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Bryan Matthew Tom, Student

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ABSTRACT OF THESIS

A COMPARISON OF NONINVASIVE SURVEY METHODS FOR MONITORING MESOCARNIVORE POPULATIONS IN KENTUCKY

Harvest data are typically used to evaluate mesocarnivore population dynamics in many states, including Kentucky. While relatively easy to collect, these data are subject to reporting biases, and inferences about population trends can often only be made at coarse spatial scales. Gray fox (*Urocyon cinereoargenteus*), bobcat (*Lynx rufus*), and coyote (*Canis latrans*) populations in Kentucky are managed primarily through harvest data used to establish future harvest quotas. Increasingly, noninvasive survey methods have been used to characterize a number of population parameters for a variety of species; however, successful use of these methods is often site-specific. We assessed the efficacy and cost-effectiveness of two noninvasive survey methods, scat detection dogs and rub-pad hair snares, for surveying mesocarnivore species at two sites in the mixed-mesophytic forest of northeastern Kentucky. We sampled 100 hair snares covering approximately 100km^2 and 27 transects covering approximately 27km^2 from which 7 hair samples and 261 scat samples were collected respectively. Hair snares cost $397/sample at 6.4 hours/day, while scat detection dogs cost $47/sample at 4.9 hours/day. Genetic methods were used to identify biological samples to species and individual. Our findings should prove useful to state wildlife managers in comparatively evaluating methods for future mesocarnivore monitoring.

KEYWORDS: Mesocarnivore, Noninvasive, Genetic, Hair Snare, Scat Detection Dog

Bryan M. Tom

1 August, 2012

A COMPARISON OF NONINVASIVE SURVEY METHODS FOR MONITORING MESOCARNIVORE POPULATIONS IN KENTUCKY

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture
at the University of Kentucky

By

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Lexington, Kentucky

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and Dr. Paul J. Kalisz, Associate Professor of Forest Soils

Lexington, Kentucky
2012
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CHAPTER 1: ECOLOGICAL ROLE OF MESOCARNIVORES

Buskirk (1999) defines mesocarnivore as a predatory mammal weighing between 1 and 15 kg. Mesocarnivore has also been used in a dietary classification context to characterize mammals whose diets consist of 50-70% vertebrate flesh (Van Valkenburg 2007). These definitions are restrictive but can be appropriate for class delineation in certain types of analyses. A more flexible definition, with broad ecological applications, may be desired. Within this framework, a mesocarnivore, or mesopredator, may be considered any predatory mammal which occupies intermediate trophic levels in a given community (Prugh et al. 2009). This type of generalization allows for an animal to be a mesocarnivore in one ecosystem and an apex predator in another (e.g., coyote, *Canis latrans*). However, this fact illustrates the challenge involved when attempting to classify a group so wide ranging and ecologically diverse. Regardless of which definition you use, mesocarnivores vastly outnumber large carnivores in species richness and the diversity of their behavior and ecology (Roemer et al. 2009).

Regardless of their relatively low densities, large carnivores can have profound top-down impacts on ecosystems structure (Ripple and Beschta 2004; Ray et al. 2005). Yet despite the increasing recognition of their ecological importance, overexploitation coupled with habitat loss and fragmentation by humans has caused the eradication of large carnivores from much of the globe (Laliberte and Ripple 2004), restricting the remaining populations to more wild and remote areas. Expanding on Laliberte and Ripple (2004), Prugh et al. (2009) found that recent range contractions of large carnivores have been countered by range expansions of many mesocarnivore species; seven species classified as apex predators experienced some degree a range contraction, while 60% of

mesocarnivore species exhibited range expansions. Historically, mesocarnivore species outnumbered apex predator species by no more than 9:1 and only in select areas of North America, a ratio that has since changed dramatically to 17:1 as mesocarnivores filled niches vacated by large carnivores throughout large portions of the United States (Prugh et al. 2009). This phenomenon termed mesopredator release (Soule et al. 1988) occurs in the absence of or negative change in apex predator density or distribution and subsequent expansion of density, distribution, or changes in behavior of mid-trophic predators in a trophic system (Brashares et al. 2010). Brashares et al. (2010) also point out that mesopredator release is often hypothesized to have negative effects on species occupying lower trophic levels, but this should not be considered a requirement for the definition.

Mesopredator Release

The concept of mesopredator release is worth examining in greater detail due to its complexity and global implications. As large carnivores continue to decline and trophic webs are disrupted, insights gleaned from mesopredator release may inform future wildlife management decisions. Since the late 1980's, researchers have used observational and empirical studies to examine the effects of mesopredator release in terrestrial, marine, and freshwater ecosystems. A recent study of mesopredator release that exemplifies the Brashares et al. (2010) definition took place in the Greater Yellowstone Ecosystem (GYE). Based on the traditional trophic cascade model, one could expect the survival of ungulate prey such as pronghorn (*Antilocapra americana*) to decrease as sympatric wolf densities increase. However, Berger et al. (2008) found an opposite trend; pronghorn were thriving in areas of high wolf density and declining in areas of low wolf density. To explain this observation, the authors compared coyote

densities among study sites and found that high wolf densities correlated to low coyote densities, due to top-down regulation through interference competition and intraguild predation. As coyotes are known to prey heavily on pronghorn fawns in the GYE, the absence of top-down forces (i.e., low wolf densities) released coyotes and allowed them to prey on pronghorn fawns without restrain. Unfortunately, few places remain with a large predator guild as complete as the GYE and in many systems coyotes act as the lone apex predator.

In the absence of large carnivores, coyotes across much of North America have ascended to the role of, quite literally, top dog (Gompper 2002). Their range has expanded by 40% in North America over the last few centuries, more than any other mesocarnivore species (Prugh et al. 2009). Coyotes are intelligent, adaptable generalist predators, capable of flourishing in close proximity to humans (Gompper 2002). There are more terrestrial studies of mesopredator release with coyotes acting as apex predators than any other carnivore species (Brashares et al. 2010). A heavily cited example of this comes out of coastal southern California. Crooks and Soule (1999) used the principles of trophic cascades and island biogeography to conclude that mesopredator release was, in part, responsible for sharp declines in sage-scrub dependent bird populations. In this example, the absence of coyotes released gray fox (*Urocyon cinereoargenteus*) and domestic cats (*Felis silvestris catus*) to prey on scrub-breeding birds. Within sage-scrub fragments, coyote abundance was positively correlated to scrub-breeding bird abundance and negatively correlated to mesocarnivore (gray fox, domestic cat, etc.) abundance. The Crooks and Soule (1999) study was more than just a compelling case for mesopredator release, it highlighted some of the inherent complexities surrounding the concept.

Our understanding of trophic cascades has deepened considerably since the simplistic, 3-level (decomposer, producer, and consumer) perspective provided by Hairston et al. (1960). As an extension of trophic cascade theory, mesopredator release is also influenced by varying degrees of ecological factors. Two system-specific factors have been shown to exert the most influence over mesopredator release: the productivity of a landscape and the strength of ecological interactions among trophic levels (Brashares et al. 2010). These factors not only affect the outcome of mesopredator release, they make it extremely difficult to determine if mesopredator release has actually occurred. In theory, landscape productivity will determine a trophic level's dominance over a given system. For example, a system of low productivity is expected to limit mesocarnivore abundance through prey availability instead of predation (Brashares et al. 2010). Only two studies have been successful at separating the effects of landscape variation and apex predator decline on mesocarnivore abundance (Crooks and Soule 1999; Elmhagen and Rushton 2007). Using long-term wolf, Eurasian lynx (*Lynx lynx*), and red fox (*Vulpes vulpes*) abundance data throughout Sweden, Elmhagen and Rushton (2007) demonstrated the strength of mesopredator release was dependent on landscape productivity. The authors found top-down effects to be strongest in highly productive regions while bottom-up effects controlled areas of low productivity. Crooks and Soule (1999) found that coyote abundance regulated mesocarnivore predation of ground nesting birds. However, this study was conducted in a highly fragmented landscape, resulting in sage-scrub habitat "islands" restricted to steep slopes. Using the principles of island biogeography, Crooks and Soule (1999) were able to correlate habitat fragment size to coyote abundance. Thus, concluding that mesopredator release combined with habitat

fragmentation was responsible for structuring the community. Landscape variation should certainly be considered when developing a mesopredator release study, but the productivity or fragmentation of an area will not independently predict an ecological response.

The strength of ecological interactions among trophic levels is critical to identifying mesopredator release. The strongest ecological interactions occur between predators with a highly specialized diet and their primary prey species. Therefore, the removal of a dietary specialist from a system is expected to result in a considerable increase in primary prey species abundance. In contrast, dietary generalists would exhibit weaker ecological interactions because their influence is spread out among many prey species. The strength of ecological interactions among trophic levels, in relation to dietary plasticity, helps to explain the increase in lower trophic level predation pressure resulting from mesopredator release (Brashares et al. 2010). Apex predators are typically hypercarnivorous, effectively restricting their diet to a certain degree of specialization. Conversely, mesocarnivores tend to be omnivorous or dietary generalist allowing for the exploitation of many prey species. As mesocarnivores are released from top-down regulation, their flexible diets facilitate high population densities (Roemer et al. 2001). This strengthens their ecological influence and in turn, suppresses a wide variety of prey species.

A deep understanding of dietary niche breadth among all trophic levels is important when attempting to explain mesopredator release, but there are a myriad of other factors that will significantly complicate the process. To add further complexity, these factors can be system-specific, and it is essential to consider multiple species per

trophic level. A study may focus on only 1 or 2 species but, depending on the system, the behavior and habits of target species will be influenced by other members of their community. A classic example supporting the community structuring potential of behavioral change comes from Ripple and Beschta (2004) working in the northern range of Yellowstone National Park (YNP). Wolves were extirpated from YNP in the mid 1920's and then reintroduced to the park in 1995. Elk (*Cervus elaphus*) were being culled at an increasing rate from the mid 1920's until 1968, when it was decided that "natural regulation" should dictate their numbers. After 1968, elk populations grew steadily and as a result, riparian vegetation, such as cottonwood (*Populus* ssp.), aspen (*Populus* ssp.), and willow (*Salix* ssp.) recruitment essentially stopped due to over-browsing. As streams in the northern range became denuded of riparian vegetation, erosion increased and beaver (*Castor canadensis*) populations suffered. After wolf reintroduction, elk populations began to decrease while beaver populations grew and riparian vegetation flourished. Profound changes in elk behavior were also noted. Before wolf reintroduction, elk leisurely browsed creek banks for extended periods of time, but after wolf reintroduction elk moved quickly and vigilantly through riparian corridors, only stopping briefly to browse. The steep creek banks prevented early predator detection and could act as a funnel, narrowing escape routes during an attack. It was the presence of wolves and the subsequent fear of predation that altered elk behavior, with rapid and drastic effects on community structure.

In the previous example, prey behavior was altered by the presence of an apex predator. However, behavioral adaptations among predator species can also affect the strength of ecological interactions and the outcome of mesopredator release. Intraguild

predation and interference competition are significant drivers for space use and resource partitioning among predators, both within and among trophic levels. Intraguild predation occurs when one predator species consumes another predator species with whom it also competes for shared prey (Vance-Chalcraft et al. 2007). Two or more predator species can be affected by intraguild predation and each to a varying degree. For example, two predators may be capable of consuming each other, or multiple predators may be consumed by one or more predators occupying a higher trophic level. These interactions will primarily influence space use, causing spatial or temporal avoidance by the exploited species (Brashares et al 2010). Interference competition occurs when a species out-competes another species for a given resource (e.g., prey species, habitat type, optimal den site, etc.) (Case and Gilpin 1974). The effects of which, can play a significant role in limiting a species abundance and distribution. Tannerfeldt et al. (2002) believe that arctic fox (*Alopex lagopus*) distribution was restricted by the presence of red fox, through interference competition for den sites. While the authors admit that other factors could be partially responsible, they were able to show arctic fox breeding success was negatively correlated to red fox presence.

The interrelated complexities of trophic interactions are numerous. Other factors such as prey switching, territoriality, exotic species introduction, climate variation, and anthropogenic pressure further complicate the ecological process. As apex predators continue to decline in both abundance and distribution, it may be necessary to predict the system-specific consequences of mesopredator release. To achieve these predictions, observation alone may not be enough. Henke and Bryant (1999) used a controlled experiment and continuous monitoring to document the effects of mesopredator release in

west Texas. The authors measured relative faunal abundance between control and treatment sites for 3 years; one year prior to coyote removal followed by 2 years of continuous coyote removal from only treatment sites. The absence of top-down regulation by coyotes on treatment sites resulted in statistically significant increases in mesocarnivore (bobcat, *Lynx rufus*, gray fox, and badger, *Taxidea taxus*) abundance.

Any situation in which the targeted removal of a species is deemed necessary, such as nuisance control, should be carefully considered beforehand. An incomplete knowledge of the system coupled with species extermination can have deleterious ecological effects. Such was the case in east-central Florida, where raccoons (*Procyon lotor*) were removed from the beaches to prevent nest predation and increase endangered sea turtle (family *Cheloniidae*) survival. Unfortunately, the successful removal of raccoons allowed ghost crab (*Ocypode quadrata*) abundance to increase, resulting in even higher nest predation rates (Barton 2005). Ignoring the ecological potential of unregulated mesocarnivores can have far-reaching implications. A better understanding of trophic cascades in relation to mesopredator release will help guide appropriate management decisions and ultimately protect biodiversity.

Prior studies collectively suggest that instances of mesocarnivore overabundance are likely to spread with the decline of apex predators. In general, large carnivores are predisposed to the impacts of humans because they occur in relatively low densities, avoid fragmented landscapes, and frequently conflict with human interests (Buskirk 1999). In contrast, mesocarnivores thrive in habitat fragments and can attain high densities in close proximity to human development. Furthermore, human dominated landscapes, such as agricultural lands or suburban sprawls, can actually promote

mesocarnivore overabundance by providing readily accessible food sources in a large-predator-free environment. Efforts to regulate mesopredators are often met with a great deal of resistance. Henke and Bryant (1999) reported that decreasing the coyote population by 50% in a 10 km^2 area of west Texas required the lethal removal of 354 animals over a 2 year period. High densities, recruitment rates, and dispersal rates make mesocarnivore control programs costly, and therefore unattractive (Palomares et al. 1995). Perhaps the most efficient and cost-effective approach to preventing widespread mesopredator release is the reintroduction of large carnivores to their native ranges.

Studies of mesopredator release have provided substantial evidence for the community restructuring potential of mesocarnivores. This potential is amplified by the near ubiquity of human disturbance and the global decline of apex predators. As a result, mesocarnivores have ascended the trophic hierarchy and now occupy the apex consumer position in many systems. However, occupying an apex position does not necessarily translate to filling an apex ecological role. Mesocarnivore species tend to retain their behaviors regardless of trophic promotion, and few examples exist to demonstrate the contrary (Prugh et al. 2009).

One such example by Gese and Grothe (1995) documented a behavioral change in coyotes in the absence of wolves. While conducting a forging ecology and carcass utilization assessment, the authors observed several predation events targeting large ungulates, the typical prey of wolves. In these cases, coyotes formed packs of 5-7 individuals and worked together to bring down elk and white-tailed deer (*Odocoileus virginianus*). However, further observation helped to illustrate the ecological differences between an apex predator and the mesocarnivore attempting to occupy an apex role.

Despite a relatively large pack size, only 2-3 coyotes were actually responsible for making the kills. This is in sharp contrast to wolf attacks, where many pack members participate through various, synchronized roles (Gese and Grothe 1995). Additionally, the manner through which these coyote attacks occurred differed from typical wolf attacks. Wolves generally attack from behind, targeting the rump or hamstrings of large ungulate prey, whereas coyotes in these cases were observed targeting the throat. It could be argued that a small mammal-dependent diet has prevented coyotes from evolving a sophisticated pack hunting system (Kleiman and Eisenberg 1973). Fundamental differences such as these highlight the evolutionary, and therefore behavioral, dissimilarities between the ecological roles of apex predators and mesocarnivores.

