GLM). GLM analysis was conducted in R statistical package version 2.9.0, with function "lm" (2009 The R Foundation for Statistical Computing). The response variable was the number of successfully amplified microsatellite nuclear loci, ranging from 0-4. The number of successfully amplified loci can be used as a surrogate measure of the quality of DNA found in a given sample. Independent variables included scat age, scat odor, scat diameter, confidence in dog alert, and latrine/non-latrine. Additionally, individual variables for the date of extraction, and dog/handler team were included. While 'date' and 'team' may not help develop culling criteria, 'date' controls for any day-specific laboratory variation in genotyping success and 'team' controls for variation in team target-specificity.

GLM diagnostic plots of the residuals revealed a right-skew. Log-transformation of the response variable resulted in a reasonably normal residual distribution. R^2 and Adjusted R^2 were calculated for each of the candidate models. Individual parameter estimations, standard deviations, and significance based on t-test, were calculated for all parameters in the top-performing models.

Table 2.3. Independent Variables and sample size (N) used for General Linear Model analyses of Scat Sample Genotyping Success.

Scat Odor		Date of DNA Extraction		Detector Dog/Handler		Confidence in Dog 'Alert'		Scat Diameter		Latrine	
LEVEL	N	LEVEL	N	LEVEL	N	LEVEL	N	LEVEL	N	LEVEL	N
none	16	Dec, 23	16	J.S./B	13	Low	13	0-1cm	13	Latrine	31
light	19	Jan, 6	12	J.W./CJ	69	Med	10	1.1-2cm	55	Single Scat	53
med	17	Jan, 8	25	Total:	82	High	17	2.1-3cm	10	Total:	84
strong	19	Jan, 14	31			Total:	40	3.1<cm	3		
very strong	12	Total:	84					Total:	81		
Total:	83										

Objective 3. Quantitative evaluation of the effects of environmental variables on scat detection by detector dog teams.

Field Methods: Bobcat scats were collected from three Michigan zoos throughout the summer, 2006. Tomahawk Wildlife Park, GarLyn Zoo, and DeYoung Family Zoo house 5 bobcats collectively. Tomahawk feed their two bobcats zoo-animal feed, GarLyn Zoo primarily fed their two bobcats chicken, and DeYoung's bobcat was primarily fed road-killed white-tailed deer (*Odocoileus virginianus*) meat. Zoo staff voluntarily collected scats during regular enclosure cleanings approximately once a week. GarLyn and DeYoung zoos immediately froze the scat samples at -20°C, while Tomahawk zoo stored samples dry at room temperature. Following collection, zoo scats were randomly assigned to artificial 'aging' treatments. Ages included: one week desiccation (in a green-house condition (Figure 2.3), two week desiccation, one week precipitation (watered 3x day), and two week precipitation, and fresh (from either GarLyn or DeYoung, and frozen from collection until field trials began). Aging treatments were chosen to approximate the level of degradation observed in 'wild' scats. One week of the precipitation visibly changed the scat samples to appear more degraded than the 'fresh' samples. Two weeks of precipitation treatment removed the majority of fecal material, while leaving the less-soluble scat contents.

Figure 2.3: Artificial Scat Aging Treatments. (*Left*): Greenhouse condition desiccation. (*Right*): Precipitation with automatic sprinkler 3times a day.

Scats were placed at GPS recorded locations marked along five triangular transects 3 km long (1 km each leg). Transects had five samples on each leg for a total of 15 samples per transect. Locations for the five transects were chosen in order to capture maximum landscape heterogeneity (based on MI IFMAP datalayer) so that we could examine the effect of all representative vegetation classes within the study area. Samples were placed at three distance classes from the linear triangular route walked by the handler (distance classes: 0-5m, 5-20m, 20-50m) (See Table 2.8). Distance classes were chosen based on average distance between dog and handler observed first-hand during the preceding training and

field trials. Subsequent to this study, "buffer" distances surrounding detector dog paths have been reported between 30- 50 meters (Wasser et al. *in review*, Vynne 2010). Therefore, distance classes we assigned in this study represented a conservative measure of the effect of distance on detection. The artificially 'aged' scats were randomly distributed between transects, distance classes, and vegetation characteristics.

Two types of vegetation characteristics were recorded at each sample location: local understory density (LUD) and microhabitat. LUD was classified as: open (open field), medium (scattered woody stems, handler could not always maintain straight path, but could move efficiently), and high (vegetation caused significant reduction in handler progress along transect). LUD was qualitatively judged based on the woody stems and coarse woody debris within the surrounding 30 m of the scat sample. These qualitative categories made it possible to quickly assess the LUD without necessitating measurements of stem density or other quantitative measures, which is particularly useful for future NLP projects which may need to quickly decide whether an area is too dense for a detector dog search. Samples were placed in each LUD category according to the naturally occurring frequency of each category. For example, there were more samples placed in LUD of low (n=56) and medium (n=50) than in open (n=14) or high (n=30). Microhabitat was defined as the physical structure of the understory vegetation in the immediate vicinity (<5 m) of the sample. Microhabitat was classified as hidden, open, mound, corridor, tree base, or raised (Figure 2.4). These microhabitat classifications were chosen based on reports of detector dog search

patterns from Pack Leader Dog Training and UW Conservation Canines, as well as previous microhabitat classifications for bobcat radio-telemetry locations (Kolowski and Woolf 2002).

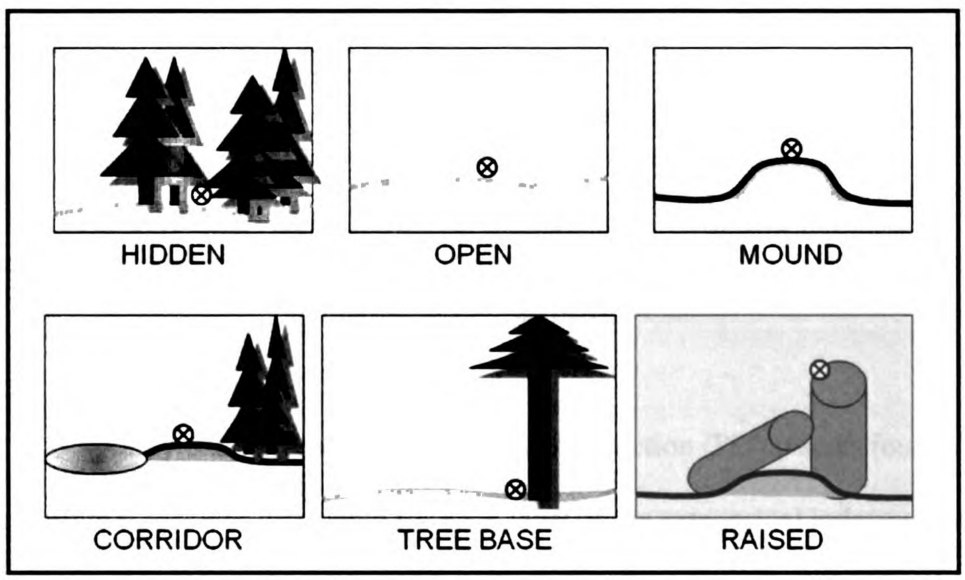

Figure 2.4: Microhabitat Classifications. Scat sample (dot) location in relation to immediate surrounding vegetation.

Five experimental transects were established and sampled by both teams independently between December 12 and 23, 2006. One handler set up two of the five transects, while the other handler set up the other three transects. Samples were left untouched for two days prior to the searches to allow for human/artificial scent to dissipate. Climactic variables were recorded at every 0.25 km during field trials. Wind was ranked as none, breeze, mild, gusty, or strong/consistent. Categories were chosen based on training received regarding wind speed and direction affecting odor movement during handler-training.

Weather condition was classified as heavy rain, rain, light rain, foggy, cloudy, clear (no clouds but not warm to handler), or sunny (handler felt significant warmth from sun). Weather categories were chosen to reflect the possible weather of the NLP and enable future projects to assess the efficacy of conducting a search based on qualitative observation of the weather. The two dog-handler teams each completed all 5 transects, with a minimum of one day between the teams on each transect to allow for scent dissipation. However, the dogs may be able to cue in on scents from the previous dog's search. Therefore, one team was the first to search 3 transects, and the other was first on the other 2 transects.

Analytical Methods: Probability of detection (PD) (#scats found / # possible scats) was calculated for each level of the categorical independent variables. Long et al. (2007a) used program CAPTURE to perform a GLM to examine the effect of various environmental/team factors on species 'detection' for three carnivores. We modeled scat detection (0, 1) using generalized mixed effect model (GLMM) within R statistical package version 2.9.0 (2009 The R Foundation for Statistical Computing). A generalized model was used due to the binomial response variable of scat detection. GLMM was performed within R using the "lmer" function from the package "lme4" with the binomial family to account for the binomial distribution of the response variable. Independent variables included: distance from handler path, weather, wind, leg of transect (1-3), which person setup the transect, scat age treatment, which zoo the scat came from, LUD, microhabitat, and dog/handler team. Candidate models were

compared using Akaike's Information Criterion (AIC) (Akaike 1974, Burnham and Anderson 2002). Parameter estimates were analyzed using z-test within the lmer function (Bates 2007). The lmer function uses the method "Laplace" for all fixed effect approximations (Bates 2007).

Two random effects, "sample ID" and "transect ID", were considered for inclusion in the models. Transect ID would account for the variance due to pseudoreplication within each transect. Sample ID would account for the variance due to repeated sampling of each sample by the two teams. Likelihood Ratio Tests were used to assess the improvement of the models by the inclusion of these random variables. Diagnostic plots showed no outliers greater than Cook's distance of 0.5, and an error structure suitable for a binomial distribution based on residual Q-Q plots. The quasibinomial family (no assumption of binominal error distribution) did not reveal any cases of overdispersion (variance within the data beyond what is expected by a binomial error distribution given a certain model).

RESULTS:

Objective 1. Compare the efficacy of detector dogs versus hair snares

Laboratory Genotyping: We had a 1.2% success rate for obtaining a multilocus microsatellite bobcat genotype from hair samples, and 9.5% success rate from scat samples. These success rates are highly conservative, as genotyping was attempted for all samples regardless of remarkably poor sample quality. The bobcat hunting season (62 days) resulted in 49 bobcat tissue samples from the study site counties. All tissue samples yielded multilocus microsatellite bobcat

genotypes. Probability of Identity (PID) across the three FCA loci was 0.004996. (Table 2.4). Applying the FCA loci only, there remained multiple matches between tissue samples collected from individual harvested bobcats. Therefore, assuming that the tissues came from different animals, the PID from the three FCA loci proved insufficient to distinguish individuals from the NLP population. Four additional microsatellite loci (6HDZ) resulted in a PID of 1.7223E-05 and no matches between tissues samples. The suite of all 7 microsatellite loci resulted in no matches between noninvasive samples and tissue samples, which negated the possibility of a CMR population estimate.

Table 2.4: Allele Frequencies and Probability of Identify (PID). Based on assumption of Hardy-Weinberg equilibrium.

	Fca026		**Fca043**		**Fca090**		**6Hdz056**		**6Hdz057**		**6Hdz700**		**6Hdz610**	
	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.
	130	0.3000	116	0.0196	103	0.0392	165	0.0405	92	0.8137	134	0.2979	162	0.0106
	132	0.3000	120	0.0196	105	0.1863	167	0.1622	94	0.1863	138	0.0745	164	0.1489
	134	0.2700	122	0.5000	107	0.2745	169	0.1622			140	0.5745	168	0.2872
	136	0.1300	124	0.4608	109	0.0392	171	0.4324			142	0.0213	170	0.5532
					111	0.4118	173	0.1757			150	0.0319		
					113	0.0294	175	0.0270						
					125	0.0196								
PID:	0.1238		0.3213		0.1256		0.1116		0.5315		0.2457		0.2366	
	PID FCA Loci Only = 0.004996													
	Overall PID = 1.722E-05													

Hair Snares: Hair snares generated 168 samples and 2 bobcat genotypes. Those two samples had matching genotypes (were from the same individual) and were collected from the same hair snare (Transect 6, Station 1) on the same day (Oct 4th) (Figure 2.5). The majority (148 of 168) of hair samples contained DNA but did not successfully amplify at felid-specific microsatellite loci (Figure 2.6). This could be caused by degraded DNA, or the samples coming from non-felid mammals. However, they were not from local carnivore species based on allele ranges for non-target sympatric carnivore species (Table 2.2). The track plates surrounding the hair snares were not highly successful at identifying visiting species due to snow and rain disturbance. However, several non-target species were observed, including black bear, coyote, raccoon, mustelid, and squirrel. Bobcat tracks were positively identified only once, and there was no bobcat DNA found from hair samples at that station.

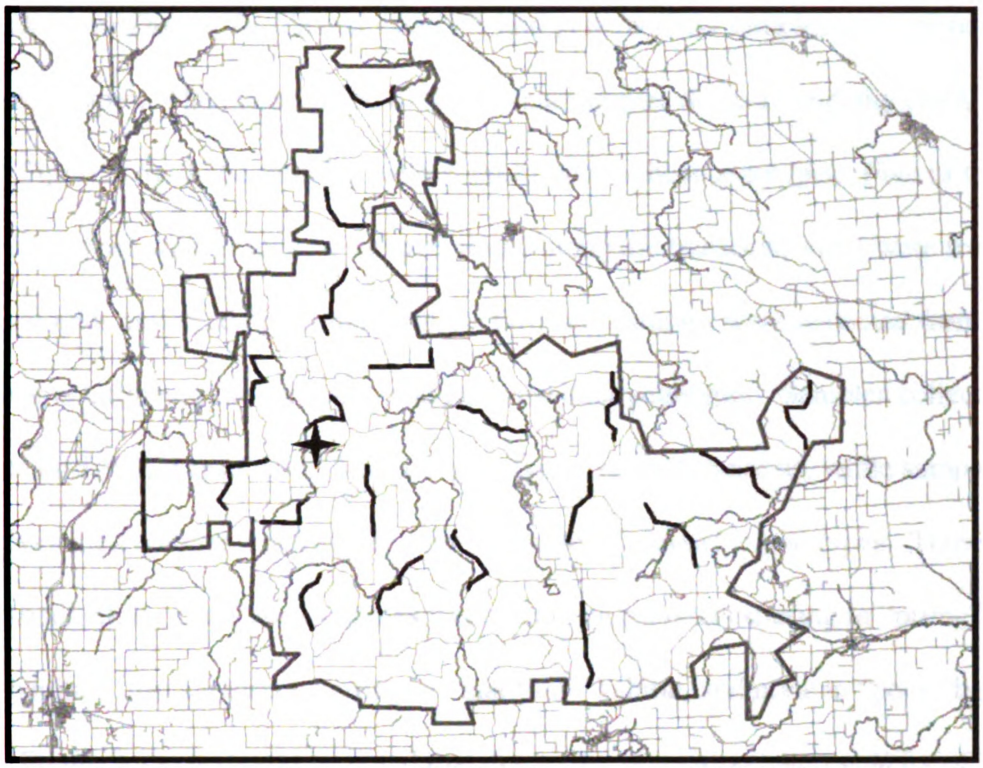

Figure 2.5: Hair-snare Transects. Five km linear transects (black lines) within the Pigeon River Study Site. Each transect contains 5 hair snare stations and was checked weekly. Bobcat genotype obtained from hair sample from Transect 6 (Tin Bridge Rd.), Station 1 (star).

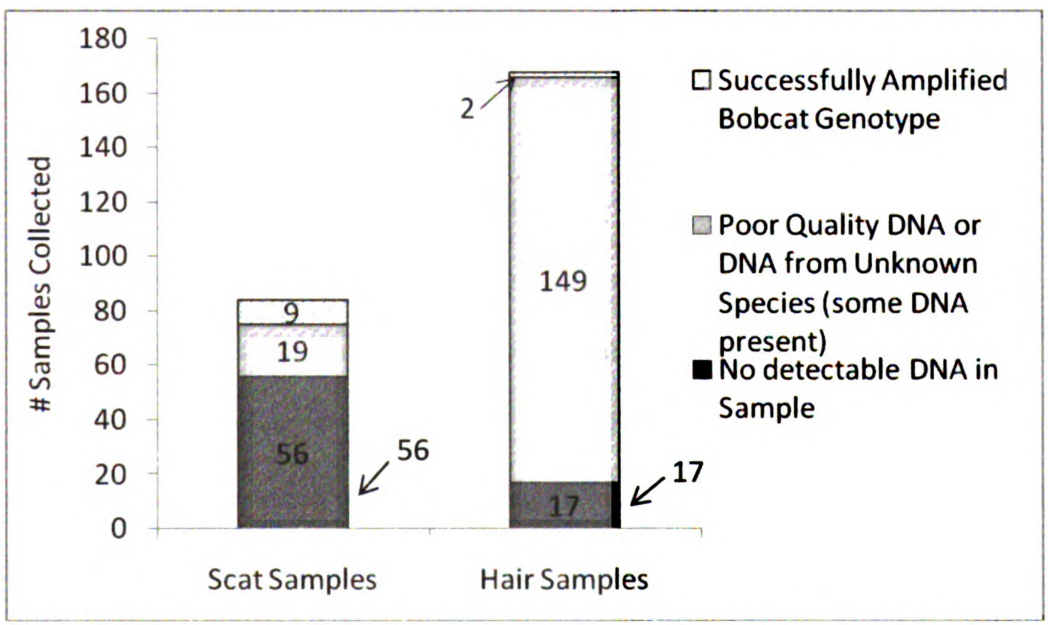

Figure 2.6: Scat Sample Collection and Genotyping Success. Comparison between detector dogs and scat samples versus hair-snares and hair-follicle samples. Total number of samples collected, samples with no detectable DNA, DNA present but either of poor quality or from non-target species, and successfully genotyped bobcat samples.

Detector Dogs: The detector dog method generated 84 samples and 9 bobcat genotypes. The majority (56 of 84) of scat samples did not yield any DNA (Figure 2.6). Nine samples yielded multilocus bobcat genotypes. Five of those genotypes were from scat samples found along the transects, and 4 were from samples collected opportunistically. Two scat samples were collected from the same latrine on the same day and had matching genotypes. Samples collected by team J.W./CJ resulted in 3 bobcat genotypes over 17 transects, while samples collected by team J.S./Bruiser over 18 transects yielded 2 genotypes. Three of the successfully genotyped samples were found on transects starting in "prime" habitat, 1 on a transect starting in "marginal" habitat, and none in "poor" habitat (Figure 2.7). It should be noted that due to the vegetation heterogeneity of the study area, only the start points of the transects can be categorized accurately into these three habitat classes. Three of the opportunistically collected samples that resulted in a bobcat genotype were collected while searching for captive scat on experimental transects. One additional genotype was obtained from a sample found by the canine, CJ, when he accompanied J.White on a final check of a hair snare station. J.White finally noticed that CJ was waiting in alert stance about 5meters away after she had been working at the hair snare station for approximately 5 minutes (Figure 2.8). The ability to opportunistically sample areas with a detector dog outside of "official" time could be considered an added benefit of the method, enabling continuous survey during any activity in the survey area when the dogs are present. Detector dogs have found target samples while on break-time walks, changing of flat tires, and while hiking to official

transect start points (J.White *unpublished data*). However, depending on the objectives of the study, it may or may not be appropriate to include opportunistically collected samples in subsequent analyses.

Based on MI Land Cover Data Set (1999), all of the successfully genotyped bobcat scat samples were found in "wooded wetland". The distance of genotyped bobcat scat samples to any road within the study area was an average of 363.6 m ± 180.7 m (MEAN ± STDEV). Two genotyped bobcat samples were found within the same Section where a different bobcat was harvested during the hunting season. The average distance between a genotyped scat sample and a Section where a bobcat was later harvested was 3.8 km ± 3.85 km.