Collective research throughout the California Channel Islands has revealed an unusual occasion in which a mesocarnivore species has filled the role of an apex predator. The island gray fox (*Urocyon littoralis*) and the island spotted skunk (*Spilogale gracilis amphiala*) are the only terrestrial predators native to the Channel Islands (Roemer et al. 2002). Of the eight Channel Islands, island gray fox occur on six and are sympatric with island spotted skunk on two, Santa Cruz and Santa Rosa (Jones et al. 2008). Before their recent population decline, island gray fox, a mesocarnivore weighing only 1.5-2.5 kg, acted in an apex capacity to effectively regulate island spotted skunk abundance. The introduction of feral hogs (*Sus scrofa*) led to a resident breeding population of golden eagles (*Aquila chrysaetos*) which, in turn, overexploited island gray fox (Roemer et al. 2001). With island gray fox numbers dangerously low, island spotted skunk and island deer mouse (*Peromyscus maniculatus*) abundance increased rapidly (Roemer et al. 2001). While this is a clear example of a mesocarnivore providing apex

level ecosystem services, landscape influences cannot be ignored. The insular nature of island systems often leads to low species diversity and simple, more linear trophic interactions (Roemer et al. 2009). Additionally, the island gray fox would not be capable of attaining apex status in mainland communities, much like their closest and larger relative the gray fox.

Allometric Ecology

Under special circumstances mesocarnivores attempt to replicate the ecological roles of apex predators. These situations can be habitat dependent, such as isolated island systems, or species-specific, like the ecological plasticity exhibited by coyotes. However, most systems are not discrete and most mesocarnivores do not display behaviors that bridge trophic levels. While abundance may spike, causing system-wide restructuring, the ecological roles of mesocarnivores remain consistent regardless of trophic status. The most apparent and perhaps the most relevant difference between mesocarnivores and apex predators in an ecological context is body size. The adult body size (i.e., weight) of mammalian carnivores has been linked to a variety of critical ecological determinants such as species richness, energetic requirements, foraging efficiency, dietary niche breadth, fecundity, population density, home range size, and dispersal capabilities. Therefore, utilizing an allometric perspective will allow us to better interpret mesocarnivore ecology.

The study of allometry, or relating body size to physical, physiological, and behavioral traits, was first described by Otto Snell in 1892 (Imam and Abdullahi 2011). Almost one century later, after advances in data collection methods, allometry became

widely popular among scientists seeking to understand the ecology of mammalian carnivores. The order Carnivora is extremely diverse, boasting 271 extant species (Bininda-Emonds et al. 1999), which span more than four orders of magnitude in body mass (Gittleman and Purvis 1998). Carnivora is further divided into two distinct suborders, Caniformia ("dog-like" carnivores) and Feliformia ("cat-like" carnivores). Using the sex-averaged body mass data of 240 carnivore species, pooled from a multitude of literature sources, Gittleman and Purvis (1998) reported the allometric relationship to species richness among Carnivora. They found a positive association, especially within Caniformia, between small body size and species richness. The unexpected deviation from a linear relationship between body mass and species richness was explained by variations in habitat productivity and metabolic rates or by extinction events that left gaps in the Carnivorian assemblage. The latter explanation could have important conservation implications, helping to predict future extinctions. Regardless, species richness within the mesocarnivore guild can provide insights into their ecological diversity.

Other allometric relationships, such as those dealing with diet and energy demand, adhere to much stricter and more universal associations. Body size determines a predatory mammal's energetic requirement, which in turn determines its prey selection and hunting strategy (Carbone et al. 1999). Large carnivores must ingest copious amounts of prey biomass to sustain their physiology. To do so, they select large prey which allow for maximum net energy gain (Carbone et al. 2007). Recent examples from Africa support this trend for large carnivores, showing positive associations between predator body mass and both prey body mass (Radloff and Du Toit 2004) and proportion of large prey selected (Owen-Smith and Mills 2008). Among carnivores, as body size

decreases the energy expenditure and inherent risk of attempting to attack larger prey becomes too great. This cut-off point occurs at approximately 21.5 kg and predators weighing less exhibit an overwhelming tendency for smaller prey (Carbone et al. 1999). Interestingly, 21.5 kg is close to the upper limit for coyote body mass and may help to explain their propensity for both large and small prey.

Most mesocarnivores weigh in below the threshold described by Carbone et al. (1999) and have adapted generalist or omnivorous feeding strategies. These strategies allow mesocarnivores to utilize a broad prey base which is represented by a variety of taxa (Roemer et al. 2009). In addition, low cost dietary subsidies such as fruit, vegetable matter, and insects produce an energy buffer against the high cost of capturing and subduing prey or failed predation attempts (Carbone et al. 2007). Due to energy constraints, large carnivores typically do not compete with mesocarnivores for prey. Some of these prey species, like seed dispersers or seed hunters, can be very influential to producer-level trophic functions, leaving mesocarnivores to regulate their abundance (Roemer et al. 2009). Small body size has directly affected the dietary niche breadth and foraging efficiency of mesocarnivores, allowing them a flexible energy budget and solidifying their vital ecological roles.

Body size in carnivores has also been shown to limit physical capabilities and establish demographic parameters. These types of data are especially valuable for managing populations and assessing the feasibility of reintroduction programs, or monitoring their progress. Carnivore litter size and rate per individual, known as fecundity, can help to explain the persistence or rarity of a species. Allaine et al. (1987) have shown that, among mammals, carnivores exhibit one of the strongest associations

between body mass and fecundity. Their results indicate that reproductive potential decreases sharply as body size increases. Based on these results, it can by hypothesized that population density (n/km^2) will follow a similar trend. Several studies have addressed this question, with results that corroborate the prediction that the negative allometric relationship to population density remained as mammals were classified according to dietary strategy and geographic location (Peters and Raelson 1984; Currie 1993; Silva and Downing 1995). Peters and Raelson (1984) showed that carnivores and omnivores in North America, South America, Africa, and Asia all exhibited this same pattern. Silva and Downing (1995), though, revealed that as carnivore body size reached its upper limits, the negative association to population density began to level off. This suggests the existence of some apex-level trophic equilibrium through which the population density of the largest carnivores may be maintained by factors such as habitat productivity or dispersal capability. Mesocarnivores, on the other hand, exhibit a clear, negative log-linear allometric association to body size. This implies that mesocarnivore population densities are heavily reliant on body size, which can be explained by another allometric relationship discussed earlier. Dietary niche breadth, as a product of body size, has afforded mesocarnivores with the dietary plasticity to efficiently exploit a given system. As a result, mesocarnivore density has a much greater potential than that of large carnivores, and in the absence of top-down regulation, as seen in mesopredator release, small bodied predators can reach extremely high densities.

Mesocarnivore abundance may also be explained by another allometric association that imparts physical limits to their home range size and dispersal capabilities. Certain morphological features (e.g., short limbs) may prevent some animals from

traveling long distances within various temporal scales, but morphology alone will not dictate space use. This point is exemplified by the wolverine (*Gulo gulo*), a short, stocky mustelid weighing on average 15-20 kg, which has been documented traversing a home range of up to 900 km^2 (Kyle and Strobeck 2001). Allometric equations of home range size would place the wolverine in the same class as the puma, an animal weighing over five times greater (Lidstedt et al. 1986). Despite the few exceptions, carnivores follow a general trend opposite to that of population density: as body size increases, home ranges get larger (Lidstedt et al. 1986; Swihart et al. 1988). This suggests that home range size is determined by the energy requirements for a given species. However, the influences of diet breadth, foraging efficiency, and habitat productivity must be considered (Carbone et al. 2005). Species that exploit a wider variety of prey, employ low cost hunting strategies, or inhabit highly productive systems will require less area to obtain their caloric needs. The body size of mammalian carnivores has been shown to dictate a range of essential ecological processes. In addition, each of the trends discussed are interrelated and all stem from fundamental physiological constraints. Collectively they form a crucial trophic component, the mesocarnivore, which effectively bridges the gap between large and small.

Trophic Linkage

Mesocarnivores not only link trophic levels, they also play an important role in linking trophic systems. Many mesocarnivores are opportunistic and have readily adopted scavenging as part of their foraging strategy. Mesocarnivores occurring in coastal regions frequently subsidize their diets with marine derived nutrients through scavenging or direct predation (Carlton and Hodder 2003). In Baja California, Rose and

Polis (1998) analyzed the diets of coastal and inland coyote populations. They found an average of 47.8% of all items in coastal coyote scats originated directly from the sea, compared to < 1% from inland populations. While conducting this study, the authors also observed other mesocarnivore species, such as gray fox, kit fox (*Vulpes macrotis*), and ringtail (*Bassariscus astutus*) consuming marine prey. Carlton and Hodder (2003) compiled studies involving terrestrial species preying on marine organisms and found accounts of 16 different mesocarnivore species utilizing marine resources. In addition to relative diet, Rose and Polis (1998) estimated relative abundance between coastal and inland coyote populations. Their results showed that coastal coyote populations were on average 4.7 times the density of inland populations. The trend of increased abundance in terrestrial carnivores occupying coastal systems is likely global in scale (Rose and Polis 1998; Carlton and Hodder 2003). Opportunistic mesocarnivores facilitate the flow of nutrients from marine to terrestrial systems, which alters the structure and complexity of their trophic interactions.

As mesocarnivores have the ability to promote nutrient flow among systems, they too have the potential to impede well established nutrient cycles, with community altering effects. A profound example of this comes out of the Aleutian archipelago, Alaska. Historically, the archipelago supported dozens of breeding seabird species with populations reaching tens of millions of individuals (Maron et al. 2006). The islands were ideal seabird breeding grounds, despite their moderate productivity, because of an abundant marine food supply and predator free environment. Over a century ago, the collapse of the maritime fur trade led to the introduction of non-native arctic foxes to >400 islands as a supplemental fur source (Croll et al. 2005). Foxes persisted on

approximately 100 of these islands and did so by predating seabirds to the point of near extirpation (Maron et al. 2006). With time, the islands described as “fox-infested” displayed a clear shift in floral composition. Islands that remained fox-free were classified as dense grassland, with a diversity of lush grasses and sedge species typically found at high latitudes. In contrast, fox-infested islands contained a much higher proportion of dwarf-shrubs, forbs, mosses, and lichen which closely resembled the community structure of high latitude tundra (Maron et al. 2006). To explain the producer-level differences observed between fox-infested and fox-free islands, Croll et al. (2005) and Maron et al. (2006) compared the nutrient profiles of plant and soil samples among islands. The samples revealed phosphorus levels in soils on fox free islands were almost one-quarter those on fox-infested islands. In addition, plants of fox-free islands contained substantially more nitrogen than those of fox-infested islands. The authors concluded that arctic fox predation on seabird communities resulted in a 60-fold decrease in nutrient-rich guano deposition, which ultimately transformed lush grassland to barren tundra.

Nutrient flow between ecosystems can alter community composition, structure, and function. The ecological role of mesocarnivores to either facilitate or impede this flow has recently become apparent. Perhaps more importantly, toward the advancement of ecological theory, are the discovery of new pathways through which predators directly or indirectly influence trophic processes. The means through which arctic fox predation cascaded into a producer-level composition shift was not predicted by theorists like Hairston et al. (1960) or their successors (Croll et al. 2005). As such, further study of

mesocarnivore ecology is likely to have implications beyond conservation and management, leading to a more complete, global trophic paradigm.

Project Species Accounts

The discussion of mesocarnivore ecology thus far has been broad in scope, and has dealt with trends common to many species as well as their ecological implications. However, my research focused on three mesocarnivore species: gray fox, bobcat, and coyote. To provide context for my research, I will briefly discuss the natural history and conservation status of these species individually, with emphasis on North American populations. In addition, I will summarize the status and management of these species in Kentucky, as communicated through the Kentucky Department of Fish and Wildlife Resources (KDFWR).

Gray Fox

The genus *Urocyon* is considered the oldest lineage among Canidae, and is believed to have diverged from other fox species early in the family's history, approximately 12 million years ago (Mya) (Graphodatsky et al. 2008). Currently, *Urocyon* is comprised of only two remaining species, the gray fox and the island gray fox (*U. littoralis*); the latter is endemic to the Channel Islands, California, while the former can be found across the United States, excluding the northwestern quarter of the country, and south through Mexico into northern South America. As a result of its distribution, the gray fox is highly polytypic with 16 subspecies recognized (Fritzell and Haroldson 1982).

In the eastern United States, gray fox weight 2 – 5.5 kg and are closely associated with deciduous forests. However, the species can inhabit a variety of systems, from brushy or rocky arid lands in the western U.S. to subtropical and tropical forests in Central and South America (Fritzell and Haroldson 1982). Gray fox have uniquely flexible limbs and are accomplished tree climbers, adaptations that improve foraging efforts and allow for predator avoidance (Fritzell and Haroldson 1982). The diet of gray fox is regarded as the most omnivorous of all North American canids, which is especially true in spring and summer with the increased availability of fruits, nuts, and invertebrates (Fritzell and Haroldson 1982). Rodents, lagomorphs, and a variety of other small vertebrates make up a greater proportion of fox diets during fall and winter (Fritzell and Haroldson 1982). Gray fox have also been documented exerting strong top-down control on weasel (*Mustela* spp.) populations (Hensley and Fisher 1975).

While gray fox are predated by species such as coyote, bobcat, golden eagle, and puma, their populations are influenced primarily by fur trapping (Fritzell and Haroldson 1982). Disease, especially canine distemper, also plays a localized role in regulating gray fox numbers. Despite these multiple sources of mortality, the gray fox is currently listed by the International Union for Conservation of Nature (IUCN) Red List as a species of least concern (Cypher et al. 2008) because it is abundant throughout its range with stable population trends. The population status of gray fox in Kentucky is currently under review (Laura Patton, KDFWR, pers. comm.). Throughout the region, gray fox harvest rates have declined, but this trend has not been confirmed, nor has a specific cause been determined in Kentucky. The KDFWR believes this trend may be due to an increase in coyote and bobcat abundance or a result of distemper outbreaks transmitted by dense

raccoon populations. The state is in the process of updating harvest data and trapper surveys to examine long-term trends in gray fox harvests. Additionally, KDFWR plans to track future distemper outbreaks among raccoon populations to assess their impact on gray fox numbers (Laura Patton, KDFWR, pers. comm.).

Bobcat

The bobcats is one of four extant members of the genus *Lynx* (Hansen 2007) that is believed to have diverged from the Puma lineage approximately 7 Mya (Johnson et al. 2006). This cat is on average the smallest species within this clade and arguably the most abundant (Hansen 2007). Bobcats are moderately polytypic with 12 subspecies recognized (Macdonald et al. 2010). The distribution of the bobcat is primarily in the United States with gaps in the upper Midwestern and northeastern states, but they can also be found along the southern border of Canada and the northern half of Mexico (Macdonald et al. 2010).

The bobcat, weighing 7.3 – 10 kg, is a habitat generalist that successfully occupies a range of systems, form arid or rocky deserts to dense tropical forests, a factor that has allowed it to persist where other more specialized felids have declined or been locally extirpated by humans. Prey availability and adequate cover appear to be the most important factors to bobcat distribution (Hansen 2007). Lagomorphs are the primary prey of bobcats throughout their range, although they are known to predate rodents, squirrels, birds, lizards, and insects (Hansen 2007). In comparison to their only sympatric relative, the Canada lynx (*Lynx canadensis*), bobcats demonstrate a much more generalist and opportunistic dietary strategy (Macdonald et al. 2010).

The IUCN Red List cites the bobcat as a species of least concern due to its general abundance and widespread distribution (Kelly et al. 2008). Although habitat destruction, with emphasis on prey decline and reduced cover, and competition with coyotes pose serious threats to bobcat persistence in a given location, the greatest cause of mortality remains fur trapping, as bobcat are the leading felid in the global fur market (Kelley et al. 2008). Its popularity as a furbearer species has caused the bobcat to be protected under Appendix II of the Convention on International Trade in Endangered Species (CITES), which monitors and regulates their trade.

In accordance with CITES, bobcat harvest in Kentucky has been closely monitored for over 2 decades. State law requires all harvested bobcats be registered with the KDFWR in order to regulate harvest. KDFWR uses this spatially and temporally explicit harvest data to predict harvest trends and adjust season length, season timing, and bag limits accordingly (Laura Patton, KDFWR, pers. comm.). In addition, the state considers the total number of licensed trappers, the proportion of successful trappers, current fur prices, and in-state research to guide management decisions. Bobcat abundance has been estimated at between 2.3-3.5 million individuals throughout the entire United States, with an increasing trend (Roberts and Crimmins 2010). This trend has been reflected in Kentucky as bobcat harvest zones have expanded from exclusively eastern Kentucky in the late 1980's to the current statewide harvest (Laura Patton, KDFWR, pers. comm.).

Coyote

Canis, which includes wolves, jackals, and the coyote, split from the South American canid linage approximately 11 Mya (Wang et al. 2004). Coyote distribution blankets North America and they are found everywhere except extreme northeastern Canada and the Atlantic coast of Central America (Gese et al. 2008). The species is highly polytypic with 19 subspecies currently recognized.