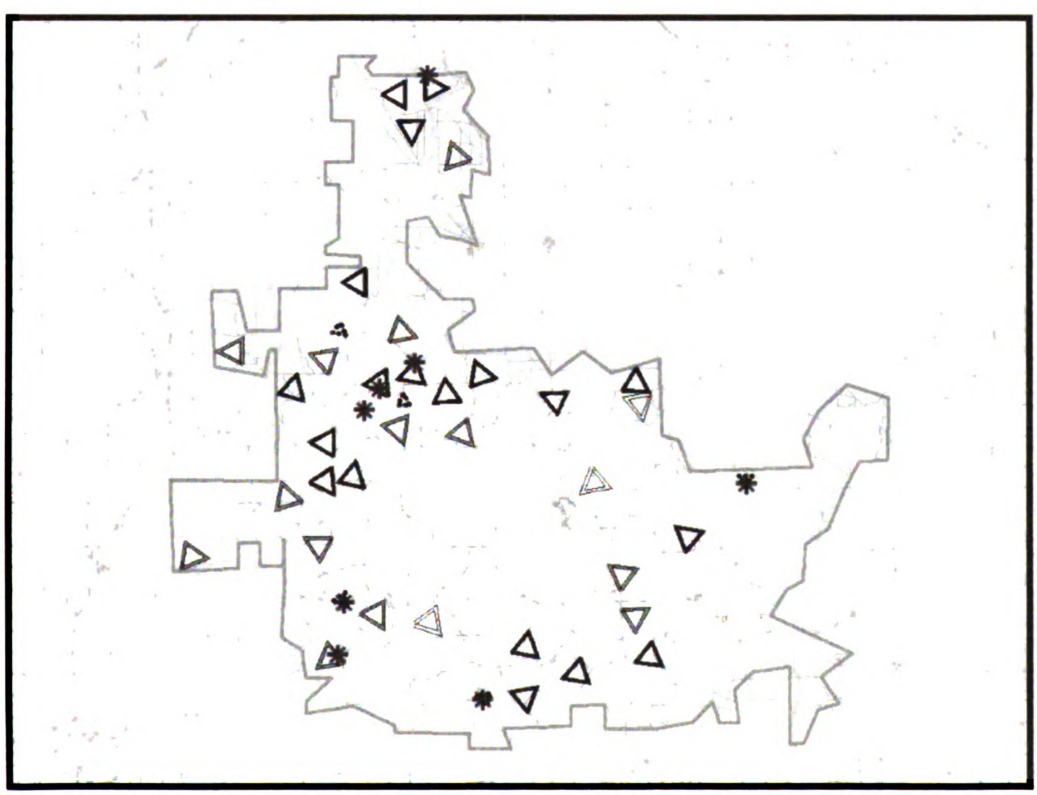

Figure 2.7: Detector Dog Transects. Triangular transect locations by bobcat habitat: Prime (black), marginal (grey), poor (lt.grey). Experimental transects with captive bobcat scat (dashed small triangles). Bobcat genotypes obtained from scat found at asterisks.

Figure 2.8: Example of Detector Dog Efficiency. Hair snare in foreground (indicated by black arrow) was unsuccessful in yielding a bobcat genotype after 3 months. A detector dog located a bobcat scat which yielded a multilocus nuclear genotype on the mound approximately 5 m away (indicated by dashed arrow) while accompanying handler to check hair snare.

Comparison of Cost Efficiency: The initial capital investment / materials cost was much higher for detector dogs ($9,055) compared to hair snares ($600) (Figure 2.9, Table 2.5). The initial investment in the detector dog method primarily consists of handler training. This is a fixed cost; whether a study then employs the dogs for one month, or 5 months following training. Operational costs for the duration of the study were higher for hair snares ($6,750) versus detector dogs ($5,150). This was largely due to the time spent surveying with the different methods. Hair snares were monitored for 3 months, while surveys with detector dogs were complete within less than 1 month. For the amount of time and area we sampled, total cost of detector dogs ($14,205) was nearly double that of hair snare ($7,350).

Two analyses of CPUE and Cost-per-Genotype were performed, one including opportunistically collected samples, and the other with only the 5 scat samples found during official transect searches. In both analyses of cost effectiveness, detector dogs had a higher CPUE than for hair snares (Table 2.6). The Cost-per-Genotype was lower for detector dogs than for hair snares in both analyses, even despite the much higher total field cost of detector dogs. When including opportunistically collected samples, the Cost-per-Genotype was more than twice as much for hair snares than for detector dogs.

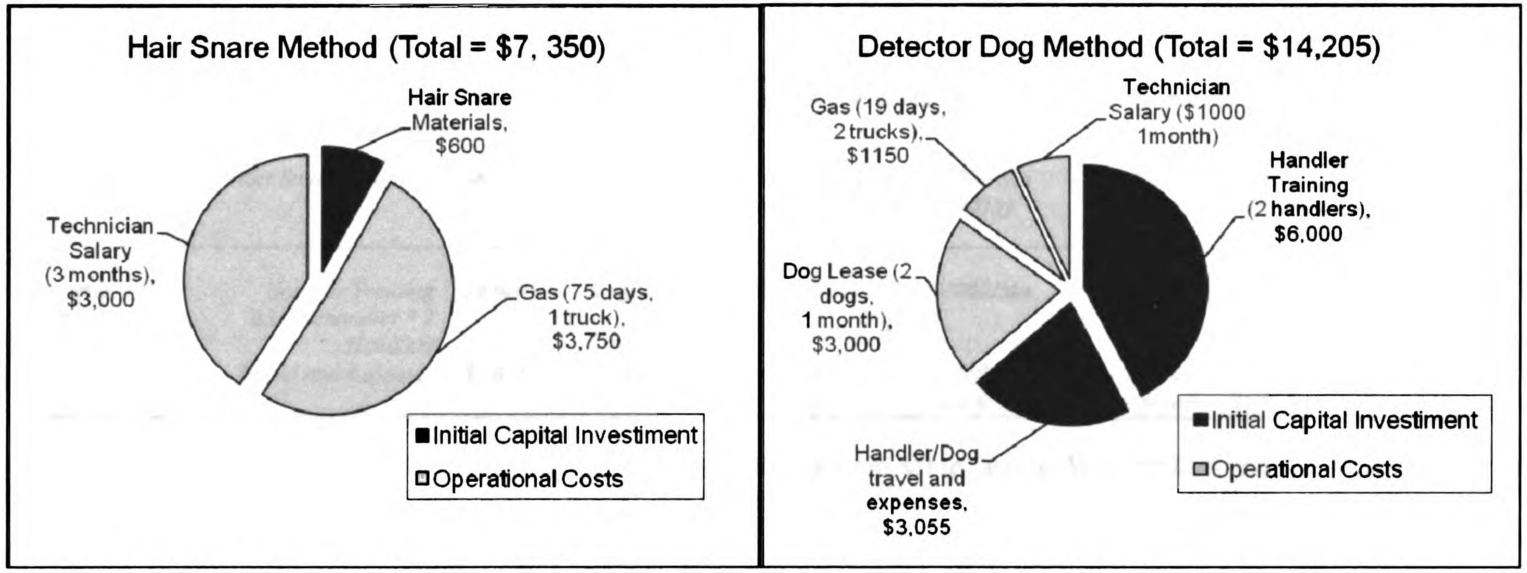

Figure 2.9: Field Costs of Hair Snare and Detector Dog genetic sample collection methods. Breakdown by Initial Capital Investment (materials, training) and Operational Costs (gas, salaries, lease fees)

Table 2.5: Field Costs of Noninvasive Sample Collection Methods: Hair Snares vs. Detector Dogs

Method	Initial Capital Investment / Materials Cost		Operational Costs per Unit Time		Total Operational Costs		Total Cost
Hair Snares:	*Hair Snare Materials*	***$600***	*Technician Salary*	***$1000/Mo***	*3 Months*	***$3,000***	**$7,350**
			*Gas $50/day/truck * 1 truck*	***$50/Day***	*75 Days*	***$3,750***	
Detector Dogs:	*Handler Training $3,000/handler * 2 Handlers*	***$6,000***	*Dog Lease $1500/month/dog * 2 dogs*	***$3000/Mo***	*1 Month*	***$3,000***	**$14,205**
	Travel and Expenses	***$3,055***	*Gas $25/day/truck * 2 trucks*	***$50/Day***	*23 Days*	**$1,150**	
			Technician Salary	***$1000/Mo***	*1 Month*	**$1,000**	

*Travel and Expenses based on 2006 Airline fares / shipping from Detroit, MI to Seattle, WA. ** Gas prices estimated based on 2006 prices. Gas costs for Hair Snare method higher due to continuous travel while checking snares. Detector Dog method requires single trip to sampling location.

Table 2.6: Catch per Unit Effort (CPUE) and Field Cost per Bobcat Genotype. Hair snares vs. Detector Dogs. Sampling Effort = # Person-Days. Catch = # Samples Yielding Bobcat Multi-locus Microsatellite Genotype. CPUE = (Catch / Sampling Effort). Field Cost = $ Total Field Expenses for each method (Table 2.5). Field Cost per Genotype = (Field Cost/ # Bobcat Genotypes). *Field Costs do not include laboratory expenses associated with genotyping.

	All Samples, Including Opportunistically Collected Samples					Samples Collected During Official Sampling Efforts Only				
Method	Sampling Effort	Catch	Field Cost	**CPUE**	**Field Cost per Genotype**	Sampling Effort	Catch	Field Cost	**CPUE**	**Field Cost per Genotype**
Hair Snares:	75 (1 person 75 days)	2	$7,350	**0.027**	**$3,675**	75 (1 person 75 days)	2	$7,350	**0.027**	**$3,675**
Detector Dogs:	46 (2 people, 23 days ea.)	9	$14,205	**0.196**	**$1,578**	35 (2 people, 17+18 days)	5	$11,844	**0.143**	**$2,369**

Objective 2. Quantify the effect of observable scat characteristics on microsatellite genotyping success

Twenty eight candidate General Linear Models of microsatellite amplification success revealed a consistent pattern in the best-fit candidate models. Certain categories of scat diameter amplified poorly (Model 1: "2-3 cm", t=-2.709, p=.0085; "6+ cm" t=-1.745, p=0.085)(Table 2.7). The observed age of the samples had a marginal effect on microsatellite amplification, but in a counterintuitive manner. Older scat samples had a higher probability of amplification success (Model 3: t=1.844, p=0.0694). The independent parameters of odor, dog/handler team, and alert confidence were not significant predictors of genotyping success in any of the candidate models, and were not included in the top models. DNA extracted on the final day amplified poorly (Model 3: t=-2.309, p=0.0238). The latter may have resulted from the majority of latrine samples analyzed the last day of extraction. Samples from latrine sites amplified poorly, but even more so when amplified on the last day, as reflected by a significant interaction between date of extraction and latrine site samples in two of the top three models (Model 2, "date extracted * latrine" t=-2.085, p=0.041).

Table 2.7: Microsatellite amplification success GLM Results. Bolded parameters had estimates significant $p<0.05$.

	Model	R^2	Adj R^2	F-stat	df	p-value
1	**diameter** + date extracted + latrine + age + (date extracted*latrine)	0.3824	0.2825	3.828	11,68	<0.001
2	diameter + date extracted + latrine + (**date extracted*latrine**)	0.3791	0.2801	3.83	11,69	<0.001
3	**diameter + date extracted + age**	0.3184	0.2416	4.145	8,71	<0.001
Full	diameter + date extracted + latrine + age + odor + team + confidence	0.3097	0.0336	1.07	11,24	0.423

Objective 3. Quantitative evaluation of the effect of environmental variables on scat detection by detector dog teams.

The results from experimental transects with captive bobcat scats demonstrated that detector dogs found approximately half (47.5%) of the scat samples. Probability of detection (PD) was defined as the number of scats found divided by the total number of scats The PD for each level of categorical variable is summarized in Table 2.8 and Figure 2.10. J.Sayers and Bruiser found slightly more samples than J.White and CJ (PD= 0.51 and 0.44 respectively). Probability of detection declined with increasing distance of the sample from the flagged handler path, and the decline is steeper for team CJ/JW than for JS/B (Figure 2.11). Samples that were set up by J.White were found less often (PD= 0.33) as compared to those set up by Sayers. (PD= 0.57), suggesting that White may have chosen harder sample locations. There was no overall declining trend in probability of detection over transect leg (PD first =0.48, second = 0.54, and third = 0.40), suggesting that the teams did not fatigue over the course of the 3 km transect. Probability of detection was low when there was strong wind (PD= 0.15). Scats 'aged' with precipitation for two weeks were found less often (PD= 0.29) than other ages. Detector dogs found a higher proportion of the samples from DeYoung zoo (PD Tomahawk = 0.47, Garlyn = 0.45, and DeYoung = 0.67). Unexpectedly, PD increased as density of understory vegetation increased, (PD open = 0.21, med = 0.48, high = 0.53). Scat samples that were hidden (PD= 0.37) or raised off the ground (0.27) were found less frequently than those in other microhabitat locations (PD mound = 0.60, corridor = 0.60, treebase = 0.50).

Differences between these raw PD data should be interpreted with caution due to the complicated nature of the detection dog method, and the potential covariance or interaction between variables, as well as potential non-independence between samples. Therefore, it is more appropriate to view the statistical significance of these environmental variables in a GLMM framework, which can account for non-independence via the inclusion of random variables, and can investigate covariance or interactions between parameters.

Table 2.8: Probability of Detection (PD) of Scat by Detector Dogs by Environmental Variables. Environmental variables include: Person who set up the transect, handler/dog team, leg of triangular transect, weather, wind intensity, artificial scat 'age' treatment, source zoo of the scat sample, understory vegetation density, microhabitat surrounding scat sample, and distance of the sample to the handler path. n=number of scat samples, PD = probability of detection (number of scats found/total number of scats).

Transect Setup Person			Handler/Dog Teams			Transect Leg			Weather			Wind		
level	n	PD	level	n	PD	level	n	PD	level	n	PD	level	n	PD
J.S.	90	0.57	J.W./CJ	75	0.44	First	50	0.48	HeavyRain	6	0.33	None	55	0.51
J.W.	60	0.33	J.S./Bruiser	75	0.51	Second	50	0.54	Rain	17	0.53	Breeze	49	0.55
						Third	50	0.4	LightRain	29	0.52	Mild	16	0.44
									Foggy	8	0.5	Gusts	17	0.41
									Cloudy	36	0.56	Strong	13	0.15
									Clear	43	0.34			
									Sunny	11	0.54			

Scat 'Age'			Source Zoo			Understory Density			Microhabitat			Distance		
level	n	PD	level	n	PD	level	n	PD	level	n	PD	level	n	PD
Fresh	26	0.54	Tomahawk	60	0.47	Open	14	0.21	Hidden	30	0.37	<5m	60	0.7
Desic.1Wk	40	0.45	Garlyn	78	0.45	Low	56	0.49	Open	22	0.45	5-20m	60	0.37
Desic.2Wk	34	0.65	DeYoung	12	0.67	Medium	50	0.48	Mound	20	0.6	>20m	30	0.23
Precip.1Wk	15	0.47				High	30	0.53	Corridor	20	0.6			
Precip.2Wk	35	0.29							TreeBase	42	0.5			
									Raised	16	0.27			

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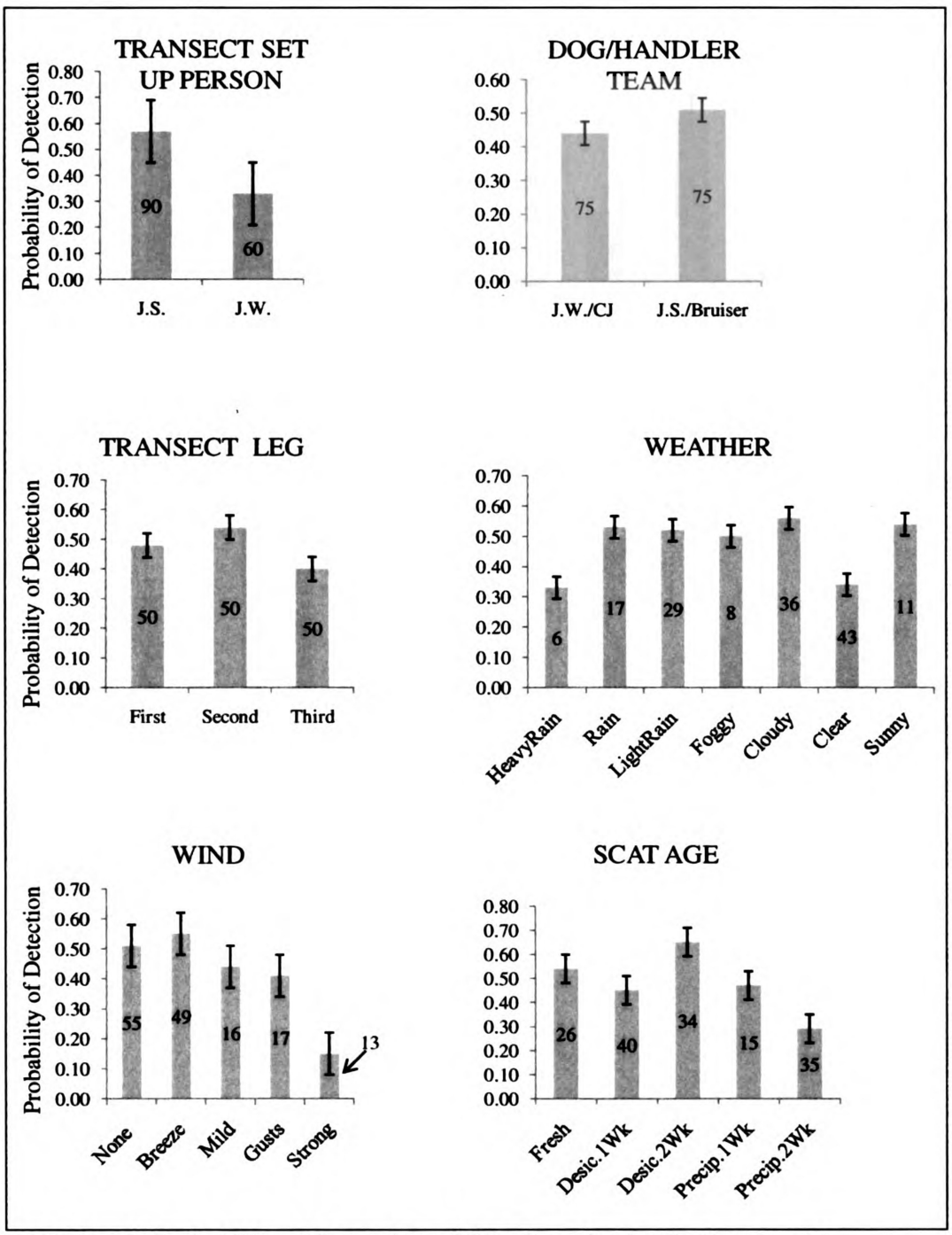

Figure 2.10: Probability of Detection of Scat Samples by Environmental Variables.