Coyotes, which weigh 9.5 – 18.5 kg, occupy almost every available habitat type within their vast distribution, including human altered landscapes like agricultural and residential areas. This species epitomizes the generalist predator as it will consume a considerable variety of items, ranging from fruit and insects to large adult ungulates (Gese et al. 2008). Coyotes are intelligent and will adjust their hunting technique according to prey species. They can exhaust large prey cursorily or utilize a slow stalk and pounce when targeting small mammals.

There are very few natural threats to coyote populations, especially those which are allopatric to wolves. In contrast, coyotes are actually more likely to pose a threat to sympatric species, either through competitive exclusion or direct predation. As a result of their widespread abundance and adaptability to human disturbance, the IUCN Red List considers coyotes a species of least concern (Gese et al. 2008). The major sources of coyote mortality are fur trapping and persecution, with many states requiring no bag limits and allowing year-round hunting.

Harvest data and nuisance wildlife control operator (NWCO) reports indicate the coyote population in Kentucky is stable to increasing (KDFWR, unpublished data).

Statewide abundance estimates are not available, but Kentucky has seen a recent increase in the reported number of human-coyote conflicts. Annually, 200-300 coyotes are removed by NWCO's in Kentucky for livestock depredation, primarily sheep and calves, or other nuisance issues (KDFWR, unpublished data). KDFWR allows year-round hunting, a limited trapping season, and offers county specific NWCO and trapper contact information to the public in its effort to manage the coyote population (Laura Patton, KDFWR, pers. comm.).

Ecological Interactions

Gray fox, bobcat, and coyote are sympatric throughout large portions of North America and, as mesocarnivores, they often compete for resources. This competition can be direct, such as interference competition through aggression, or indirect, like exploitation competition which limits resource availability (Neale and Sacks 2001). The degree of dietary overlap is a major source of competition and can determine the nature of ecological interactions among species (Polis and Holt 1992). Gray fox, bobcat, and coyote have been shown to exhibit significant dietary overlap, especially with rodent prey and lagomorphs to a lesser degree (Fedriani et al. 2000; Neale and Sacks 2001). However, as opportunistic generalists, gray fox and coyote compete year round, according to availability, for many of the same food sources (Fedriani et al. 2000; Neale and Sacks 2001). During summer and fall, these canids subsidize their diets with fruits and other vegetable matter but begin to diverge in winter and spring as coyote target ungulate prey (Neale and Sacks 2001; Thornton et al. 2004). Bobcat remain strict carnivores regardless of season, focusing on lagomorphs, rodents, and many species of birds (Fedriani et al. 2000; Neale and Sacks 2001; Tewes et al. 2002; Thornton et al.

2004). The extent of dietary niche overlap among these three mesocarnivores varies seasonally, however the competition for rodents and lagomorphs remains consistent throughout the year.

Exploitation competition for prey can lead to more aggressive forms of competitive interactions, such as interference competition or intraguild predation (Polis and Holt 1992). The dominance of coyote over both gray fox and bobcat has been well documented in the literature (Litvaitis and Harrison 1989; Fedriani et al. 2000; Farias et al. 2005). Furthermore, gray fox and bobcat abundance has been negatively correlated to that of coyote (Crooks and Soule 1999; Henke and Bryant 1999). Within this dominance hierarchy, gray fox occupy the lowest rank. However, the magnitude of and motivation for gray fox persecution differs between coyote and bobcat. Fedriani et al. (2000) reported the proportions of radio-collared gray fox mortalities resulting from coyote and bobcat at ~58% (7/12) and ~17% (2/12), respectively. Farias et al. (2005) reported very similar figures, ~67% (8/12) of gray fox deaths from coyote and ~17% (2/12) from bobcat. These data suggest coyote pose a much more significant threat to gray fox survival than do bobcat. Interestingly, bobcats were more likely to engage in intraguild predation and consume gray fox as prey, which is supported by Tewes et al. (2002). While interference competition, through interspecific killing, appeared to be the primary motivation for coyote attacks on gray fox (Farias et al. 2005). Fedriani et al. (2000) also reported 40% (2/5) of bobcat mortalities were caused by coyote and neither study, Fredriani et al. (2000) nor Farias et al. (2005), described any accounts of gray fox or bobcat killing a coyote.

The structure of dominance among these mesocarnivores may seem apparent, and the result of an encounter between a bobcat, coyote, or gray fox can certainly be lethal, especially for the latter. However, these species co-exist in many of the same habitats and can do so with remarkable stability even as coyote abundance continues to rise (Lovell et al. 1998; Gompper 2002; Prugh et al. 2009). Landscape-scale research suggests bobcat and gray fox are slightly more selective of habitat type when compared to coyote. Bobcat can occupy open, brushy, or forested areas (Litvaitis and Harrison 1989; Fedriani et al. 2000), but show an affinity for early successional habitat fragments (Constible et al. 2006). Gray fox typically avoid open landscapes and exhibit a strong preference for dense brush and forested habitats (Fedriani et al. 2000; Temple et al. 2010). Coyote can be found in a variety of habitat types, including areas of human development (Litvaitis and Harrison 1989; Fedriani et al. 2000; Gompper 2002; Riley et al. 2003; McDonald et al. 2008). Therefore, considerable spatial overlap among these mesocarnivores is likely to occur in brushy or forested areas (Neale and Sacks 2001; Chamberlain and Leopold 2005). Additionally, these species exhibit similar temporal activity patterns as nocturnal/crepuscular hunters (Fedriani et al. 2000). As such, landscape-scale analyses may not provide adequate resolution to establish spatial or temporal variations critical to the co-existence of these mesocarnivores. Fine-scale analyses of home range and core area among sympatric gray fox, bobcat, and coyote has provided insight into the survival strategies of these species. Chamberlain and Leopold (2005) used radiotelemetry to examine space use patterns of gray fox and bobcat in relation to coyote home ranges. They discovered at the home range scale, all three species demonstrated significant spatial overlap. However, at the core area scale, bobcat

and especially gray fox spatially excluded themselves from areas of high coyote activity. These results suggest coyote, despite their dominance and aggression, do not limit the distribution of bobcat and gray fox due to fine-scale spatial avoidance behaviors. Another explanation for the persistent sympatry of these species, which is poorly represented in the literature, could be the highly arboreal nature of both bobcat and gray fox as a means of competitor avoidance.

CHAPTER 2: MONITORING MESOCARNIVORES

The need for science-based wildlife management strategies was pioneered nearly a century ago by the influential naturalist Aldo Leopold (Leopold 1933). Since Leopold, the number of methods for managing animal populations have proliferated, become more empirically-based, and incorporated new technologies. As such, the population status of species can be characterized by short-term standardized assessments, or by repeated assessments over time, known as monitoring (Gese 2001).

Population monitoring, for mesocarnivores or any vertebrate, will follow a simple, basic framework: 1) determine species distribution, 2) estimate species abundance, and 3) repeat abundance estimates over time to establish a population trend (Gese 2001). Before implementing a mesocarnivore population monitoring program, an investigator should consider what is known about the species of interest within the area of study (Gese 2001). For example, a rare species occurring at low densities would require more precise and accurate population estimates to detect a true change in abundance than would a common species occurring at high densities. The species of interest should be considered in all aspects of study design, including choice of survey method, timing and duration of survey, study area size, sampling design, and detectability or catchability. Additionally, a monitoring program should have achievable objectives which can provide answers to questions being asked. Mesocarnivores, specifically furbearer mesocarnivores such as gray fox, bobcat, and coyote, are managed though harvest by either trapping or hunting. With these and other furbearers, population management can be directed towards one of three principle objectives: conservation, sustained yield, or control (Wolfe and Chapman 1987). For example, in Kentucky, gray fox and bobcat are managed to

maintain sustained yields for harvest, whereas coyote management falls under the population control objective. However, these monitoring objects are broad and more specific objectives, like an absolute population estimate, may be desired. A monitoring program should be carefully designed and a pilot study implemented to assess method feasibility, labor, and cost to achieve relative or absolute population estimates.

Methods to Determine Species Distribution

Habitat Modeling

The large-scaled distributions of many species in North America have been well established for decades (Prugh et al. 2009). Although, within these large-scaled distributions, landscape level variation can exclude a species of interest, validating the need for distribution assessments. The development of remote sensing through Geographic Information Systems (GIS) and an increasing availability of satellite imagery has led to methods for predicting species distribution known as habitat modeling or occupancy modeling (Prugh et al. 2009). Satellite images are stratified, at various scales, according to habitat type or land-use class and used to predict occupancy based on previous knowledge of target species habitat preference. Habitat models have been used to successfully predict gray fox, bobcat, and coyote distribution on regional scales (Hackett 2008; McDonald et al. 2008). While this method has broad applications and continues to increase in popularity, results should be confirmed using field methods that address both the accuracy of habitat classes and the occupancy of target species.

Presence of Sign

Many mesocarnivores are secretive and nocturnal, making visual detection difficult or impossible. Under these circumstances, surveying for animal sign (e.g., tracks, scats, hair, dens, pug marks, etc.) can be an effective and low-cost method for establishing species presence and, therefore, distribution. A search for the presence of animal sign can be conducted passively, by walking transects, hiking trails, and forest roads, or actively with the use of baited hair snares or trained detection dogs (Heinemeyer et al. 2008). Sign surveys have been used successfully to detect gray fox (Cunningham et al. 2006), bobcat (Gompper et al. 2006; Ruell and Crooks 2007), and coyote (Murray et al. 1994; Kohn et al. 1999). It should be noted, however, that an inability to detect sign should not be considered an absolute measure of species absence. Misidentification of sign can be another source of error but can be alleviated through molecular techniques or the use of multiple trained observers. Passive searches for sign are further subject to sampling bias resulting from trail or road based transects, however active searches by detection dogs can reduce this bias (MacKay et al. 2008b).

Track Stations

There are several different styles of track station, which require varying degrees of preparation and which are best suited for detecting different species (Ray and Zielinski 2008). Surveys for smaller mesocarnivores (e.g., mustelids) typically use enclosed track plates, while the majority of track station surveys for larger mesocarnivores (e.g., felids and canids) use two styles, track plots and scent stations (Ray and Zielinski 2008). Track plots are prepared with natural substrates smoothed out into a circular or square shaped

plot along an existing travel route. No baits or attractants are used with track plots, relying on passive carnivore movements for detection. Scent stations can be prepared identically to track plots with the exception of a bait or attractant placed in the center of the plot. Both styles have been used to detect gray fox (Crooks 2002; Sinclair et al. 2005), bobcat (Crooks 2002; Harrison 2006a), and coyote (Crooks 2002; Gompper et al. 2006); however, scent stations were used considerably more often. Track stations are a fairly efficient and cost-effective method for detecting species presence, especially when attractants are species-specific. However, weather events can distort tracks resulting in misidentification or erase tracks, leading an observer to believe no detections have been recorded. Scent stations may attract too many individuals which can result in "overtracking" that covers the tracks of target species. Seasonal considerations when planning a track station survey and frequent station checks can reduce these potential pitfalls (Ray and Zielinski 2008).

Camera Traps

Remotely triggered cameras have increased in popularity since their first large-scale scientific applications in the 1990's (Cutler and Swann 1999). While camera traps have a long history with wildlife applications, early models were bulky and expensive, thus limiting their ability to cover large areas. As technologies progressed, camera size and cost decreased as picture quality and triggering mechanisms improved (Kays and Smith 2008). Camera trapping can fulfill a variety of study objectives and has been consistently ranked as a high quality method for carnivore research (Kays and Smith 2008). Cameras are typically attached to trees along trails, creek beds, or expected travel routes, resulting in photographic evidence of species presence. However, there are many

camera options available, with various features, and an investigator should consider the species of interest before making a purchase. Film cameras provide the cheapest option but require frequent visits to replace film and additional time for photo development. Digital cameras can be expensive but they offer high quality images, adequate memory, and, with some models, the ability to wirelessly download images as they are captured. Digital video cameras are typically the most expensive option. They provide all of the benefits of digital still cameras plus the capability to record animal behaviors which may otherwise remain unknown (Kays and Smith 2008). The triggering mechanism should also be considered in relation to the target species. Early camera trapping surveys used pressure pads or bait tied to a string to trigger cameras. While these options still have species specific applications, many modern cameras offer two types of sensor systems, active and passive, to remotely trigger cameras. Active systems behave much like a motion detector by triggering the camera when an animal breaks an infrared beam. However, active systems can require additional set up time and are prone to false captures resulting from wind-blown vegetation. Passive systems use an infrared beam to detect a change in temperature and trigger the camera. These systems are typically more user friendly but their placement must be considered in relation to the sun. Direct sunlight can cause the temperature change necessary for triggering and result in false captures (Kays and Smith 2008). Furthermore, night captures utilizing a visible flash can reduce the probability of recapture for shy or apprehensive species (Wegge et al. 2004). Trap shyness from visible flash can be largely avoided with the use of an infrared flash. However, trap shyness can also result from the actual presence of an unfamiliar object, such as a remote camera (Sequin et al. 2003). Despite the tradeoffs, remote cameras have

been used to survey gray fox (Moruzzi et al. 2002; Stake and Cimprich 2003; Zielinski et al. 2005), bobcat (Moruzzi et al. 2002; Heilbrun et al. 2006), and coyote (Moruzzi et al. 2002; Gompper et al. 2006; O'Connell et al. 2006). Camera trapping requires a substantial initial investment and the potential for costly theft or malfunction must be considered. Regardless, this method provides relatively low biased results for a variety of objectives and generally requires minimal labor past the initial deployment.

The methods previously described, with the exception of habitat modeling, have the ability to both determine species distribution and estimate relative abundance. However, in order to accomplish the latter, the survey method and the amount of survey effort must be standardized and consistent (Gese 2001). In addition, as with determining species distribution, methods to estimate relative or absolute abundance can be species-specific and require some degree of knowledge prior to their implementation.

Methods to Estimate Absolute Abundance

Methods to estimate absolute abundance require direct counts (i.e., actual counts of individual animals, alive or dead), which is in contrast to indirect counts (i.e., counts of animal sign) which can only be used in relation to one or more study sites and in turn, provide estimates of relative abundance (Gese 2001). Of the methods described for determining species distribution, only remote cameras can estimate both relative and absolute abundance for select species (Kays and Smith 2008). I will discuss remote cameras as a method for absolute abundance later in this section. Once the target species distribution has been established, an investigator can begin to estimate species abundance. When choosing a method to estimate abundance, careful consideration

should be given to the amount of precision and accuracy a method can provide in relation to what is already known about the species of interest (Gese 2001). For example, a rare species occurring at low population densities would require more precise and accurate methods to detect a true change in population. Thus, an investigator should examine the assumptions inherent to each available method and choose a survey technique that is appropriate for both the study objectives and the species of interest.

Harvest Reports

Examining harvest reports is a general way to gain insight into the abundance of game species. Records of furbearer harvest have been maintained for centuries in the United States (Ray 1987). However, the collection of detailed harvest data (hunting and trapping) by state fish and wildlife agencies was not emphasized until relatively recently (Connelly et al. 2005). Information such as the age and sex of harvested animals can be used to construct models for estimating absolute abundance (Paloheimo and Fraser 1981). However, when harvest data are used independently they are subject to socioeconomic and environmental biases that result in unreliable population estimates (Gese 2001). For example, fur prices, variable harvest methods, and incomplete hunter or trapper reports can influence harvest intensity or success and reported totals, resulting in biased abundance estimates. Statewide harvest data are relatively easy to collect as hunting and trapping licenses as well as reporting harvest totals for many species are required by law. However, an investigator should be cautious when attempting to use harvest data to guide management decisions. Selective harvest can influence abundance estimates from harvest data, and the means by which selection has occurred can vary this influence. Selection can either be direct, if hunters or trappers target specific genders, age classes, or

species disproportionally, or indirect, as a result of variable harvest probabilities both among and within species (Lancia et al. 2005). Abundance estimates from harvest data are further complicated by inconsistencies related to harvest-per-unit-effort. Specific harvest method and especially harvester effort are not always known and, therefore, not reflected in harvest data (Gese 2001). As such, attempts to standardize harvest-per-unit-effort occur *post hoc* and often result in inaccurate abundance estimates. Harvest reports can provide general, but subjective, information regarding abundance trends, even after standardization. Therefore, additional methods which offer greater precision and accuracy should be used in conjunction with harvest data to validate abundance trends.