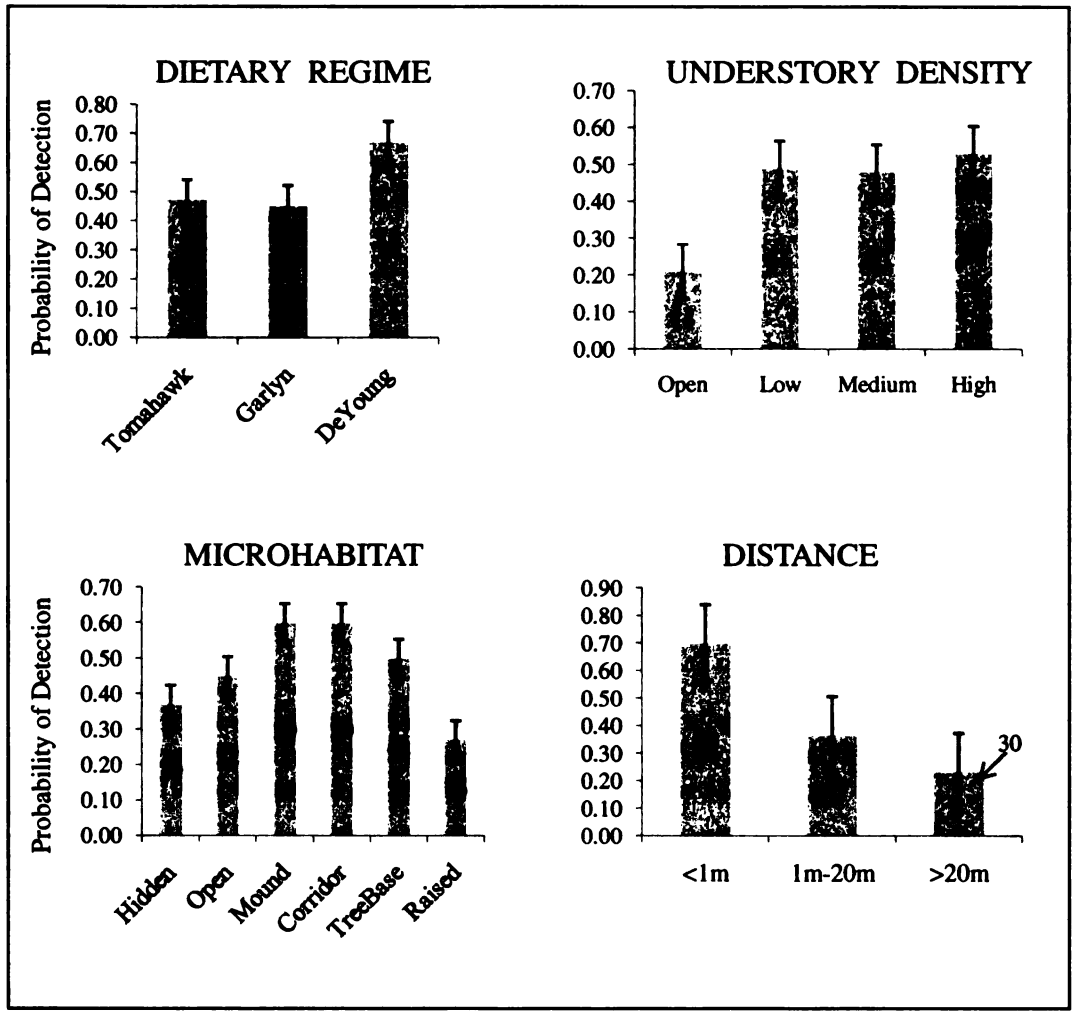

Figure 2.10 *continued.* Probability of Detection of Scat Samples by Environmental Variables.

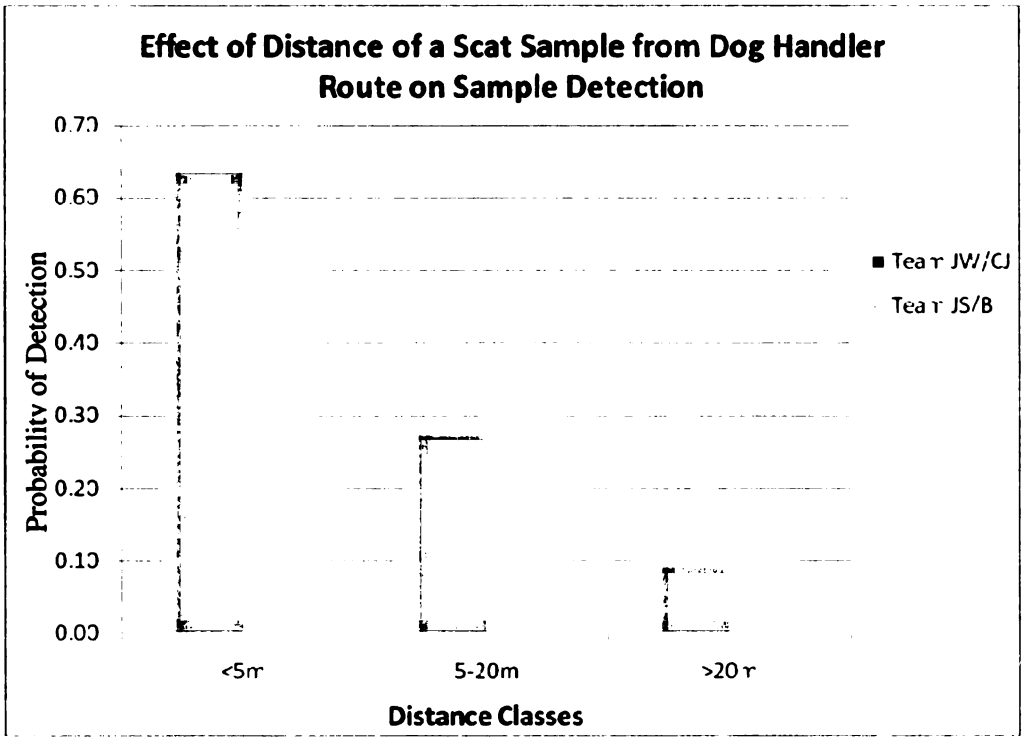

Figure 2.11 Effect of Distance of a Scat Sample from Handler Path on Scat Detection. Compared between two dog/handler teams (JW/CJ and JS/B). Probability of Detection = (number of samples found at specific distance class/total scats found)

The results of the Likelihood Ratio Tests (LRT) between candidate GLMMs with and without the random effects of "transect ID" and "scat ID" demonstrated improvement of the models with the inclusion of scat ID, but not transect ID. Using the top performing model as an example, adding scatID was significant improvement (χ^2= 9.83, df=1, p = 0.0017) over the model with fixed effects only. Adding transect ID alone was not a significant improvement (χ^2= 0.744, df=1, p = 0.7850). Having both random variables as compared to transect ID only was a significant improvement (χ^2= 9.76, df=1, p = 0.0018) but was not an improvement compared to scat ID only (χ^2= 0.00474, df=1, p = 0.9451).

Having both random variables as compared to fixed effects only was a significant improvement (χ^2= 9.84, df=2, p = 0.0073).

These results suggest that the random variable of transect ID does not significantly improve the models, therefore, individual transects were not a significant source of variation in PD. On the other hand, the significance of the scatID parameter suggests that there was variation due to the re-sampling of the same sample, once by each team. In other words, it was more likely that both dog/handler teams would find a particular scatID than random, perhaps because the second dog detected the scent of the first. There is one caveat to the results of this analysis; the models with fixed effects only were estimated using REML, while the GLMM were estimated using Laplace for binomial distribution. Ideally, LRT should compare two models estimated using the same method. Despite the different methods of estimation, the LRT provides an indication that scat ID improves the models, supporting the biological reasoning for its inclusion.

Forty five candidate models were chosen for the GLMM analysis of scat detection. The results from the GLMM analysis (Table 2.9) revealed that distance of the scat from the handler path was included in all of the top (< ΔAIC = 2.0) models and parameter estimation was significant as determined by z-score (z=-3.416, p<0.001). Scat age of "fresh" had a positive effect on probability of detection in one of the top models (z=2.003, p=0.045). The wind strength variable was included in all top models and had a negative parameter estimate, however, that estimate was not statistically significant unless the model was without the setup person by wind interaction term (AIC=173.8, ΔAIC =7.3), then

stronger wind had a significant negative effect on probability of detection (z=-1.949, p=0.0512). The top performing models also included variables "leg of transect", "local vegetation", however neither of these parameters were significant in any of the models.

There was a significant interaction between the parameters of "setup person" and "team". The interaction term was positive when the same person who set up the transects was also the person who searched for the samples (setupJW*teamJWCJ: z=3.000, p=0.003). This suggests that the dog handlers were not able to maintain objectivity when directing the dogs' search on the transects that they, themselves setup. The terms were also independently significant in the majority of the top models, with team JW/CJ having a lower probability of detection (z=-3.431, p<0.001) and transects setup up by JW having a lower probability of detection (z=-1.888, p=0.059). However, setup person by wind interaction was also significant with stronger wind negatively interacting with setup JW (z=-2.101, p=0.036). A second analysis was conducted using only the data from trials where the setup person and handler were different. The same top performing models arose with the more limited data set, and the parameter estimates were in the same positive or negative direction. However, the statistical significance of those parameter estimates was reduced, most likely due to the reduced data set (n = 150 for the full data set, versus n = 75 when only considering transects set up by the other person). The models including an interaction term between setup-person and team successfully accounted for the

variation in the data due to this effect, and the full data set should be considered when interpreting the biological significance of our data.

Table 2.9: Environmental Effects on Scat Detection by Detector Dog Teams GLMM Results. Based on known locations of captive bobcat scat. Legend: ***p< 0.001,** p<.01, * p<0.05, ` p<0.1, "NS" = categorical variable included in model but with an NS effect, ↓=direction of effect, JW=team J.White/CJ, P2 = Precipitation for two weeks.

Model	Distance	Wind	setupperson	team	Scatage	leg	localveg	(setup*team)	(setup*wind)	AIC	ΔAIC	(w_i)
1	↓***	↓ns	JW↓`	JW-***	Ns			(JW*JW+)**	(JW*wind)-*	166.2	0	0.33
2	↓***	↓ns	JW↓*	JW-***	Ns	ns		(JW*JW+)**	(JW*wind)-*	166.6	0.4	0.27
3	↓***	↓ns	JW↓*	JW-**	F+`		ns	(JW*JW+)***	(JW*wind)-**	167.0	0.8	0.22
4	↓***	↓ns	JW↓*	JW-**	F+*	ns	ns	(JW*JW+)***	(JW*wind)-**	167.5	1.3	0.17
Full	distance + wind + setupperson + team + scatage + leg + localveg + weather + zoo + microhabitat + (setup*team) + (setup * wind)									169.1	2.9	-

DISCUSSION:

Objective 1. Compare the relative efficacy of detector dogs versus hair snares

Hair snares generated more samples than detector dogs. However, out of the 149 hair samples that yielded DNA based on PCR amplification of the sexing locus, only two yielded a bobcat genotype. The other hair samples did not yield a bobcat genotype either because the DNA was too degraded to amplify successfully, or more likely, they were from non-carnivore species, possibly small mammal species attracted to the scent lures. In comparison, the vast majority of scat samples did not contain any viable DNA, target or otherwise. However, of the 19 samples that did contain some amount of DNA, nine yielded a bobcat microsatellite genotype (5 collected from transects, 4 collected opportunistically). These results suggest higher target-specificity of detector dogs than hair snares.

The hair snare by which CJ found the opportunistically collected scat sample was on the same transect as the only successfully genotyped hair samples (approximately 5 km away). Since the hair genotypes did not match the scat genotype, it demonstrates that one hair snare transect spanned the movement areas of at least two bobcats, but was unable to detect more than one animal over 3 months. On the other hand, there were three detector dog transects in close proximity to the successfully genotyped hair sample, and no bobcat genotypes were obtained from samples found on those transects. The average distance between successfully genotyped bobcat scats to a road was 363.6 m ± 180.7 m, suggesting that our placement of "off-road" hair snares 250 m off of a road was adequate to account for any road-avoidance behavior by bobcats.

The detection of matching genotypes from noninvasive sources and the ability to distinguish those samples from harvested individuals demonstrates that the genotyping protocol developed within this study is able to provide a sufficient PID to distinguish individuals, as well as to detect matching genotypes. These protocols are a necessary step toward implementing a genotypic CMR population estimation. Greater collection effort for noninvasive samples would increase the number of 'marked' individuals within the population, and thereby increase the 'recapture' probability from harvested tissue. It is also possible to add 'recapture' sessions by noninvasive sample collection (opposed to harvest samples) which provides more robust abundance estimation (Mowat and Strobeck 2000, Wasser et al. 2004).

The matching hair samples were from the same trap, and collected on the same day. The matching scat samples were two deposits at a single latrine. It is impossible to tell if the two hair samples were left at the same time or on repeat visits. Conversely, each scat sample represents a single deposition event and therefore, repeated visitation of a single individual to a site. This type of detailed information is valuable when studying population dynamics, behavior, or resource selection.

Detector dog teams found more samples on transects that started in "prime" or "marginal" bobcat habitat as opposed to "poor" habitat and all genotyped scat were located in "wooded wetland". This suggests that our *a-priori* habitat classifications were useful for allocating the limited time with the dogs. This result may simply reflect the stratified design of the survey of more transects

in better habitat types, however, Pruess and Gehring (2007) also found preferential use of lowland forest by bobcat in the NLP using radio-telemetry locations. Future projects should critically examine any *a-priori* assumptions of resource selection and effects of censoring marginal habitats when designating transect locations. If managers have confidence in their understanding of resource selection, they may optimize scat detection by focusing surveys in areas of established high resource use areas (Wasser et al. *in review*). If confidence is low, a random or grid sampling regime is suggested in order to avoid a biased representation of the population in the marked individuals. An additional consideration for study design is sex-differences in space use. For example, the core areas for both sexes are more likely to be found in lowland forest than other habitat types, and female bobcat home-ranges are generally smaller than males (Pruess and Gehring 2007). Therefore, if there is a sex difference to use of 'marginal' habitat, a stratified design with greater effort placed in lowland forest may reduce capture bias between sexes by limiting sampling to areas frequented by both sexes. These guidelines could be applied to hair snare locations as well as areas to be sampled with detector dogs.

Remote areas are easier to survey with detector dogs rather than hair snares, making it a more desirable method for animals with possible road-avoidance behavior such as the NLP bobcat. The difficulty in surveying remote areas with hair snares stems from the necessity to frequently check and re-bait the stations, whereas detector dogs need only pass through an area once to locate previously deposited scat samples. Therefore, areas with limited access can be

sampled more completely with dogs than with hair snares. Dogs can be transported by canoe in water-logged areas or by sled in winter.

The cost-efficiency analysis demonstrated higher CPUE for detector dogs, likely due to target specificity of the method as demonstrated by our genotyping results. Previous studies have also found detector dogs to be highly accurate to target species. For example, in a kit fox (*Vulpex macrotis*) study, over 400 samples detected by canines were genetically tested, and of the ones that yielded DNA, 100% were from the target species. The study area of their study had several sympatric carnivore species including badger, coyote, and skunk (*Mephitis mephitis*) (Smith et al. 2005).

Surprisingly, the detector dog method also resulted in a lower Cost-per-Genotype even though the total cost of detector dogs was approximately twice that of hair snares. These results hold true regardless of whether opportunistically collected samples were considered part of the "Catch". It should be noted that if the duration of the field season was extended in order to increase the number of "marked" animals, the overall cost difference between the methods would probably become less pronounced, since the vast majority of the hair snare method costs were "Operational Costs" whereas the majority of the costs incurred for the detector dog method came from a one-time "Initial Investment" in training and travel. Therefore, with a longer duration of both field methods, it is likely that the Cost-per-Genotype would become even more in favor of detector dogs.

Objective 2. Quantify the effect of observable scat characteristics on microsatellite genotyping success

Scat samples in certain diameter categories amplified poorly compared to those in other diameter categories. These results suggest that future projects may lower their laboratory costs by excluding scat size classes that are unlikely to be from their target species. However, due to the small sample size of amplified scat samples (n=10) these data do not conclusively define size ranges for bobcat scat. Based on the data from this study, scats of all diameters should be processed for bobcat DNA. *A priori* determination of target scat size classes is extremely difficult due to variation in scat size caused by age of the animal and diet; it is ill-advised to use captive scat size as a proxy for wild scat due to dramatically different diets. A better option is to train the dogs off of non-target species in areas where there are high densities of non-target species so that they are not collected to begin with.

Unexpectedly, "older" scats had a higher genotyping success than fresher samples. While initially this seems counterintuitive, the age of the scats were primarily based on the perceived moisture content. Moisture is a leading cause of DNA degradation (Wasser et al. 1997); therefore the DNA of older, drier scats may actually have been better preserved. These results suggest that all effort should be taken to remove moisture from scat samples immediately following collection. Additionally, scat samples should not necessarily be culled simply because they "look old". Previous studies have suggested that the odor of scat is a

good predictor of extracting high quality DNA (Wasser et al 2004). However, our results showed no correlation of genotyping success with odor.

Since detector dogs are trained for species-specific search, it is tempting to use the strength of the dog alert to prioritize sample culling. However, our results show that the strength of the dog's alert behavior was not a significant predictor of genotyping success. This is most likely because dogs are cuing on species-specific molecular compounds in the scat other than DNA, and therefore the dog's "confidence" may not be correlated with the amount or quality of DNA in the sample.

There was a lower success rate on the last day of DNA extraction. It is unlikely that this was due to the extent of time between the first extraction day to the last, when considering the extent of time the samples were likely exposed to the elements in the field prior to collection. The poor success from the samples extracted on the last day was most likely due to those samples being from latrine sites and in generally poorer condition than non-latrine samples. However, this supposition is only partially supported by the GLM analysis; the "latrine" parameter was not a significant predictor of genotyping success, but there was a significant interaction between latrine and extraction date. Collection and laboratory analysis of samples found in latrines may have diminishing returns if the project goal is to identify as many individuals as possible, since many scat samples may come from the same individuals. However, information regarding the use of latrines by one or related individuals may provide valuable ecological and behavioral data under different project goals.

Objective 3. Quantitative evaluation of the effect of environmental variables on scat detection by detector dog teams.

Four major factors were found to affect scat detection. Contrary to our prediction, the strongest variable affecting scat detection was not wind strength, but distance between the sample and handler. As distance increased, dogs were less-likely to detect scat samples, similar to a sight-ability distance function. Unfortunately, quantifying a universal olfactory-detection versus distance relationship is not possible considering the many factors that impact odor trail movement, such as wind direction and speed, vegetation, humidity, and topography. Our results suggest that strength by which distance affects scat detection may vary by dog/handler team. This observation is supported by observations of the two dogs' search behavior in the field; CJ maintained a closer search pattern to his handler while Bruiser ranged over larger distances.

Overall success rates also vary between dog/handler teams. Team JS/B had a higher probability of detection than did Team JW/CJ when searching for captive scat, but found fewer "wild" scats over the course of the survey (JS/B =17, JW/CJ = 67). Long et al. (2007b) also found significant variation between detector dog team success. Variation between teams is critical to the practical application of this technique in the field. Geographically proximate sample locations, or repeated sampling occasions should be conducted by different teams to account for variation in team success.

The third factor affecting scat detection was the extreme ages of the samples themselves. Specifically, "fresh" scat samples (in this case, frozen within

3 days of deposition and thawed in the field immediately prior to trials) were found more often than other age treatments. Our data also suggests that samples subjected to daily precipitation for two weeks were found less often. While the effect of our experimental scat age on scat detection was stronger than predicted, it is not difficult to imagine that the strength of the odor trails emitted by fresh vs. degraded samples could influence a dog's ability to find a sample. However, there are numerous first-hand stories from detector dog handlers describing the poor quality and tiny size of scat samples found by detector dogs.

Finally, our results suggest that the probability of detection decreased as wind strength increases, but this conclusion is only partially supported by the GLMM analysis. The low statistical support for the effect of wind was surprising, and could potentially be due to low sample size or poor categorization of wind strength. However, even with our limited data set, we were able to detect a negative trend in the effect of wind strength on scat detection, highlighting the importance of understanding air flow between sample and dog. Handler training is paramount for understanding how vegetation and topography affect air-current flow. Handlers must be able to predict air-currents and direct the dog's search accordingly.

The results dismiss some of the common arguments against the use of detector dogs. The results from the GLMM analysis demonstrated that the source zoo was not a significant predictor of scat detection, even though the raw data showed a higher PD for Garlyn zoo. While there are many possible differences between the scat samples from different zoos (eg. hormone levels within the scat),

the most pronounced difference between zoos was the diet fed to their bobcats. Therefore, the lack of predictive power of the zoo variable, suggests that diet does not significantly impact scat detection by dogs. There was no trend in the effect of weather on detection, implying that dogs' performance was neither positively or negatively affected by the weather experienced during the experimental transects. Over the distance we evaluated, the PD did not from the first to the last leg of the transect, suggesting that the teams' performance was equal at the start and end of each transect. This is not surprising given each transect was only 3 km long, and detector dogs can cover 8 to 12 km per-day depending on heat and terrain.

The impact of understory vegetation density was not as predicted. Quite the contrary, probability of detection increased with density of understory vegetation. One possible explanation for this observed trend is the pace that the handler was able to maintain. As understory vegetation increased, the handler pace was slowed, often more dramatically than for the dog. Therefore, dogs may have had more time to search the surrounding area while the handler attempted to move along the route. These results suggest that the vegetation of the NLP is not a significant hindrance to the efficacy of detector dogs finding bobcat scat. The handler has a greater challenge than the dog when moving through alder thickets and swamps. Survey days through thick vegetation or challenging terrain may take longer, or necessitate a shorter route, but are not impossible. Open sphagnum low-lands did pose a challenge to both dog and handler when the ground became unsteady.

Microhabitat had an inconclusive effect on probability of detection. All models including microhabitat were far beyond ΔAIC =2. However, the raw data suggest that raised or hidden samples have a lower probability of detection as compared to other microhabitats. The effect of microhabitat most likely depends on training the dog receives specific to the behavior of the target species, as well as search direction given by the handler. For example, studies of arboreal species, such as fisher, have successfully trained detector dogs to target their search along downed tree trunks (UW Conservation Canines, *unpublished data*). For the purpose of this project, dogs were encouraged to search low to the ground, due to the assumption that bobcats defecate on the forest floor.