Capture-Mark-Recapture

Wildlife biologists have used capture-mark-recapture (CMR) methods to estimate population abundance for decades (Lincoln 1930). Within this time, CMR methods have been adapted to meet rigorous statistical standards and applied to a variety of data collection techniques for many different species (Lancia et al. 2005). As a result, an extensive body of literature exists which provides evidence for the reliability and accuracy of CMR methods. The basic framework of CMR is quite simple. A population is sampled, through live trapping or other means, and all captured animals are uniquely marked then released (n_1). Later, the population is sampled again, capturing previously marked individuals (m_2) from a total sample of both marked and unmarked individuals (n_2). The ratio of previously marked individuals to total individuals captured during the second sampling occasion is equivalent to the ratio of total individual animals marked to total population size (N): $m_2/n_2 \sim n_1/\mathrm{N}$. This equation is known as The Lincoln-Peterson model and it has provided the foundation for more complex models which incorporate the

effects of data collection bias and account for different sources of variation in capture probabilities (Lancia et al. 2005). Adaptations of this model have been used to successfully estimate abundance for gray fox (Conner et al. 1983), bobcat (Heilbrun et al. 2006), and coyote (Kohn et al. 1999).

The evolution of CMR models is a direct result of applying CMR concepts to a variety of sampling methods, each with their own inherent biases. Traditionally, CMR utilized live capture, through cage or foothold trapping, to physically handle and mark individuals. There are many types of marks available but only several can be applied to furbearers. These marks can be relatively noninvasive, such as collars, dyes, and ear tags, or rather invasive, like radioactive markers, passive integrated transponder (PIT) tags, and tissue removal (Silvy et al. 2005). However, physically capturing an animal introduces a sampling bias known as trap avoidance, which can influence an individual's probability of recapture and thus affect abundance estimates (Woods et al. 1999).

Within the last two decades, advances in sampling methodologies have provided researchers with low bias sampling techniques that are not reliant on physical capture to distinguish individuals. One such method, which has been previously described to assess distribution, is camera trapping. Remote cameras utilize natural variation in pelage patterns to identify individuals and create capture histories that can be subjected to CMR models (Kays and Smith 2008). However, survey design for remote camera studies will vary based on project objectives. For example, when assessing distribution, only one camera is needed per trap site. Whereas, abundance studies will require two cameras per trap site to simultaneously capture both flanks of an individual. Furthermore, systematic random trap placement with consistent spacing between camera traps is critical for CMR

analysis (Kays and Smith 2008). Remote cameras have been used to estimate bobcat abundance through their unique spot patterns (Heilbrun et al. 2003; Heilbrun et al. 2006; Larrucea et al. 2007a). Species with little variation in pelage, such as coyote and gray fox, will be more difficult to reliably distinguish individuals from photographs and may require previously applied marks, such as collars (York et al. 2001; Larrucea et al. 2007b). However, remote cameras have been used to estimate red fox (*Vulpes vulpes*) abundance by using careful camera placement to focus on limb markings (Sarmento et al. 2009). The low inherent bias of camera trapping can be further optimized by using unbaited trap locations and infrared flash systems, resulting in a near ideal method for CMR models.

Advances in molecular techniques applied to wildlife biology have revolutionized the CMR concept. All previous CMR methods required visual confirmation of marked animals. Whereas, molecular identification through DNA collected from hair, scat, or various other sources can be used to identify individuals without the need for capture. These methods will be discussed in greater detail later but the CMR related aspects should be noted here. As with any CMR method for estimating abundance, a random and consistent sampling design is fundamental to reducing sampling bias (Lancia et al. 2005). For example, repeated sampling of hair snares placed in a systematic fashion beginning at a random start point can be an effective method of collecting DNA for CMR analysis (Murphy 2011). Molecular identification can be costly and require specialized knowledge of laboratory techniques. Furthermore, individual identification is not always certain due to high rates of genotyping error associated with noninvasively collected DNA samples (Taberlet et al. 1996). Genotyping error results in what is essentially “lost

marks", a violation of CMR models. However, laboratory techniques specific to noninvasive DNA samples are rapidly being developed to reduce genotyping error and the bias it creates (Pompanon et al.2005; Waits and Paetkau 2005).

Radiotelemetry

The concept of radiotelemetry as a means to locate animals for research was conceived in the late 1950's, after drawing inspiration from miniature transmitters ingested by pilots to monitor the physiological effects of flight (LeMunyan et al. 1959). The prototype transmitter was surgically implanted into woodchucks (*Marmota monax*) with a maximum effective range of ~ 23 m. Several years later, Cochran and Lord (1963) developed a smaller, more powerful transmitter collar and successfully used it to track the movements of rabbits (*Sylvilagus floridanus*), striped skunks (*Mephitis mephitis*), and raccoons. Since the birth of the radiocollar, transmitter units have undergone significant technical advancements and now provide one of the most accurate estimates for home range, density, and population abundance of any method available (Gese 2001). This is especially true for carnivores, which are generally secretive and occupy well defined territories.

Radiotelemetry requires study animals to be live captured and fitted with radio transmitters (collars are typically used for mesocarnivores) where each emit a unique radio frequency. If a sufficient number of individuals are tracked over time, the average home range size (accounting for intersexual variation) in conjunction with percent home range overlap and the proportion of transients, can be used to estimate density and therefore abundance (Gese 2001). Radiotelemetry has not been used to estimate gray fox

abundance (Fuller and Cypher 2004), however Trappe (1978) attempted to estimate density based on 4 individuals. Additionally, Chamberlain and Leopold (2005) appeared to have had an adequate sample size and the appropriate data to perform density calculations but did not do so. Both bobcat (Bailey 1974; Rolley 1987; Rucker et al. 1989) and coyote (Babb and Kennedy 1989; Gese et al. 1989) abundance have been successfully measured using radiotelemetry.

While radio tracking can provide explicit and valuable data, it requires a substantial investment of time, effort, and capital. Furthermore, there are several sources of location error associated with radiotelemetry that, if not accounted for, can seriously bias space use and density estimates (Saltz 1994). Location error can be especially influential for studies which examine fine-scale habitat selection (Otis and White 1999). However, new technologies, such as Global Positioning System (GPS) tracking, can provide continuous monitoring with relatively low error (Fuller et al. 2005). Currently, GPS tracking collars are limited by unit weight and battery life, but they have been utilized to track male bobcats and can be programmed to extend the power supply (A. Shipley, in press). Before conducting a telemetry study, an investigator should consider the study objectives and determine if radio or GPS tracking is an appropriate method. In addition, the capabilities and limitations of tracking equipment, in relation to the species of interest and the study area, should be considered when designing a telemetry study (Fuller et al. 2005).

After species distribution has been assessed and the appropriate method for estimating abundance has been chosen, a monitoring program can begin. The critical element to successfully monitoring population status is standardized consistency. Survey

methods, timing, duration, data collection, and analyses must all remain consistent each time a population is sampled. Over time, a population trend (e.g., increasing, decreasing, or stable) will emerge. Only after a trend is revealed through repeated, fixed methodology can an investigator begin to make appropriate management decisions.

Noninvasive Genetic Methods

Before examining noninvasive methods, it is important to distinguish the terms *noninvasive* and *invasive* as they apply to methods for wildlife investigation. These terms hold an inherent connotation which may be misleading and perceived as synonymous with good versus bad. The terms noninvasive and invasive were adapted from a medical context (MacKay et al. 2008a). An invasive medical procedure is one which requires penetrating the skin through incision or injection as opposed to a noninvasive procedure which does not. Thus, a noninvasive survey method is one which does not require the study animal to be physically handled, chemically immobilized, or even seen by an investigator (MacKay et al. 2008a).

Early survey methods relied on the morphological characteristics of noninvasively collected samples to distinguish among species or, in fewer instances, individuals. Examples of which include interspecific variation in hair shaft or root morphology (Hilton and Kutscha 1978) and physical measurements of scat (Danner and Dodd 1982). These species identification methods were either subjective or relatively inaccurate. Danner and Dodd (1982) found that coyote scat diameter could almost completely overlap with that of gray fox. Furthermore, early methods to noninvasively identify individuals were even more prone to subjectivity and misconception. Smallwood and

Fitzhugh (1993) developed a method to indentify individual puma by recording multiple track measurements and comparing them through statistical analyses. While the authors boasted exceptional success rates, the ideal conditions required, coupled with individual track variation resulting from countless sources (e.g., substrate, slope, movement speed, etc.), severely limit the method's feasibility and accuracy. As such, noninvasively collected samples were restricted to basic assessments of diet and distribution or as indices of relative abundance. However, recent advances in wildlife genetics have allowed for the expansion of noninvasive methods to a broader range of applications. In the following discussion I will review the noninvasive survey methods and molecular techniques used both within and beyond the scope of my research.

Hair Snares

Hair collected from wildlife has long been a valuable source of information for outdoorsman and scientists. Hunters and trappers used an intimate knowledge of the natural world and the physical characteristics of hair discovered in the field to track and locate game. Early researchers took a different approach, by painstakingly examining microscopic variations and cataloging them according to species to create keys for identification (Mayer 1952; Short 1978; Clement et al. 1981). While these identification keys are still useful for low cost dietary assessments, they have largely been replaced by modern genetic techniques. However, before a hair sample can be identified to species, regardless of method, it must be collected.

Hair collection methods have been broadly categorized into two distinct groups, baited and passive (Kendall and McKelvey 2008). Baited hair collection devices rely on

the use and placement of baits, lures, or rub-behavior elicitors to either guide an animal's movement across a collection device or induce a behavioral response which results in hair collection. Passive devices do not require attractants or a change in target animal behavior. Instead, they utilize either carful placement along travel routes or existing natural rub objects to collect hair samples. Both passive and baited hair snares are further subdivided according to the style of collection device, each of which has varying degrees of species specificity (Kendall and McKelvey 2008). Despite the bias introduced by the use of an attractant, baited hair snares are more commonly used among researchers.

Baited Hair Collection

Rub Pads

Rub pads are a type of baited hair snare most commonly used to survey felids and canids. These devices use lures, such as beaver (*Castor canadensis*) castoreum and catnip oil, to elicit a cheek-rubbing response in felids (Mellen 1993) or a neck-rubbing response in canids (Bullard et al. 1983). Two common styles of rub pads exist, adhesive rub stations and barbed rub pads. Adhesive rub stations are typically used to target canids and used less frequently than barbed rub pads (Kendall and McKelvey 2008). An adhesive rub station is constructed by wrapping a block of wood with double-sided tape and applying a species specific lure. Barbed rub pads have been primarily used to target felids however several studies reported cases of significant canid by-catch, of which included coyote and gray fox (Harrison 2006; Ruell and Crooks 2007). Downey et al. (2007) set out to survey margay (*Leopardus wiedii*) in Mexico and puma in California using barbed rub pads but recorded gray fox success rates at ~20% and ~48% respectively. Several styles of barded rub pads have been tested. Harrison (2006a)

assessed metal mending plates and Ruell and Crooks (2007) used pieces of unpainted welcome-mats. However, the most common style was pioneered by McDaniel et al. (2000), and used extensively for the National Lynx Survey (NLS). The NLS rub pad hair snare design was a 10 x 10 cm piece of closed-loop, natural fiber carpet with 8-12 small nails pushed through the back side. Rub pad hair snares are typically nailed to trees at a height specific to the target species. For example, McDaniel et al. (2000) used a height of 0.5 m to survey lynx, while Weaver et al. (2005) lowered hair snares to 0.3 m for ocelot (*Leopardus pardalis*). However, the exact height of snare placement may be less influential to other species such as bobcat, which have been successfully sampled at heights ranging from 0.3-0.6 m (McDaniel et al. 2000; Shinn 2002). Generally, barbed rub pads are accompanied by a visual attractant to induce predatory curiosity. Aluminum pie plates, compact discs, and turkey feathers are examples of commonly used visual attractants (McDaniel et al. 2000; Ruell and Crooks 2007).

Finally, the NLS program was one of the most successful rub pad hair snare surveys conducted and it provided some important hair snare design considerations for future studies. McDaniel et al. (2000) determined that both carpet color and style were critical to efficiently locating hair samples on a rub pad. Shag carpet will easily fray under field conditions, resulting in hair-like carpet fibers mixing with actual hair samples. The use of closed-loop carpet will minimize fray and increase sample collection efficiency and accuracy. Synthetic carpets have an unnatural, chemical smell that may inhibit visitation by apprehensive species. Therefore, the authors recommend natural fiber carpet, such as wool, to reduce snare avoidance. Additionally, neutral and multi-colored carpets can make sample detection difficult. A carpet of uniform color that

contrasts with animal hair will allow investigators to easily locate and collect hair samples. McDaniel et al. (2000) also made recommendations on the type of nails that should be used to maximize hair collection. Nails specifically designed for use in nail guns are ideal for barbed rub pads. These nails are connected by a copper wire which, when clipped and individually pushed through carpet, create barbed protrusions near the nail point that facilitate sample collection. Hair snares used for my research were constructed directly from the NLS design.

Hair Corrals

Within the baited hair snare group, the hair corral method has proven particularly effective at collecting hair samples from ursids (Boulanger et al. 2006; Dreher et al. 2007; Hast 2010; Murphy 2011). Hair corrals are constructed by stretching barbed wire around a group of trees or posts to create an exclosure. Typically, one strand of wire is used, positioned at 50-60 cm above the ground (Woods et al. 1999; Boulanger et al. 2006). However, some studies have found that two parallel strands, positioned at 25 and 50 cm above the ground, can increase the number of samples collected (Kendall and McKelvey 2008). To attract study animals, bait or lure is suspended over the center of the exclosure, which should be ≥2 m from the perimeter (Murphy 2011). If constructed properly, an individual will deposit hair samples regardless of whether they crawl under or step over the barbed wire. By design, hair corrals should be most effective with large, curious species such as black bear (*Ursus americanus*) and grizzly bear (*Ursus arctos*). However, Frantz et al. (2004) modified the hair corral to successfully estimate Eurasian badger (*Meles meles*) abundance.

Post Snares

Post snares are a relatively new form of baited hair collection device used almost exclusively to sample wolverine (Kendall and McKelvey 2008). They are constructed by setting a 2-2.5 m long, 10 x 10 cm wooden post into the ground and wrapping it with barbed wire (Mulders et al. 2007). Bait, such as caribou (*Rangifer tarandus*) meat, is secured to top of the post to encourage a target species to climb. As an animal climbs the post, it brushes across the barbed wire and leaves behind hair samples. However, non-target sampling can be expected when food baits are used. Fisher (2004) collected hair samples from 11 different species, including several canids. As mentioned earlier, wolverines occupy a disproportionally large home range in relation to body size, which makes noninvasive estimates of abundance difficult (Kyle and Strobeck 2001). Although, Mulders et al. (2007) demonstrated that the systematic deployment of 284 post snares, covering >2500 km^2, was a feasible noninvasive survey design for estimating wolverine abundance.

Cubbies

Lastly, cubbies are an early form of baited hair snares used primarily to survey mustelids, but have also been successful with small canids (Kendall and McKelvey 2008). While there are many variations of the cubby snare, they all follow the same basic design principles. A long, thin enclosure is created using a wooden box, plastic culvert pipe, etc., leaving only one end open. Bait is placed inside the cubby, against the back wall and some form of hair collection device (e.g., barbed wire, gun brushes, or adhesive) is strategically positioned to contact an animal as it enters. Cubbies, in various forms,

have successfully sampled fisher, *Martes pennanti* (Belant 2003), American marten, *Martes Americana* (Zielinski et al. 2006), short-tailed weasel, *Mustela ermine*, and long-tailed weasel, *Mustela frenata* (Mowat and Paetkau 2002). In addition, Bremner-Harrison et al. (2006) developed a cubby snare which successfully collected samples from the endangered San Joaquin kit fox (*Vulpes macrotis mutica*) in California and swift fox (*Vulpes velox*) in Colorado. The efficacy of cubby snares has been attributed to two primary advantages. Cubbies, by design, restrict target animal movements such that they must encounter a snaring device and, therefore, increase the potential for hair collection (Kendall and McKelvey 2008). Additionally, cubbies provide a degree of species specificity from both a design and behavioral standpoint. Large species simply cannot fit inside and some mustelids are more likely to respond to bait that is located within a covered structure (Foresman and Pearson 1998).

Passive Hair Collection

Natural Rub Objects

Many species have been documented scent-marking landscape features through various means (Bullard et al. 1983; Mellen 1993; Hutchings and White 2000; Green and Mattson 2003). These chemical signals are an important form of communication which can convey information such as territorial boundaries, social rank, and reproductive status. In terms of hair collection, scent-marking through glandular secretions are of particular interest because they require animals to rub against an object. In doing so, hair is often left behind. However, for an investigator attempting to locate these natural rub objects, the task can be daunting. That is, unless the species of interest is either conspicuously large or affixed with a tracking system. Green and Mattson (2003)

presented a case in which both aforementioned luxuries were accommodated as they examined tree rubbing behavior in grizzly bears. The authors used radiotelemetry and keen observation to conclude that time of year and tree size were the variables that best predicted grizzly bear tree rubbing in the GYE. However, Kendall et al. (2008) took this information and combined hair collected from hair corrals and natural rub trees to estimate grizzly bear density throughout Glacier National Park (GNP), Montana. The success of Kendall et al. (2008) led to the development and implementation of a GNP-wide bear monitoring program which incorporated barbed wire around rub trees, much like a post snare (Stetz et al. 2010).

Natural rub objects provide investigators with an additional, low cost noninvasive source of DNA, but natural rub objects are not always natural. Green and Mattson (2003) collected bear hair samples from trail markers, cabin posts, and barn siding. Additionally, Karamanlidis et al. (2007) monitored an expansive network of power poles to document brown bear distribution in Greece. The formal use of natural rub objects for scientific endeavor has largely been limited to ursids, until recently. Schmidt and Kowalczyk (2006) applied the NLS protocol to Eurasian lynx in Poland and used snow tracking to locate natural rub objects. The authors compared sampling yield (i.e., number of hairs) between natural rub objects and hair snares placed directly on natural rub objects. They found lynx were more likely to both cheek-rub and leave high yield samples at natural rub sites which included hair snares. The use of natural rub objects as a source of noninvasive sampling is likely to increase as new applications continue to be discovered.

Travel Route Snares

Travel route snares rely on careful placement along existing game trails or other high activity locations to collect hair samples. The snaring device can be a body snare or foot-hold trap, commonly used by fur trappers, that is modified to collect hair without actually trapping an animal. To sample brown bear in Alaska, Beier et al. (2005) modified wolf neck-snares by attaching a rubber break-away component to complete the snare loop. As a bear walks through the snare, it tightens to collect hair and then breaks, releasing the bear. The same principle has been applied to foot-hold snares. DePue and Ben-David (2007) attached wire brushes to one jaw of foot-hold traps and welded metal spacers to prevent the jaws from completely closing. An animal caught in this trap will be able to pull its foot out and leave behind hair samples in the wire brushes. The authors successfully used this modified foot-hold trap to collect hair from river otter (*Lontra canadensis*), but note its potential to sample many other species.

Travel route snares may also be a strategically placed strand of barbed wire. This passive hair collection method has been used extensively to sample Eurasian badger population demographics and abundance (Scheppers et al. 2007; Huck et al. 2008; Balestrieri et al. 2010). Scheppers et al. (2007) tested adhesives but ultimately determined a single strand of barbed wire, 20 cm from the ground and strung between to metal posts, was an effective method for sampling badgers. Furthermore, Scheppers et al. (2007) and subsequent badger studies placed these simple snares across known badger trails and directly outside of dens to maximize sampling potential. While passive travel route snares provide low bias sampling, they require an intimate knowledge of localized animal movement patterns and possibly a pilot study to be successful.

Hair collection methods, whether baited or passive, have displayed a certain degree of species specificity. For example, methods which successfully sample mustelids, like cubbies, are simply not suitable for ursids or felids. Choosing an appropriate hair collection method will be heavily dependent on the species of interest, however, the study design and objectives should also be considered. Before molecular advances, the information which could be acquired from hair was limited to basic assessments such as species distribution, habitat preference, and diet. With the progression of genetic analyses and careful survey design, hair can be used to identify individuals for population monitoring through estimates of relative or absolute abundance. Furthermore, noninvasive hair sampling can address population genetics issues like genetic variation, population connectivity, inbreeding, and hybridization (Kendall and McKelvey 2008).

When designing a noninvasive hair snare study, there are practical considerations which must be addressed before sampling can begin. The survey methods previously described have been successful because they were designed to exploit certain behavioral responses in target species. Baited methods use lures to induce scent-marking or food rewards to guide an individual across a snaring device. Passive methods rely on habitual movement patterns resulting from territoriality or den location. Therefore, the behavioral response of a target species, in relation to a given survey method, will strongly influence survey design. For instance, Mulders et al. (2007) found that probability of capture varied for wolverine, both between sexes and among sampling-grid cell size. Additionally, Schmidt and Kowalczyk (2006) discovered lynx were significantly more likely to rub hair snares during the breeding season. Study design may be further

complicated by interspecies interactions. Downey et al. (2007) hypothesized that non-target gray fox marking at snare sets was responsible for low target species success rates. As such, the behaviors of a target species are directly linked to either the success or failure of hair snare study.

Noninvasive hair snare surveys which utilize baited methods introduce a degree of sampling bias that can have a serious influence on certain types of studies. In particular, CMR studies to estimate population abundance are highly susceptible to sampling bias as it alters the probability of capture (Kendall and McKelvey 2008). Food baits can create a trap-happy response which increases capture rates. Whereas lures may cause trap avoidance, as no reward is offered, and decrease capture rates. To reduce sampling bias, Boulanger et al. (2006) suggested relocating snare sites between sampling occasions to increase their novelty. Passive methods, such as natural rub objects, may be used more frequently by different sexes or age classes, resulting in sampling bias. Boulanger et al. (2008) combined multiple data sources, from both passive and baited CMR methods, to estimate grizzly bear abundance with reduced bias. Ultimately, this form of sampling bias can be explained by species specific behavioral responses. A deeper understanding of target species behavior will allow for optimal survey design.

In summary, noninvasive hair collection methods are diverse and adaptable. They can be used to survey large areas which include various habitat types, are capable of simultaneously targeting multiple species, and can address relevant conservation or management objectives. Typically, DNA analysis is required to identify hair samples but snaring devices are comparatively inexpensive, which helps to offset project costs. When

surveys are designed correctly, accounting for target species behavior, hair snares provide a cost effective and efficient means for noninvasive sampling.

Scat Detection Dogs

As humans transitioned from hunter/gatherer to an agriculturalist lifestyle, the role of domestic dogs became increasingly diverse (Boyko 2011). Their extraordinary abilities were applied to a variety of tasks, such as herding, protection, tracking, and hunting (MacKay et al. 2008b; Boyko 2011). In particular, the scenting ability of domestic dogs has been a valuable resource to both early and modern humans. Many large breed dogs have 50 times more olfactory receptors than humans, and are capable of detecting concentrations as low as 500 parts per trillion (MacKay et al. 2008b). As such, the repertoire of domestic dogs has expanded in modern times with applications in the fields of law enforcement, military, rescue, public safety, biosecurity, and wildlife biology (Browne et al. 2006). Within each field, detection dogs perform a variety of jobs. As assistants to wildlife research, detection dogs have been trained to locate bird carcasses (Homan et al. 2001; Paula et al 2011), Mojave desert tortoises, *Gopherus agassizii* (Nussear et al. 2008), black-footed ferrets, *Mustela nigripes* (Reindl-Thompson et al. 2006), brown treesnakes, *Boiga irregularis* (Savidge et al. 2011), and bumblebee (*Bombus* spp.) nests (Waters et al. 2010). While the detection of wildlife has been useful for assessing the distribution of endangered species, such as the black-footed ferret, or invasive species, such as brown treesnakes, the majority of detection dog research has targeted wildlife sign. Among wildlife sign, scat detection has proven especially useful for wildlife research.

Scat detection dogs are trained through a process similar to that of narcotics or explosives detection dogs. However, before detection training can begin, a dog which is appropriate for the job must be selected based on several fundamental characteristics. An ideal scat detection dog will be obedient, physically capable, high energy, focused, and obsessed with its favorite play toy (Lucas Epperson, Ecodogs, pers. comm.). These attributes are essential and dogs that lack any one of them are not suitable for the task. Training begins with basic obedience, allowing the dog and handler to build a relationship. Next, the dog is exposed to a target odor, immediately given a detection command, and rewarded. By giving a detection command during training, a dog learns what should be done when it encounters a target odor in the field. These commands are not given during field surveys. Typically, detection dogs sit, lie down, or bark to indicate a target odor source has been located. As a reward, dogs receive a short (1-2 min) play time with their favorite toy. Eventually, nontarget odors are introduced into the training process, allowing detection accuracy to be monitored. Finally, training, with both target and nontarget odors, is reinforced in a variety of field locations which expose the dog to different environmental distractions. This process can be repeated for multiple target odors. A detection dog may be trained to locate the scat of as many as ten species simultaneously, including those which are closely related (Dr. Todd Steury, Ecodogs, pers. comm.). Detection dogs have been trained to locate scat from a variety of taxa including felids (Beckmann 2006; Harrison 2006b; Long et al. 2007a; Kerley 2010; Vynne et al. 2010; Karmacharya et al. 2011), canids (Smith et al. 2003; Smith et al. 2005; Beckmann 2006; Smith et al. 2006; Dematteo et al. 2009; Vynne et al. 2010), ursids (Wasser et al. 2004; Beckmann 2006; Long et al. 2007a), mustelids (Long et al. 2007a),

rodents (Duggan et al. 2011), and cetaceans (Rolland et al. 2006). Furthermore, detection dogs have been trained to successfully match the scents of scat samples, allowing for individual identification without the need for molecular techniques (Kerley and Salkina 2007; Wasser et al. 2009).

Once a scat detection dog has been trained to indicate the location of target scats and ignore nontarget scats, field surveys can begin. A field team should include three members: a detection dog, the handler, and an orienteer. Typically, the detection dog works off-lead and ahead of the other team members. The dog will independently work a pattern perpendicular to wind direction, allowing for maximum coverage. As a target odor is detected, the dog will exhibit a change in behavior. For example, a wide undulating search pattern will change to focused circling of the odor source. When the dog locates a target odor source it will perform the detection behavior learned during training. It is important this detection behavior is passive, such as sitting or barking, so the dog never comes in direct contact with the target scat. Once the handler confirms a target scat has been located, the dog is rewarded with play time.

The handler may be the most critical component to a successful field survey. They must be able to visually assess dog behavior at all times and respond appropriately with precise timing. A handler should have an intimate knowledge of their dog's personality and behaviors to ensure the dog's safety and maximize their detection potential. In addition, the handler must be aware of the dog's physical condition and limitations. Since a handler must focus so much of their attention on the dog, it is the orienteer's job to follow behind and keep them on transect. An orienteer may also be responsible for sample collection while the handler administers the reward.

Studies which utilize scat detection dogs can address all of the same basic and advanced objectives as hair snaring (MacKay et al. 2008b). Scat can be used to assess species distribution, habitat preference, and diet. In addition, DNA extracted from scat samples can be used for population estimates, population genetics assessments, and monitoring programs. However, the acquisition of animal feces provides research objectives which are not possible with hair samples. For example, steroid levels present in scat can be quantified to compare physiological stress and reproductive output between protected and hunted populations (Foley et al. 2001; Gobush et al. 2008) or guide management decisions (Hayward et al. 2011).

Scat detection dogs not only allow for a wide variety of study objectives, they can reduce sampling bias, increase capture probabilities, and outperform other noninvasive survey methods in both sampling yield and accuracy. Traditional scat surveys involved human observers walking transects and collecting any scat they could visually locate and identify as of target origin. However, dense vegetation or leaf litter can obscure even large scats, causing observers to supplement their searches in areas more amicable to visual detection, such as forest roads. In contrast, the olfactory-based detection employed by scat dogs allows for random transects to be actively searched with minimal sampling bias. Additionally, tests designed to compare search efficacy between human observers to detection dogs have revealed dramatic results. Meadows (2002) reported detection dogs outperforming human observers by 900%, while human observers in deOliveira et al. (2012) were not able to find a single scat. Detection dogs can survey a larger area than human observers in a fraction of the time (Homan et al. 2001; Nussear et al. 2008). Olfactory detection provides another significant advantage in that a scent can travel great

distances from its source. Cablk et al. (2008) recorded detection distances of up to 62 m for dogs successfully locating desert tortoise. However, while searching for North Atlantic right whale (*Eubalaena glaciali*) scat in the Bay of Funda, Canada, detection dogs were able to guide researchers to floating scats from up to 563 m away (Rolland et al. 2006).

As a relatively new method, scat detection dogs have been directly compared to other noninvasive survey techniques. Under a mark-recapture framework, Wasser et al. (2004) used radiotelemetry, hair snares, and scat detection dogs to survey grizzly and black bears in Canada. The authors sampled fewer individuals during one season of hair snaring and 2 seasons of live capture combined than during 8 weeks of scat dog surveys. Harrison (2006b) compared the efficacy of camera traps, hair snares, scent stations, and scat detection dogs at sampling bobcat in the forests of central New Mexico. His results showed that scat detection dogs produced almost 10 times more bobcat detections than the other three methods combined. Finally, in Vermont, Long et al. (2007b) tested the efficiency of camera traps, hair snares, and scat detection dogs at sampling black bear, bobcat, and fisher. The authors concluded that scat detection dogs yielded the highest rate of detection, the highest probability of detection, and greatest number of individuals sampled for each species.

The sampling efficiency of scat detection dogs holds great potential for the future of wildlife research; however this method is not without limitations. While detection dogs have been employed in habitats ranging from tropical rainforests (Javier Carrazo, Panthera, pers. comm.) to the Arctic tundra (Lydersen and Gjertz 1986), certain local terrain elements may limit or prevent their use. Excessively steep slopes or cliff habitats

should be avoided as these areas significantly increase the potential for serious or fatal injuries. Wetlands and regions of open water are generally not feasible for detection dog surveys unless specific training and study design considerations are addressed prior to deployment. Additionally, dense vegetation can limit a detection team's ability to efficiently survey an area by impeding movement and requiring dogs to remain close to the handler.

Study area climate should be considered when designing a detection dog survey. Aside from the physical stress of working in extreme conditions, certain climate variables have been shown to limit a dog's ability to detect target odors. Among these variables, wind speed and precipitation appear have the greatest effect on sample detectability (Smith et al. 2005; Cablk et al. 2008; Hunter 2011; Reed et al. 2011). Wind speed will influence detection distance. Using controlled experiments under low wind speed conditions, 1.6 m/s, Reed et al. (2011) found that detection rates decreased sharply from ~90% at 10 m to ~50-60% at 20 m. Whereas, Cablk et al. (2008) recorded wind speeds up to 9 m/s and used fine-scale GPS tracking coupled with observation to demonstrate detection distances >60 m. The amount of precipitation has been shown to influence detection rate, however the mechanism by which this occurs has not been clearly resolved. While rainfall has been shown to accelerate scat degradation (Hunter 2011), it has also been proposed to wash away scent-causing bacteria (Syrotuck 1972). Reed et al. (2011) measured the number of days since a significant precipitation event (>5 mm) and compared it to scats located per km for 2 dogs. The authors found that as the number of days since a precipitation event increased, detection rates increased. Hunter (2011) corroborated these findings by measuring detection rates in relation to the number of days

scat was exposed to natural degradation, accounting for rainfall. Results suggested while some scats can be detected over 3 months after deposition, the overall frequency of detection decreases with time. Other environmental factors, such as temperature, relative humidity, and vapor pressure, have been examined but appear to exhibit minimal influence on detection rates (Reed et al. 2011).

Scat deposition and degradation rates, specific to the target species, should also be considered as they have been shown to influence detection rates. Rare species present a unique challenge to scat detection dog surveys. Detection dogs may become less motivated to search when scat frequency is low, because they associate target scats with play time. To overcome this loss of interest, search team members can discretely place known target samples in the field and reward the dog as though they had located a study sample. Additionally, by training a detection dog to find the scat of a common species along with that of a rare species, the dog is less likely to lose interest. The target species may also influence detection dog behavior. For example, dominant detection dogs may urinate near wolf scats (MacKay et al. 2008b), while submissive dogs may simply avoid them (Beckmann et al. 2006). Scat degradation will affect detection rates and subsequent genetic analyses, but the rate of scat degradation can be influenced by other factors. As mentioned, precipitation increases the rate of scat degradation however this rate can be altered spatially, through microhabitat variations. Scats deposited in areas with little or no canopy cover will degrade faster than those found in hardwood or pine forests (Tsaparis et al. 2009; Hunter 2011). Furthermore, the rate of degradation can also be influenced by scat size and composition. Small or loosely packed scats, such as those from many omnivores, can rapidly be broken down by environmental stimuli, thereby

reducing their probability of detection (Hunter 2011). As with hair snaring, or any survey method, the species of interest should be thoroughly considered when designing a detection dog study.