CONCLUSIONS:

The choice between hair snares or detector dogs ultimately depends on the project goals, resources available, and species of interest. The detector dog method was more cost-effective for generating unique nuclear genotypes than hair-snares. The cost efficiency of detector dogs holds true despite the low quality DNA found in scat samples and larger total budget needed for detector dogs compared to hair snares, primarily due to training the dogs to search for scats from target species. However, the low initial costs of setting up hair snares make them an attractive option if the species of interest has behavioral characteristics that allow for hair-snares to be target-specific (e.g. mustelids and squeeze boxes mounted on trees [Williams et al. 2009]), or if project goals include surveying for species diversity. However, if project goals include

detection of a suite of target species, detector dogs can also be trained to survey for many species at once (Long et al 2007a, Vynne 2010).

The costs incurred by the hair snare method are primarily time and travel to cover sufficient ground to obtain enough samples. Hair snares cannot distinguish multiple visitations if they occurred within a single check time period, while scat deposits are temporally independent even if found at the same time. Hair snares are also fixed locations, whereas detector dogs do not require any prior set up to move to a new sample location. Detector dogs also continually improve as the dogs adaptively learn where they are likely to find a scat of the target species, and therefore receive their ball reward.

Previous studies have also found hair snares to be unreliable for obtaining samples from secretive and reclusive felid species such as cougars and bobcats (Long et al. 2007, Ruell and Crooks 2007). Conversely, prior studies have found detector dogs to be successful for obtaining samples from bobcats (Harrison 2006, Long et al. 2007ab) as well as for a number of other carnivore species including kit fox (Smith et al. 2005), grizzly bears (*Ursus arctos*) (Wasser et al. 2004), and fishers (*Marten pennant*) (Long et al. 2007a). Detector dogs are emerging as a highly efficient, cost effective way of gathering information on species that are logistically difficult to study. For example, in a study of swift fox abundance, scat collection followed by genetic analysis was found to be more cost-effective than scent station, trapping, track counts, spotlighting, or auditory surveys (Smith et al. 2005). The use of scat samples is particularly appealing when monitoring endangered or threatened species characterized by solitary lifestyles, wary

behavior, or cryptic predation tactics because scat is an extremely abundant biological material and its collection is completely noninvasive.

If the choice is made to proceed with detector dogs for scat collection, it will be tempting to cull scat samples prior to laboratory analysis based on observable characteristics or confidence in the dogs' alert. However, this study demonstrates it is ill-advised to cull bobcat samples based on observed age, odor, or strength of the dog's alert. In fact, what we might consider an "old" sample may ultimately be a 'well-preserved' sample that yields higher quality DNA than a moist sample. These results echo similar recommendations to fully dry scat samples as soon as collected (Murphy et al. 2002). It should be noted that there are many different scat collection and storage methods suggested, and the optimal method will depend on the species (e.g.: carnivore vs. herbivore) and environment in which the study is conducted (e.g.: arid or humid). For species that defecate in latrines, there may be limited utility in collecting all samples from a latrine, especially for CMR where all samples may be from the same individual. On the other hand, if latrines are locations where multiple individuals defecate, then they may be particularly valuable locations to CMR or behavioral studies. The decision whether to collect and analyze samples from latrines will ultimately depend on the research objective and the behavior of the target species. One potential method of culling bobcat samples is by diameter. *A-priori* knowledge or a pilot season may be used to determine target scat diameter from sympatric species. Diet may affect scat diameter, so it is ill advised to use scat from captive animals for this purpose.

This study found four factors that affect scat detection by dogs: 1.distance of the sample from handler with increasing distance lowering probability of detection, 2. variation between dog/handler team, 3. extreme ages of the samples, with freshly deposited scat being found at a higher rate, and samples extremely degraded by precipitation found less often, and 4. increased wind speed decreasing the probability of detecting an odor trail. The variable detection of scat samples adds an additional level of complexity to the capture probabilities of CMR population estimation. Capture probabilities of traditional (or genotype CMR from hair snares) will vary with the individual animals' behavior (e.g. trap-shy / trap-happy response). The analogous capture heterogeneity for scat samples comes from individual differences in defecation location/rate. However, in addition to the capture heterogeneity due to individual defecation behavior, the detection of the samples once deposited will vary by those environmental and team parameters listed above. Future work should include the development of statistical methods to incorporate this unique source of capture heterogeneity into genotype CMR methods.

Given the effect of distance on probability of detection, it is tempting to conceive of a distance-function of olfactory detection similar to sight-ability curve. However, it is unlikely that there is a definitive point beyond which samples have a zero probability of detection even though handlers must maintain a line-of-sight to dog, because wind may bring the odor of a sample to a dog from far away. Detector dogs have a 'change in behavior' between when they are

simply searching, and when they are 'locked on' to an odor trail. The handlers are trained to recognize this change in behavior, and they will follow the dog in order to maintain line of sight when the dog is pursuing an odor trail. Temporally dependent factors such as wind speed, as well as the wandering search path of the dogs preclude the development of a standardized distance-function. Additionally, the large variability across teams decreases the likelihood of developing a standardized function.

The variation in success between dog/handler teams is critical to planning surveys, especially when transects or grids will be searched repeatedly. There is also variation in the amount of area surveyed between teams due to differences in dog search behavior. Certain dogs will maintain a closer search pattern with the handler while other dogs may wander farther from the handler while searching. There is no clear indication that one behavior is preferable to another, but the variation should be accounted for when planning survey days. Project managers should rotate teams when surveying areas of close proximity or the same cells over multiple sessions.

Wind strength also has direct impact to project planning. It may be more important to plan sample days around wind speed than weather events. For example, a rainy day with mild wind may be a better day to sample than a clear day with strong, gusty wind. Wind direction should also be considered when searching an area. Handlers must be trained to account for wind direction when directing the search of the dog to maximize the amount of area effectively searched and avoid missing upwind samples.

The factors that were not significant predictors of probability of detection in this study included: 1. diet of the animals, 2. fatigue (over 3 km), 3. microhabitat and, 4. density of understory vegetation. Whether detector dogs are trained with captive scats or wild samples an adjustment period of a few weeks may be needed for a dog to connect the scent of the training scat with wild scat of the target species. However, this study suggests that it is not the diet of the animal that affects probability of detection. Dogs selected for wildlife detector work have both extreme play drive and high energy level. This often makes them undesirable as domestic pets, but means that the dogs may have higher endurance than their handlers in certain conditions. However, fatigue may become important in areas of extreme weather conditions or topography. Extremely hot temperatures affect the hydration levels of the dogs, thereby impacting their scent-tracking abilities. Deep snow may also tire dogs before their handlers, especially when snowshoes allow the handler to stay above loose snow (UW Conservation Canines, *pers. comm.*).

The vegetation of the NLP does not significantly impact scat detection by canines. The understory density did not inhibit scat detection, and dense understory vegetation even had a marginally positive effect on scat detection, likely due to the amount of time it takes the handler to move through dense vegetation. While our microhabitat classifications did not have significant effect on PD, microhabitat may be important when the target species defecate in specific locations such as atop logs, in depressions, or water edges. Detector dog training can easily be modified given specific species behavior. This study included

regions of alder thickets, swamps, dense cedar, and areas of extreme coarse woody debris. The dog/handler teams were able to survey throughout all vegetation types. However, extreme vegetation, such as secondary growth of tropical forest, may pose a limitation to canine movement, and thereby limit the utility of detector dogs in select cases. In these cases we would expect many wildlife species would also avoid these impermeable areas as well, and both wildlife and detector dog would be able to use linear features as movement corridors.

Realization of a cost-effective method for collecting noninvasive genetic samples is extremely valuable to the field of wildlife ecology. Information from noninvasive genetic samples can be used to investigate a myriad of ecological questions including population demographics, conservation genetics, reproductive biology, and resource selection. Our field trials have provided both financial and biological guidelines for choosing between hair snares or detector dogs for noninvasive genetic sample collection. It has also provided guidelines for laboratory and field methods when employing detector dogs to search for wildlife scat. Our data support the practicality of the detector dog method for monitoring elusive species in a broad range of landscapes.

FUTURE RECOMENDATIONS:

Hair Snares: The two samples collected that yielded DNA were from the carpet patches with tacks. Numerous configurations of scent, bait, and visual attractants were used during this study. Our results suggest that alternate configurations of these attractants and snare elements will not increase the sample collection success. It is possible that the frequency with which the snares were checked prevented sample deposition due to human odor and re-baiting activity. However, care was taken to minimize odor in the area (no smoking or perfumes and no contact with the snare other than with forceps or swabs) and the potential for DNA degradation necessitates frequent checks of the stations.

Detector Dogs: Adequate training of both dog and handler are absolutely essential for the successful application of this method. Abbreviated training, or the selection of a dog that does not possess optimal behavioral characteristics (especially with a suboptimal play drive) may lead to poor performance in the field and high laboratory costs due to higher proportion of non-target samples. Anyone employing detector dogs in the field should absolutely consult a professional that specializes in wildlife detector dogs. Companies that currently run wildlife detector dog and handler training programs are University of Washington Conservation Canines (http://conservationbiology.net/conservation-canines/), Pack Leader Dog Training (http://www.packleaderdogtraining.net/), and Working Dogs for Conservation (http://www.workingdogsforconservation.org/). Services available and prices

vary over time; project planners should consult their websites to evaluate which group may meet their needs, and contact each group for up-to-date rates.

Training to avoid non-target species may be important in areas of high sympatric carnivore abundance. Beginning search periods in areas with high target density will help reinforce the target-specificity. This recommendation applies to the first few days of sampling sessions, as well to days where target scat density will likely be low. Non-target samples may also be included in training "problems" in the evenings during sampling sessions. However, professionals should always be consulted regarding the proper application of non-target training. Mishandled non-target training can result in a confused dog, frustrated handler, and disappointed project managers.

Many of the professional detector dogs are trained on numerous wildlife species and are employed on projects across the globe. Therefore, detector dog sample collection success can often be improved by the inclusion of a "burn-in" period (recommended maximum of two weeks). This will increase the cost of applying the detector dog method, but should result in a greater number of samples in hand. Field samples often smell somewhat different than training samples that have been stored in plastic and have gone through multiple freeze-thaw cycles. The burn-in period provides the dog sufficient opportunity to encounter field samples and be rewarded for finding these new-smelling targets, quickly locking the smells into the dog's repertoire. The burn-in period also acclimates the dog and handler to local conditions (e.g. climate, heat, urban noise,

local flora and fauna) prior to search initiation (UW Conservation Canines, *pers. comm.*).