In summary, detection dogs provide a relatively new, but powerful, tool for the study of wildlife. While this method can be costly, it has proven both efficient and accurate at collecting large quantities of scientifically valuable scat samples from multiple species simultaneously. When studies are designed correctly, according to the target species, survey area, and objectives, detection dogs offer one of the most effective noninvasive survey methods available.

Molecular Methods

Discoveries in the field of human genetics during the 1980's have laid the foundation for molecular studies of wildlife. In 1985, through the study of hypervariable regions (sections of DNA comprised of multiple short sequence repeats) in human DNA (Jeffreys et al. 1985a), it was discovered that each person contained a unique genetic "fingerprint" (Jeffreys et al. 1985b). By using a method known as the Southern blot, these hypervariable regions were excised from the genome and aligned in order of size. This fingerprinting method was first applied to the field of wildlife in 1987 to study house sparrow (*Passer domesticus*) demography with great success (Wetton et al. 1987). However, the Southern blot method requires a considerable amount of DNA for barcode patterns to be visible. To achieve this, blood or tissue samples must be taken through invasive means.

At the time when fingerprinting was being discovered in England, researchers in the United States were refining a method to amplify (i.e., copy) extremely small quantities of DNA. This method, known as the polymerase chain reaction (PCR), revolutionized the field of genetics (Saiki et al. 1985; Mullis et al. 1986). Essentially, PCR is an enzymatic reaction which utilizes temperature changes to exploit the natural replicative tendencies of DNA. By using short primer sequences to initiate DNA replication, PCR allows for the targeted amplification of specific, known sequences. This is contrary to the Southern blot method, which simply cuts many variable length regions of DNA and aligns them without indication of their genomic origins. As such, the final discovery which allowed for individual identification through PCR was a new class of primers referred to as simple sequence length polymorphisms (SSLPs) (Tuatz 1989; Weber and May 1989). SSLPs, also known as simple sequence repeats (SSRs), short tandem repeats (STRs), and microsatellites are similar to hypervariable regions except they can exhibit a range of diploid variation (Guichoux et al. 2011). Simply stated, microsatellites are short blocks of tandem (i.e., two alleles) DNA repeats (Schwartz and Monfort 2008). With the discovery of microsatellite markers, combined with PCR, wildlife biologists could take a noninvasively collected DNA sample and produce a single locus barcode of known genomic origin. By repeating this process for multiple microsatellite markers, a genetic fingerprint, or genotype, is revealed.

As PCR became available to the field of wildlife biology, new possibilities for genetic sampling emerged. While hair and scat remain the most popular types of noninvasive samples, genetic material can be acquired from a variety of sources, such as saliva, urine, regurgitates, feathers, and museum specimens (Schwartz and Monfort

2008). However, the quantity and quality of DNA from a given species fluctuates both among and within these sample types. As a result, amplification success rates vary widely, regardless of whether studies use the same sample type from the same species (Schwartz and Monfort 2008). There are many environmental and methodological factors which contribute to these inconsistencies. Three environmental factors have been identified which appear to strongly influence DNA amplification success. Sample freshness, or the amount of time a sample is exposed to natural elements, has been directly linked to DNA quality (Fernando et al. 2000; Murphy et al. 2007; Santini et al. 2007). Frequency of exposure to water, through precipitation events, humidity, or dew, decreases amplification success by breaking down DNA molecules through hydrolysis (Farrell et al. 2000; Piggott 2004; Murphy et al. 2007). Lastly, high temperatures facilitate molecular degradation, whereas samples collected frozen in snow showed higher amplification success (Lucchini et al. 2002). Furthermore, when dealing with scat samples, the location on a scat from which a sample is taken can affect the quantity and quality of DNA collected (Stenglein et al. 2010). Once collected, researchers have many options for sample storage, especially for scat. A review of these methods by Schwartz and Monfort (2008) revealed a general lack a consistency, as no single storage method could be considered optimal.

Laboratory methods also present researchers with many protocol options which can influence DNA amplification success. There are a variety of DNA extraction protocols available that generally correspond to specific sample storage methods. However, regardless of storage method and sample type, studies which perform DNA extractions within 3 months of sample collection typically demonstrate higher

amplification success rates (Schwartz and Monfort 2008). Amplification protocols are designed to meet specific objectives. Although, researchers have many options for reagent/enzyme manufactures as well as their quantities and concentrations used for PCR. Therefore, a pilot study which compares storage, extraction, and amplification methodologies specific to the sample type and collection method for a target species is highly recommended (Valiere et al. 2007; Schwartz and Monfort 2008).

Despite the challenges, noninvasive genetic sampling can address a wide range of ecology, management, and conservation based study objectives. Many of these objectives require the same laboratory procedures: species, gender, and individual identification. With this information, researchers can infer fundamental population parameters such as distribution, survival, dispersal, abundance, viability, relatedness, and hybridization to monitor population trends and guide management decisions

Species Identification

As discussed earlier, a reliance on morphological characteristics to identify noninvasive samples can be difficult and prone to error. However, molecular techniques offer a reliable and efficient means for species identification. Two common methods are typically employed to achieve species identification from noninvasive samples. Both methods utilize mtDNA, due to its high degree of variation among species but conserved nature within species. First, PCR is used to amplify a short (<1000 bp) polymorphic region of mtDNA. Several markers have been developed which can amplify a given region for multiple species, eliminating the need for species specific amplification tests (Paxinos et al. 1997; Mills et al. 2000a; Bidlack et al. 2007). Once the mtDNA region

has been amplified, a researcher typically has a choice between two methods depending on the available laboratory equipment and project budget. The direct and generally more accurate method is to sequence the amplified region and compare it to known sequences. Sequencing requires access to expensive equipment but often provides clear results. The cheaper, indirect option is to perform restriction enzyme digests on the amplified region. Restriction enzymes cut DNA at specific, known locations, called recognition sites. For example, the restriction enzyme *Hpa*II will only bind to the sequence, 5'...CCGG...3' and it will always cut between the cytosines (Bidlack et al. 2007). By digesting the amplified mtDNA with one or more restriction enzymes, a species specific barcode pattern can be visualized.

Gender Identification

To determine gender, markers specific to nuclear DNA sex chromosomes are used to amplify either the SRY gene found only in males or the zinc-finger (ZF) region in both males (ZFY) and females (ZFX) (Murata and Masuda 1996; Pilgrim et al. 2005). The SRY method uses SRY-specific primers to amplify a region a DNA which is only found in the Y-chromosome. When visualized, one band present indicates a male and no band indicates a female. The SRY method is less commonly used as it is prone to false negatives (i.e., no bands). To check for false negatives, a microsatellite region from each sample is co-amplified. If the microsatellite does not amplify, the sample is considered poor quality and culled from the data set. Zinc fingers reduce the need for microsatellite co-amplification. In felids, and many other mammals, the ZF region of the Y-chromosome is slightly shorter than the ZF region of the X-chromosome. Therefore, by amplifying and visualizing these regions, a male will display two bands (one X and one

Y) whereas a female will display one band (two X's of equal length). Both methods should utilize a selective or random sub-set of replicates to ensure accuracy.

Individual Identification

Since their discovery, microsatellites have become the most popular markers for assessing kinship and identifying individuals from noninvasively sampled populations (Schwartz and Monfort 2008). Within the nuclear genomes of most taxa there are non-coding regions of DNA, typically < 300 bp, which consist of short (1-6 base) repeats (Selkoe and Toonen 2006). Furthermore, these short-repeat regions occur in tandem, as alleles from both maternal and paternal origin are present in diploid organisms. Therefore, one microsatellite locus can have two alleles of equal length (homozygous) or two alleles of variable lengths (heterozygous). In order to amplify a microsatellite locus, markers for PCR must be created from the regions that flank a repeat motif (Zane et al. 2002). Markers are simply the reverse compliment sequence of flanking regions and typically 20-25 bases long. These flanking regions are conserved, or do not change, among individuals in the same species, genus, or even family. For example, Menotti-Raymond (1999) identified 246 marker pairs found within the domestic cat genome and several have been used to amplify microsatellites in bobcats (Ruell 2009), pumas (Onorato et al. 2011), leopards (Mondol 2009), and tigers (Bhagavatula et al. 2006). Multiple markers must be used to amplify independent microsatellite regions in order to distinguish among individuals. Generally, 4-10 microsatellites provide sufficient coverage to differentiate among individuals. However, isolated, small, or inbreeding populations may require additional loci due to low genetic variation (Schwartz and Monfort 2008). If an insufficient number of loci are used, samples from two or more

closely related individuals may display the same genotype. This occurrence is known as the shadow effect and will result in an underestimation of population abundance (Mills et al. 2000b).

Microsatellites have become a popular, and powerful, tool for genetic identification for several reasons. In order to amplify a microsatellite, its markers (i.e., flanking regions) must be identified and in turn, its genomic location must be known (Zane et al. 2002). This allows for direct comparisons among individuals, unlike the Southern blot fingerprinting technique. Additionally, microsatellites are relatively short sequences which make them easier to amplify and increase the likelihood they will remain intact in degraded samples (Taberlet et al. 1999). In contrast to their flanking regions, microsatellites have high mutation rates per locus per generation, resulting in allelic variation between parents and offspring (Selkoe and Toonen 2006). Furthermore, without conserved flanking regions as marker templates, microsatellite amplification would not be feasible. Finally, while some microsatellites occur within coding DNA regions, those used for individual identification originate from non-coding regions which are believed to be selectively neutral (Schwartz and Monfort 2008; Arif and Khan 2009). This neutrality is in accordance with many population genetics models that are confounded by the laws of selection (Oyler-McCance and Leberg 2005).

Genotyping Error

Despite the advantages of using microsatellites for individual identification, they are not without limitations. DNA extracted from noninvasively collected samples is typically degraded, contaminated, and present in low quantities (Taberlet et al. 1996;

Taberlet and Luikart 1999; Waits et al. 2001; Paetkau 2003). As a result, two common types of genotyping errors can occur during microsatellite amplification which significantly influences downstream analyses, such as abundance estimates, parentage reconstructions, and genetic variance.

The first type of error is called *allelic dropout*, and it occurs when one of two alleles is preferentially amplified during PCR. For individuals which are true homozygotes at the locus of interest, allelic dropout is not an issue. However, when allelic dropout occurs in a true heterozygote, the resulting genotype score will produce a false homozygote (Taberlet et al. 1999). Several sources of allelic dropout have been identified. Random pipetting error associated with highly diluted (i.e., low quantity) DNA samples can result in only one of two alleles being pipetted, amplified, and detected (Taberlet et al. 1996). If both alleles are pipetted, one allele could be incapable of amplification due to damage or degradation (Taberlet and Luikart 1999). Allelic dropout can also occur through a process known as short allele dominance, during which the shorter allele is preferentially amplified over the longer allele (Pompanon et al. 2005).

The second type of genotyping error is known as *false alleles* and is specific to dinucleotide repeating microsatellite motifs (e.g., 5'-ATATAT....-3'). False alleles are artifacts generated during microsatellite amplification that resemble true alleles (Taberlet et al. 1999). In true homozygotes, the presence of a false allele may be impossible to detect and result in a false heterozygote genotype score. In true heterozygotes, a false allele is easily detectable through the presence of a third allele. Two explanations have been presented for the creation of false alleles. Low quantities of template DNA can result in slippage events during the initial cycles of DNA amplification. When the DNA

polymerase enzyme slips, or skips over, one dinucleotide repeat the resulting product will be two bases shorter than the original DNA template. As PCR continues, both the original DNA and the shortened DNA product are used as templates for replication (Taberlet and Luikart 1999). The other explanation for false allele artifacts is contamination by nontarget DNA or cross-contamination among target samples (Taberlet et al. 1999).

Allelic dropout occurs more frequently than false allele artifacts; however both genotyping errors will result in an overestimation of population abundance (Taberlet et al. 1996). For example, Schwartz et al. (2006) compared black bear population estimates before and after error checking procedures. The authors discovered genotyping error was responsible for a 28.1% overestimation in population abundance. To overcome the errors associated with microsatellite genotyping, certain laboratory protocols and a variety of computer programs have been developed. Taberlet et al. (1996) were the first to create a multitube protocol, adapted from the theoretical findings of Navidi et al. (1992), which was designed specifically for DNA extracted from noninvasive samples. To detect genotyping errors, the multitube approach requires each sample to be independently amplified multiple times at each locus. The authors used computer simulations based on rigorous mathematical models to determine the number of replicates necessary to obtain a microsatellite score at the 99% confidence level. These simulations predicted a true heterozygote can be detected with 99% confidence if both allele scores match after two independent tests. However, to obtain this level of confidence from a true homozygote, the allele needed to be present in seven independent tests. This discrepancy is due to the overwhelming potential for allelic dropout to produce false homozygotes. The

simulations of Taberlet et al. (1996) also defined a target DNA concentration range of ~16.8-56 picograms (pg) as a 'danger zone' for genotyping error. When the concentration of template DNA for PCR is within this range, the sample is likely to both amplify and contain errors. The multitube approach can certainly detect genotyping errors but replicating samples will require additional time and capital. Furthermore, some noninvasive samples, such as hair, may not yield enough extracted DNA for this method to be feasible (Schwartz and Monfort 2008).

Several years after the multitube approach of Taberlet et al. (1996), a method was developed to quantify the amount of target DNA extracted from a noninvasive sample. Once quantified, the samples could be classified by the theoretical number of replicates required to produce a true genotype (Morin et al. 2001). Traditional methods to measure DNA concentration cannot discriminate between target and nontarget DNA extracted from a given sample. This is especially important for noninvasively collected samples because any bacterial DNA present will copurify with target DNA (Morin et al. 2001). However, this method developed by Morin et al. (2001) requires the use of quantitative, or real-time, PCR which is expensive and not available to many molecular laboratories. Furthermore, even if real-time PCR is available, the method is only designed to recommend the number of replicates based on the amount to target DNA per sample and genotyping errors may still occur (Schwartz and Monfort 2008).

Finally, several computer programs, each designed for specific project objectives, are available to identify and deal with microsatellite genotyping errors. To analyze microsatellite data collected through a CMR framework, the program DROPOUT (McKelvey and Schwartz 2005) is commonly used. DROPOUT identifies samples which

are likely to contain genotyping errors as well as loci which are likely to produce genotyping errors. In addition, this program is sensitive to the shadow effect, and can assess whether enough loci were used to accurately identify individuals.

The program RELIOTYPE (Miller et al. 2002) is essentially a computer algorithm version of the Morin et al. (2001) method for identifying samples which should be replicated. This program utilizes a maximum-likelihood model based on observed allelic frequencies to estimate the allelic dropout rate per locus. RELIOTYPE can detect genotype errors and estimate genotype accuracy to recommend samples per locus that should be replicated.

If the multitube approach of Taberlet et al. (1996) is used to genotype samples, a researchers is left with multiple genotype scores per sample per locus. To combine these scores into one consensus score, the program GIMLET (Valiere et al. 2002) can be used. In addition, this program can estimate genotyping errors and calculate important population parameters such as allele frequencies, heterozygosity, probability of identity, and population abundance.

Noninvasive sampling combined with molecular methods has provided new opportunities for estimating wildlife population parameters however it has also presented new challenges. Errors associated with microsatellite genotyping can limit the application of research findings or completely negate years of effort. The pressure to account for and report molecular errors has grown, which is reflected by a near 10-fold increase between 1989 and 2005 in the number of publications that mentioned genotyping error (Pompanon et al. 2005). Fortunately, laboratory protocols and

standardized error checking programs are available for quality control and the assurance of accurate population parameter estimates.

Project Description

Historically, Kentucky was home to a fairly robust large predator guild which included gray wolves, red wolves (*Canis rufus*), puma, and black bear (Kelly et al. 2004; Laliberte and Ripple 2004; Leonard et al. 2005). Over the last 150 years, each of these species has been extirpated from the state (Woodroffe 2001; Laliberte and Ripple 2004). Although, black bears have recently returned through natural re-colonization events (Hast 2010) and experimental reintroduction (Eastridge 2000), breeding populations remain confined to southeastern Kentucky.

In the absence of large carnivores, coyotes have risen to an apex predatory position throughout much of the southeastern United States, including Kentucky (Hill et al. 1987). The top-down influence of coyotes has been well documented, causing a decrease in sympatric mesocarnivore abundance through interference and exploitation competition (Litvaitis and Harrison 1989; Crooks and Soule 1999; Henke and Bryant 1999; Fedriani et al. 2000; Neale and Sacks 2001; Farias et al. 2005). Specifically, mesocarnivore species such as bobcat and gray fox may be at risk, as their densities have been negatively correlated to coyote abundance (Henke and Bryant 1999; Fedriani et al. 2000; Farias et al. 2005). As a result, bobcat and gray fox have exhibited fine-scale spatial avoidance, especially in forested systems where cohabitation with coyote is likely to occur (Chamberlain and Leopold 2005; Hackett 2008). Furthermore, despite seasonal

variation in diets, these three species compete year round for limited rodent and lagomorph prey (Fedriani et al. 2000; Neale and Sacks 2001; Thornton et al. 2004).

The competition among gray fox, bobcat, and coyote for critical resources has effectively linked these species within a trophic feedback system. As coyote abundance increases, sympatric mesocarnivores become excluded or exploited. As important furbearers, gray fox, bobcat, and coyote are managed through harvest but no absolute abundance estimates are available for these species in Kentucky. Harvest data and anecdotal reports suggest coyote and bobcat populations are stable to increasing, while gray fox may be in decline (Laura Patton, KDFWR, pers. comm.). However, harvest trends lack precision to reliably estimate abundance and are subject to various external biases (Gese 2001). Thus, it remains uncertain whether these trends actually represent the status of mesocarnivores in Kentucky. An argument can been made that accurate abundance estimates are labor intensive and costly, especially for multiple species. However, given the economic and ecological importance of mesocarnivores, as well as the empirical limitations of using harvest data, it is important that alternative monitoring approaches be evaluated as to their reliability, precision, and cost-effectiveness for estimating mesocarnivores in Kentucky.

In this study, I tested the effectiveness of two multi-species, noninvasive survey methods at detecting individual gray fox, bobcat, and coyote at two sites in northeastern Kentucky. First, I conducted a random systematic survey using professionally-trained scat detection dogs to locate gray fox, bobcat, and coyote scats. Then, I deployed a random systematic network of rub pad hair snares under a CMR framework. To assess

the feasibility of molecular identification techniques, I used DNA extracted from the noninvasive samples to evaluate genetic species and individual identification methods.

CHAPTER 3: STUDY AREA and METHODS

Study Area

The general study area was located in the northern portion of the Cumberland Plateau physiographic region that is characterized by steep and rugged terrain with elevation ranging from ~ 200-400 m. Forests of this region fall into the mixed mesophytic classification as described in detail by Braun (1950). In general, the mixed mesophytic classification is diverse and comprised of up to 30 co-dominant tree species, most of which are hardwoods. Additionally, understory species include mountain laurel (*Kalmia latifolia*) and rhododendron (*Rhododendron* spp.) (Braun 1950). The climate of this region is characterized by mild winters and hot, humid summers with prolonged spring and fall seasons. Average annual temperature was approximately 12°C. Winter temperatures typically range from -5° to 4°C and summer temperatures from 18° to 29°C (Hill 2005). Precipitation is distributed relatively evenly throughout the year, averaging approximately 116 cm annually, with maximum rainfall occurring in May and minimum rainfall occurring in September (Hill 2005).

Two mesocarnivore sampling sites were selected within the study area. Each was located within the 741 km^2 Cumberland Ranger District of the U.S. Forest Service (USFS) Daniel Boone National Forest (DBNF) near the city of Morehead, Kentucky (Figure 3.1). Both sites were comprised of ~95% second and third growth mixed-mesophytic forest and ~5% grassland, and were managed for multiple uses that included timber production, biodiversity management, hunting, trapping, and various types of non-consumptive outdoor recreation. Site 1 was a 44 km^2 tract within the Big Perry area (BP)

located just north of Morehead and bordered by Interstate 64 to the north and west (Figure 3.2). The Big Perry site had a road density of 0.93 km/km^2 (87.1% gravel and 12.9% asphalt) and a high degree of hunting/trapping pressure (Steve Bonney, KDFWR, pers. comm.). This site was located in Rowan County which had a population density of 32.4/km^2 in 2011 (census.gov). Site 2 was a 100 km^2 block of the DBNF that surrounded the Pioneer Weapons Wildlife Management Area (PW) located southwest of Morehead and bordered by Cave Run Lake to the east (Figure 3.3). This site had a road density of 1.47 km/km^2 (52.6% gravel and 47.4% asphalt) and a moderate degree of hunting/trapping pressure due to weapons restrictions (Steve Bonney, KDFWR, pers. comm.). The Pioneer Weapons site was located in both Bath and Menifee counties which had a combined population density of 14.4/km^2 in 2011 (census.gov). This site was surrounded by a greater proportion of small residential landholding and large grasslands/pastures than Big Perry. In addition, the Pioneer Weapons site was visited by a higher diversity of recreational users at a higher density than the Big Perry site (Jeff Lewis, USFS, pers. comm.). Study sites were approximately 14 km apart at their closest points and topographically similar, a result of dendritic erosion across the Cumberland Plateau. However, these sites differed in both geography and physiography.

Methods

I tested the effectiveness of two multi-species, noninvasive survey methods for detecting individual gray fox, bobcat, and coyote at two sites in northeastern Kentucky. First, I conducted a random systematic survey using scat detection dogs trained to locate gray fox, bobcat, and coyote scats. I then deployed a random systematic network of rub pad hair snares under a CMR framework. To assess the feasibility of molecular

identification techniques, I used DNA extracted from the noninvasive samples to test genetic species and individual identification methods.

Fundamental to the concept of noninvasive sampling is the assumption of an equal probability of detection among target individuals (Long and Zielinski 2008). Beyond the standardization of sampling devices or transect length, sampling design plays a key role in equalizing this probability of detection. The random systematic placement of snaring devices and sampling transects allows for homogenous coverage throughout a study area and fulfills the assumption of equal detectability. However, the spacing of sampling devices or transects must be appropriate for a target species. Therefore, minimum home range size is generally accepted as a determinant for the spacing of random systematic sampling devices or survey transects (Long and Zielinski 2008).

In the eastern United States, gray fox have relatively small home ranges. Estimates in southern Illinois ranged from 1.07 – 1.36 km^2 (Follmann 1973) and 0.75 – 1.24 km^2 in West Virginia (Yearsley and Samuel 1980). Given the proximity of these studies to my field sites, I felt justified that using a snare density of approximately 1/km^2 for all canid hair snares would maximize the probability of detection. Bobcat telemetry data from southeastern Kentucky was used to determine a minimum spacing for felid hair snares. Whitaker (1988) recorded bobcat home ranges from 2.8 – 66.4 km^2, and in the same general area, home ranges from 4.9 – 25.3 km^2 were later documented (A. Shipley, in press). Therefore, I placed approximately 1 felid hair snare per 4 km^2 to ensure each bobcat had an equal chance of being sampled. Canid and felid sampling grids were created using Hawth's Tools, an extension of ArcMap 9.3 Geographic Information

System (ESRI, Redlands, CA). Hair snare and detection dog survey start points were randomly chosen, allowing each possible sampling location the potential to be sampled.

Scat Detection Dogs

The use of detection dogs to survey wildlife has expanded considerably within the last decade (MacKay et al. 2008b). Researchers have applied the trusted methods for explosives and narcotics detection training to meet the needs of professional wildlife investigation. As a result, detection dogs now provide an active and efficient method for locating a variety of biologically relevant indices such as scat, with an inherently low sampling bias. Scat searches were conducted using two detection dogs contracted through Ecodogs (Auburn University, Animal Health and Performance Program, IACUC 2010-1753). Ecodogs also provided a professional trainer/handler, Mr. Lucas Epperson, to maintain the dogs and ensure their optimal performance. Both dogs (Kasey and Nitro) were Labrador retrievers between the ages of 2.5-3 years old. New and reinforcement scat detection training occurred at Auburn University prior to deployment of dogs to Kentucky. Initially, we decided on two target species, gray fox and bobcat, for both dogs. Professional detection dog agencies were hesitant to train for coyote scat detection because of this species near ubiquity throughout North America. As a result, future surveys conducted by dogs trained to locate coyote scats would encounter frequent nontarget detections. Kasey had been trained to locate long-tailed weasel scats prior to this study and, by request, she was imprinted on gray fox and bobcat using fresh scats collected from target species in Kentucky. Because Nitro was on loan from the explosives detection program at Auburn University and not anticipated to return to scat detection work, he was imprinted on gray fox, bobcat, and coyote. After identifying scats to

species we discovered Kasey had been detecting coyote scats throughout our surveys, prompting our decision to include coyote as a target species. Therefore, both dogs received reinforcement training to locate coyote scats.

The Big Perry study site was surveyed using scat dogs from 7 Feb 2011 to 18 Feb 2011; 9 full transects and two half transects were surveyed covering approximately 10 km^2 (Figure 3.4). The Pioneer Weapons study site was surveyed from 21 Feb 2011 to 4 March 2011; 17 full transects were surveyed covering approximately 17 km^2 (Figure 3.5). Scat dog search transects consisted of equilateral triangle transects of 1.5 km perimeter length; their center points corresponded to future canid hair snare deployment locations ($1/km^2$). Transect length was established to maximize the distance surveyed after considering factors which limit a detection dog's capabilities, such as topography, temperature, and expected number of transects per day. The triangular shape was chosen for practicality, as a common start and stop point minimized off-transect travel, helping to ensure against the fatigue related safety concerns of detection dog overexertion (Dr. Todd Steury, Ecodogs, pers. comm.). Transects were created using the extension Hawth's Tools in ArcMap 9.3. Due to steep, rugged terrain, ≤ 2 transects were surveyed each day, or one transect per detection dog. Each site was surveyed for 10 days, 5 days on and 2 days off to provide rest for the dogs. A continuous block of transects were surveyed at each site. When a dog indicated a scat, I used a fresh plastic tasting spoon to collect two independent ~0.5 ml samples from the scat. Each sample was placed in a 1.5 ml Eppendorf tube containing 95% ethanol used as a preservative for later genetic analysis (Panasci et al. 2011). Samples were collected from the outside, driest part of the scat (Stenglein et al. 2010), and the remaining scat was collected in a 13 oz Whirl-Pak sample

bag (Nasco, Fort Atkinson, WI) and stored at -20°C. Detection dogs were equipped with GPS collars and tracking data was recorded when a sufficient number of satellite signals were available to record a waypoint. In addition, I recorded the physical state of each scat (i.e., dry or wet) at the time of collection to correlate with species identification success using a Chi-square analysis. Furthermore, I used two proportion z tests ($p < 0.05$) to determine if detection success rates differed between dogs and between study sites.

I collected several scats opportunistically based on a visual assessment as originating from any target species. These scats were either undetected by dogs or collected from areas not surveyed by detection dogs (i.e., areas adjacent to transect start points). The purpose for these opportunistic collections was not to compare visual identification to detection dog accuracy but to maximize the total number of scats collected during field sampling.

Hair Snares

Hair snare rub pads were constructed using 10 x 10 cm squares of stiff, closed-loop wool carpet (McDaniel et al. 2000). Ten dulled nail-gun nails were manually pushed, not hammered, through the back of each square. Copper connector wires remained attached to each nail creating barbed edges near the point to facilitate sample collection (Figure 3.6). Depending on snare type, approximately 1-2 ml of olfactory attractant and/or natural rubbing-behavior elicitor was applied directly to the carpet fibers. Felid snares were nailed to a tree or log at 0.3-0.5 m from the ground (Ruell and Crooks 2007) and baited with beaver castor or catnip oil (F&T Fur Harvester's Trading

Post, Alpena, MI) and dried catnip (Figure 3.7). Canid snares were nailed to a 2.54 x 15.24 x 45.72 cm untreated pine board, placed on the ground at the base of a tree or rock, and baited with gray fox gland lure, red fox gland lure, or beaver castor (F&T Fur Harvester's Trading Post, Alpena, MI) (Figure 3.8). A visual attractant was hung within 5 m of all snare locations and approximately 1-1.5 m from the ground by fishing line and swivel. Turkey feathers were used at all felid snares and aluminum pie pans were used at all canid snares (Ruell and Crooks 2007; Kendall and McKelvey 2008). Snares were set out along game and hiking trails, near creek beds, and areas where sign (tracks, scats, etc.) from target species was observed.

Hair snare surveys were divided into five consecutive sampling occasions per site, each occasion lasting one week. Sampling occasions were set at one week each to minimize the potential for nontarget and multiple hits (Kendell and McKelvey 2008). Snares without hair samples were re-lured according to their original lure type (canid or felid). Hair samples were collected using forceps, placed into a small unwaxed manila envelope, and stored in a dry place (Ruell and Crooks 2007). The Big Perry study site was surveyed from 19 Feb 2011 to 27 March 2011 using a total of 40 hair snares; 30 canid snares coving 30 km^2 and 10 felid snares covering 40 km^2 (Figure 3.9). The Pioneer Weapons study site was surveyed from 7 March 2011 to 13 April 2011 using a total of 60 hair snares: 47 canid snares covering 47 km^2 and 13 felid snares covering 52 km^2 (Figure 3.10.

To assess the cost effectiveness of field methods I recorded labor time and total cost for hair snaring and scat detection dog surveys. Labor time was updated daily for both methods and does not include travel between sites. A fixed labor cost of $7.50/hr

was used to establish labor cost for both field survey methods. Material cost for detection dogs includes field collection materials and for hair snares, material cost includes snare construction and field collection materials. Transportation cost for detection dog surveys was included in the contract price.

DNA Analysis

Genomic DNA was extracted from all scat samples using the QIAamp DNA Stool Mini Kit (Qiagen Inc., Valencia, CA) according to manufacturer instructions. All extractions were completed within 6 months of collection in an effort to maximize DNA yield and quality (Roon et al. 2003). All DNA elutions were quality checked using a NanoDrop 2000 (Thermoscientific, Waltham, MA) spectrophotometer and immediately stored at -20°C. A small sub-set of elutions were visualized on 1.3% agarose gels to check for presence of whole genomic DNA.

Samples were identified to species using a method developed by Bidlack et al. (2007). This method is particularly useful for scat detection dog surveys because it allows for rapid identification of 7 common North American carnivore species. Two primers, HCarn200; 5'- ATTCAGCCRTARTTAACGTC-3' (Bidlack et al. 2007) and CanidL1; 5'-AATGACCAACATTCGAAA-3' (Paxinos et al. 1997), were used to amplify a short (234 bp) region of the mitochondrial (mtDNA) cytochrome *b* gene using the polymerase chain reaction (PCR). PCR was performed using 3 µl of 1:10 extraction dilution in a 30 µl total reaction volume containing 3 µl of 10X reaction buffer (Qiagen Inc., Valencia, CA), 0.6 µl each primer (10 µM each), 0.6 µl dNTP solution mix (10 mM each dNTP), 0.3 µl BSA (10 µg/µl), 0.15 µl HotStarTaq (5 U/µl) DNA polymerase

(Qiagen Inc., Valencia, CA), and 21.75 µl H_2O. Thermal cycling was initiated at 95°C for 5 min followed by 40 cycles of 94°C for 45 sec, 54°C for 45 sec, and 72°C for 30 sec with a final extension of 72°C for 10 min. One negative control, using H_2O as a template, was included in all PCR reactions to monitor for contamination. PCR products from samples that did not amplify were diluted 1:10 and used as the DNA template for a second round of PCR. Samples that did not amplify after the second round of PCR were considered poor quality and culled. Following successful mtDNA amplification, all PCR products were first digested with both *HpaII* and *DdeI*, according to Bidlack et al. (2007). These restriction enzymes cut the PCR products of bobcat, puma (*Puma concolor*), raccoon (*Procyon lotor*), and striped skunk (*Mephitis mephitis*) samples, each in a unique manner. Samples that did not cut were considered of canid origin and their PCR products were digested with *Hpy*ChV to distinguish among gray fox, coyote, and red fox (*Vulpes vulpes*). All restriction enzyme digests were 10 µl in total volume and contained 8 µl PCR product, 1X reaction buffer (New England Biolabs, Ipswich, MA), 0.5 µl H_2O, and 0.25 µl each of *HpaII* (10,000 U/ml) and *DdeI* (10,000 U/ml) for the first digest or 0.5 µl of *Hpy*Ch4V (5,000 U/ml) for the second digest. Restriction enzyme products were digested at 37°C for 4 hours, according to manufacturer instructions (New England Biolabs, Ipswich, MA). Products were visualized on 1.3% agarose gels and electrophoresed for 30 – 40 min using 0.8 µl of EZ-Vision One 6X loading dye (Amresco, Solon, OH) and 4 µl of PCR product per well.