In general, the detector dog teams in this study did not fatigue over 9km field-trial transects. Handlers were tired by especially thick vegetation, and dogs were tired when snow height required bounding. However, dogs have been successfully employed to find moose, wolf, and caribou scat in Alberta, Canada during the winter months in several feet of snow (UW Conservation Canines, pers. comm). Scat detection was not impacted by the weather conditions encountered during our experimental transects. Extreme heat conditions may impact olfactory detection if the dog is allowed to dehydrate or needs to pant excessively. However, when executed correctly, dogs can successfully survey in hot climates. For example, detector dogs have been successfully applied to find jaguar (*Panthera onca*) and puma scat in the Yucatan Peninsula of Mexico during the dry season (J. White and J. Cristobal *unpublished data*).

Budget Allocation: The primary expenses of hair snare sample collection are the operational costs of checking the snares for an extended period of time. Hair snares were employed for approximately 3 months while detector dogs were employed for less than one month. Vehicle mileage costs were not included in the analysis (only gas), but would further inflate the operational costs of hair snares over detector dogs. The primary costs of detector dogs are the initial training and travel. Total aggregate costs of the detector dogs were much higher than hair snares, not accounting for success rates of the two methods. The

difference in success of the two methods led to dramatically lower cost-per-sample for detector dogs than hair snares. The costs of these two methods will obviously be dependent on the study organism and project goals. Long term monitoring, or repeated sampling over years, could benefit from the low operational costs of detector dogs. Trained detector dogs require little time to layer on additional target species, which may enable dispersal of the initial costs of dog training across several project budgets. Professionals should be consulted regarding which species a single dog is trained on to avoid negatively impacting the search for any one species. For example, if the primary target species is rare in the study area, it is unwise to layer on a common species as a second target species (UW Conservation Canines, *pers. comm.*, Long et al 2007b).

The cost of the handler training with multiple target species will depend on whether one person is assigned the handler across several projects, or whether several people will be trained to work with a single dog. Projects that need to sample a population over small area or for a short duration will benefit from the small start-up costs of hair snares. It is important to remember that laboratory facilities may have different abilities for analyzing hair and scat samples. Therefore, the downstream costs of genetic analysis may differ between labs and will affect the overall project costs. Laboratory methods are rapidly becoming more efficient and effective for noninvasive samples, and so costs will vary from year to year.

Noninvasive Genetics and Capture-Mark-Recapture: In order to genetically mark enough animals for CMR, greater survey effort is needed with either detector dogs or hair snares. The hair snare method could potentially be improved by the implementation of a stratified design with greater effort within *a priori* designated prime habitat, and a greater density of hair snares within selected areas. Based on the results of this study, it is unclear whether greater survey effort with hair snares would dramatically increase sample size. However, the detector dogs were only employed for 35 survey days without a burn-in period, and sampled a smaller geographic portion of the study area. The addition of a burn-in period, more teams, or longer survey duration would increase the sampling effort. Given the CPUE of detector dogs during the short duration of our study, it seems like the greatest increase in marked individuals could be achieved with greater sampling effort with detector dogs.

One of the most important assumptions of CMR is population closure, both geographic and demographic. The assumption of demographic closure (no births, or death) would be easier to meet with the use of detector dogs, since a greater number of samples can be collected over a shorter amount of time (especially with the use of multiple dog/handler teams), rather than hair snares which require a longer duration of time. The entire NLP bobcat population has been genetically shown to be one interbreeding population (Millions and Swanson 2007), and the study site was only a small portion of the entire NLP bobcat population.

Geographic closure will be a difficult assumption to meet, due to the migration of individuals across the NLP, especially between the harvested area (where our study site was located) and the un-harvested area to the south west. Increasing the geographic extent of sampling to include the entire NLP would be the only way to ensure geographic closure for CMR, but would obviously increase the expense of sampling, likely above the point of feasibility. While not a perfect solution, a greater sampling area could be covered by targeting sampling using the habitat data generated by recent radio-telemetry of bobcats in the NLP (Pruess and Gehring 2007, Svoboda 2008). By focusing survey efforts in lowland forest, future project could increase the number of marked individuals as well as reduce sex-biased capture rate by limiting survey effort to areas frequented by both sexes. Targeting hair snare efforts in lowland forest across a large geographic range would likely incur high gas, labor, and mileage costs, and may have low returns based on our CPUE results. Conversely, the detector dog method is better adapted than hair snares for a large geographic study area, requiring a single pass through a given area to collect previously deposited scat samples. Matching genotypes within a single capture session (e.g. a single detector dog transect) can be utilized by specialized statistical CMR population estimation software such as CAPWIRE (Miller et al. 2005).

Even with targeted sampling, surveying the entire NLP is unlikely to be financially feasible. Therefore, future efforts will need to statistically estimate the degree of violation of geographic closure. Various statistical methods exist for dealing with the violation of geographic closure, including Bayesian hierarchical

models that explicitly account for movement distances (Gardner et al. 2009, Royle et al. 2008). Movement distances can be directly estimated from locations of multilocus genotypes between noninvasive capture sessions or between noninvasive sessions and the final harvest 'recapture' session (e.g. Williams et al. 2009). Within our Pigeon River study area, the average distance of our genotyped scat samples to Sections where bobcats were later harvested was smaller than diameter of the average MI female bobcat home-range. However, none of the genotypes obtained from scat matched harvest tissue.

Capture biases between sexes and individuals are inevitable when collecting genetic samples. However, sample collection is probably more random (less biased) using detector dogs than hair snares given that hair snares explicitly rely on the behavior of the animals to obtain a sample. Recent work with detector dogs have shown a decreased sex-bias in sample collection using detector dogs rather than visual searches by humans (Vynne 2010, K. Ayers. UW Center for Conservation Biology *pers. comm.*). However, detector dogs learn to search for the scat of target species if there are visible cues in the environment. For example, fishers often defecate on tops of logs. Experienced dogs learn to preferentially search areas where species-specific sample detection is more likely. While the ability of the dogs to learn benefits the number of samples obtained, visually-cued searches may bias sample collection if certain age classes, sexes, or individuals preferentially defecate in these locations. The independence of samples collected within the same dog transect, or at the same hair snare at

different times are not truly independent, but population estimation should be able to account for lack of independence through the use of random effects in their models.

Study design is critical to meet the assumption of sample representation of the population. A grid/uniform design makes sure that any inaccurate *a priori* assumptions of habitat association would not negatively affect the representation of the samples collected. For example, if only males move close to roads, and if we stratified our sampling with more effort in remote areas, we would be biasing our representation away from males. However, given the recent data from radio-telemetry of bobcats, a stratified design may be preferable (for a CMR) so that the majority of sampling effort is placed in areas that have an equal capture probability for males and females, namely, lowland forest.

Capture probability is likely heterogeneous between noninvasive marking sessions and harvest re-capture session. Bobcat hunters (re-capture period) likely choose their hunting locations based on both high probability of bobcat presence (lowland forest) and also accessibility. Therefore, a hybrid-estimator with multiple capture probabilities would need to be employed to account for the difference in representation from the marking session and the recapture session.

Laboratory Recommendations:

Field Collection: There are two leading considerations when collecting scat or hair samples in the field to optimize downstream laboratory success of DNA amplification: 1. Time to extraction and 2. Moisture levels within the

storage container. After a hair sample is deposited by the animal, the cells in the follicles exposed to the elements and can suffer rapid degradation. While the epithelial cells found in scat samples suffer similar rates of degradation (if not more rapid due to bacteria and other elements found in scat), when employing dogs to locate scat, they will inevitably find scat samples of extremely varying ages. If samples are stored properly, then DNA degradation should be vastly slower following collection than the time between deposition and collection. Therefore, while time to extraction should always be minimized, time between sample collection and DNA extraction may not be as crucial when dealing with scat samples collected by dogs.

The handling of collected samples is a crucial piece to optimization of this method. There have been numerous studies regarding alternative methods of hair and scat sample storage (Wasser et al. 1997, Roon et al. 2003, Nsubuga et al. 2004, Waits and Paetkau 2005, Santini et al. 2007). The results of this study suggest that the most important condition for successful PCR amplification following scat sample storage is low moisture. Drying the samples or keeping samples dry will minimize DNA degradation between collection and extraction. Optimally, samples should be dried out completely prior to storage. Freezing samples is recommended but controlling moisture is the most important recommendation. For example, it may be better to store completely desiccated samples dry at room temperature than to store moist samples frozen in plastic bags. Protocols are being developed for swabbing the outer surface of scat

samples rather than extracting the entire sample, and may yield higher amplification success than previous storage techniques (Rutledge et al. 2009).

Molecular Markers: The microsatellite markers used in this project were chosen for their high level of polymorphism and short fragment length. On average, FCA loci amplified more cleanly than the 6HDZ loci. None of the loci were multiplexed (multiplexing increases analysis efficiency but also increases the likelihood of allelic dropout). Our results also demonstrated the utility of using the mammalian sexing primer as a first-stage culling of samples that do not contain DNA.

APPENDIX I. LABORATORY PROTOCOL DEVELOPMENT:

Each 10μL polymerase chain reaction (PCR) contained:

- 5μL DNA template
- 1μL PCR buffer
- 200 pM dNTP
- 2 pmol forward and reverse fluorescently labeled primers
- nuclease-free bovine serum albumin (BSA)
- $MgCl_2$
- 0.5μ *Taq* DNA polymerase (New England Bio Labs, Beverly, MA)

PCR buffer contained:

- 1mM Tris-HCl at pH 8.5
- 1.5 mM $MgCl_2$
- 50 mM KCl
- 10μg/mL BSA
- 0.0025% Tween-20

Table A1.1: Locus-Specific bovine serum albumin (BSA) and $MgCl_2$ concentration.

Locus:	**FCA026**	**FCA043**	**FCA090**	**SRY/ZFX**
BSA:	1.7 μL	1.9μL	1.7 μL	1.7 μL
$MgCl_2$	0.4μL	0.2μL	0.4μL	0.0 μL

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