To test the accuracy of restriction enzyme methods and resolve any unidentified digest products, 72.8% (158/217) of samples were sequenced on an Applied Biosystems (ABI) 3730 capillary electrophoresis machine located at the University of Kentucky

Advanced Genetic Technologies Center. All PCR products were first purified using ExoSAP-IT (Affymetrix, Santa Clara, CA) to eliminate unincorporated primers and dNTPs. Clean up reactions were 7 µl in total volume and contained 5 µl PCR product and 2 µl of 1:5 diluted ExoSAP-IT. Thermal cycling was initiated at 37°C for 31 min followed by 80°C for 15 min. Sequencing reactions were performed using BigDye Terminator v3.1 (Applied Biosystems, Carlsbad, CA) and the primers HCarn200 and CanidL1. All sequencing reactions were 6 µl in total volume and contained 0.75 µl 5X buffer (Applied Biosystems, Carlsbad, CA), 0.2 µl BigDye, 1 µl primer (10 µM), 1 µl PCR product, and 3.05 µl H_2O. Thermal cycling was initiated at 96°C for 1 min, followed by 25 cycles of 96°C for 10 sec, 54°C for 5 sec, and 60°C for 4 min.

All sequences were analyzed, edited, and aligned using the program Geneious v5.5.6 (Drummond et al. 2010). Consensus sequences were compared to known sequences retrieved from the National Center for Biotechnology Information database and aligned on a Neighbor-Joining tree using the program Geneious. Extracted DNA samples identified as originating from target species were genotyped using microsatellite analysis. All samples that were unidentifiable or non-target in origin were culled. PCR fragments were fluorescently labeled using a FAM dye attached to the M13B universal tail; 5'-/56-FAM/CACTGCTTAGAGCGATGC-3', according to a method developed by Schuelke (2000). This technique allows for the cost effective incorporation of a fluorescent dye into microsatellite products.

Five microsatellite loci (GF-02, GF-07, GF-09, GF-12, and GF-14), developed by Weston et al. (2004), were used to genotype gray fox samples (Table 3.1). Four microsatellite loci (FCA026, FCA043, FCA045, and FCA057) were used to genotype

bobcat samples (Menotti-Raymond et al. 1999) (Table 3.1). PCR was performed using 1 -3 µl of either 1:10 or undiluted template DNA in a 10 µl total volume and contained 1 µl of 10X reaction buffer (Qiagen Inc., Valencia, CA), 0.1 µl M13B-tailed sequence specific forward primer (10 µM), 0.4 µl sequence specific reverse primer (10 µM), 0.4 µl FAM labeled M13B primer (10 µM), 0.15 µl dNTP solution mix (10 mM each dNTP), 0.5 µl HotStarTaq (5 U/µl) DNA polymerase (Qiagen Inc., Valencia, CA), and 4.45 - 6.45 µl H_2O. Thermal cycling was initiated at 95°C for 5 min, followed by 30 cycles of 94°C for 30 sec, variable T_A (see Table 3.1) for 45 sec, and 72°C for 45 sec, then 8 cycles of 94°C for 30 sec, 53°C for 45 sec, and 72°C for 45 sec, with a final extension of 72°C for 10 min. One negative control was included in all PCR reactions to monitor for contamination. PCR products were visualized on 2% agarose gels and electrophoresed for 50 – 60 min using 0.7 µl of EZ-Vision One 6X loading dye (Amresco, Solon, OH) and 3.5 µl of PCR product per well.

Microsatellite PCR products were analyzed on an ABI 3730 capillary electrophoresis machine located at the University of Kentucky Advanced Genetic Technologies Center. Samples for fragment analysis were 11 µl in total volume and contained 8.75 µl HiDi (Applied Biosystems, Carlsbad, CA), 0.25 µl LIZ600 size standard (Applied Biosystems, Carlsbad, CA), and 2 µl of either 1:100, 1:150, or 1:200 diluted PCR product. Microsatellites were scored using the program Peak Scanner v1.0 (Applied Biosystems, Carlsbad, CA). All coyote samples were stored at -20°C for future genetic analysis because funding and time constraints prevented genotyping.

To assess and account for microsatellite genotyping errors, a modified multitube approach was used (Taberlet et al. 1996). Samples were independently replicated per

locus until two matching scores were identified or until funds were exhausted. Consensus genotypes were generated using the program GIMLET v1.3.3 using the Threshold method (Valiere et al. 2002).

Table 3.1: Primer pair sequences and observed annealing temperatures for gray fox and bobcat microsatellite loci.

Species	Locus	Primer Sequence (5' - 3') [a]	T_A (°C) [b]
Gray Fox	GF-02 F	AATTCAATCAAAGATGGTC	52-55
	GF-02 R	CAGTCGGGCGTCATCA TTCTCCAGTTGGGTAAGT	
	GF-07 F	AAAACCCATTGAATAGTAAC	54-57
	GF-07 R	GGAAACAGCTATGACCA TGTGCCAGGAATACTCT	
	GF-09 F	CAGTCGGGCGTCATCA TACCTGGCTTGTGTTTAATG	53-58
	GF-09 R	ACTCCCCAAGGCAATATAG	
	GF-12 F	CAGTCGGGCGTCATCA TATTCTTTCTGTTGTGGCTTA	58-61
	GF-12 R	TGACCCCTGACCATAGA	
	GF-14 F	GGAAACAGCTATGACC ATGGGCCTGTATGTATCAT	54-57
	GF-14 R	AATCTTTGGGATGCAACT	
Bobcat	FCA026 F	GGAGCCCTTAGAGTCATGCA	46-50
	FCA026 R	TGTACACGCACCAAAAACAA	
	FCA045 F	TGAAGAAAAGAATCAGGCTGTG	57-60
	FCA045 R	GTATGAGCATCTCTGTGTTCGTG	
	FCA043 F	GAGCCACCCTAGCACATATACC	56-58
	FCA043 R	AGACGGGATTGCATGAAAAG	
	FCA057 F	AAGTGTGGGATTGGGTGAAA	54-60
	FCA057 R	CCATAAGAGGCTCTTAAAAACTGA	

[a]: FAM labeled M13B tail sequence not shown

[b]: Observed annealing temperature range

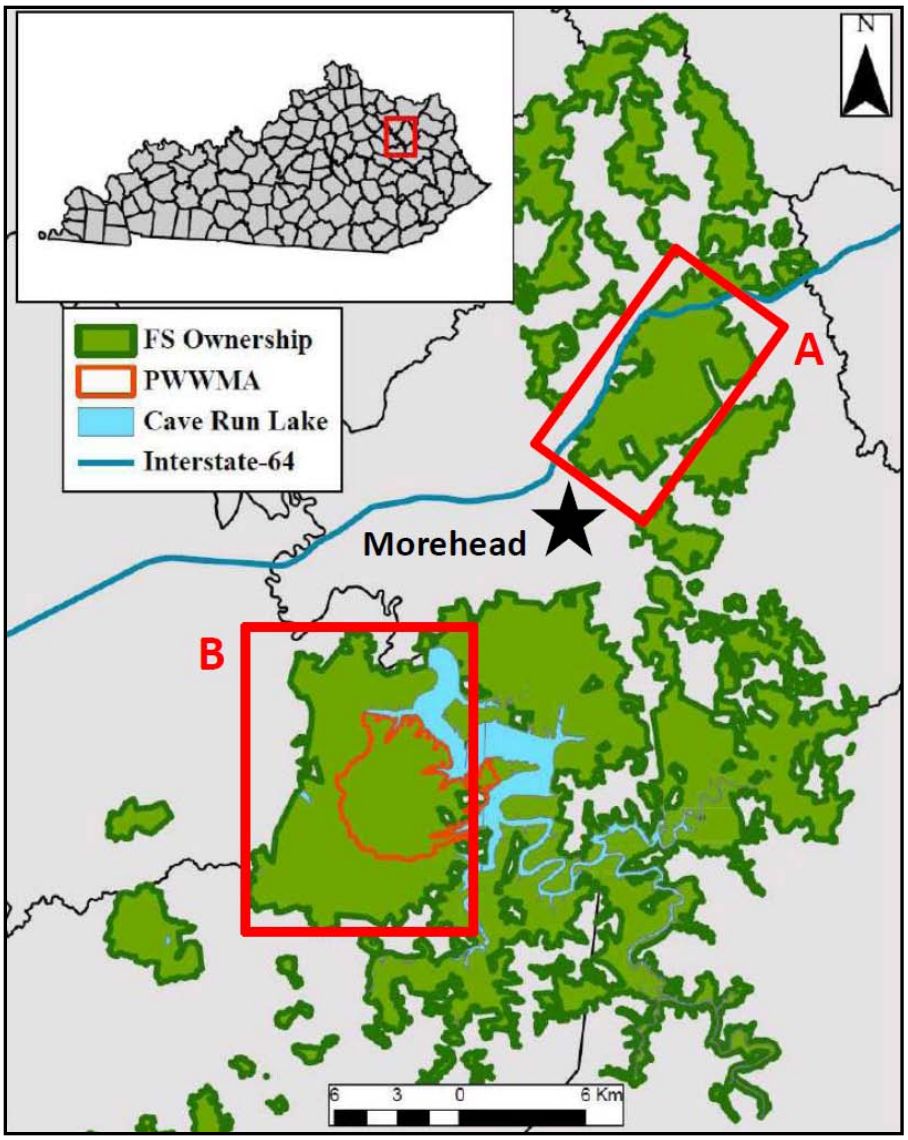

Figure 3.1: Mesocarnivore study area located within U.S. Forest Service ownership lands in the Cumberland Ranger District of the Daniel Boone National Forest, near Morehead, Kentucky. Box A indicates the Big Perry study site and Box B indicates the Pioneer Weapons study site.

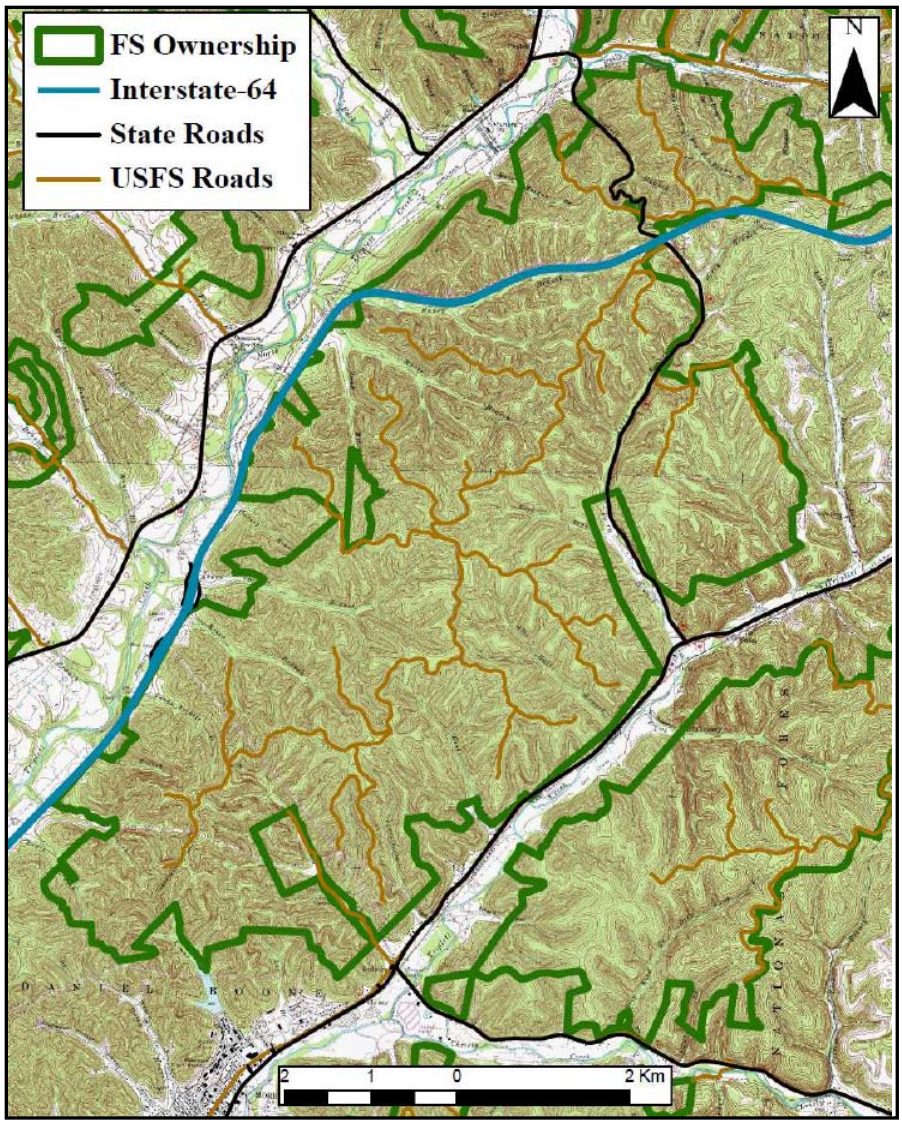

Figure 3.2: Topographic map of the Big Perry mesocarnivore study site in the Daniel Boone National Forest, Kentucky.

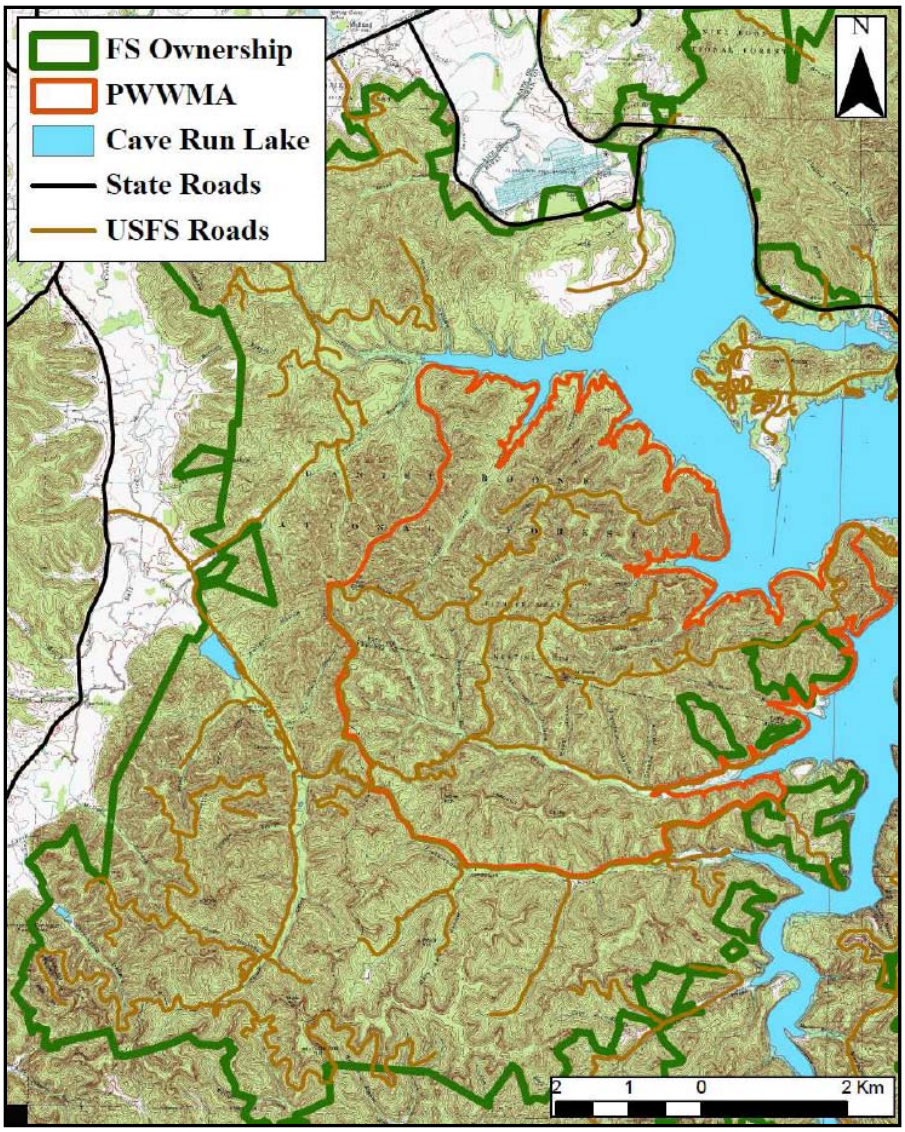

Figure 3.3: Topographic map of the Pioneer Weapons mesocarnivore study site in the Daniel Boone National Forest, Kentucky.

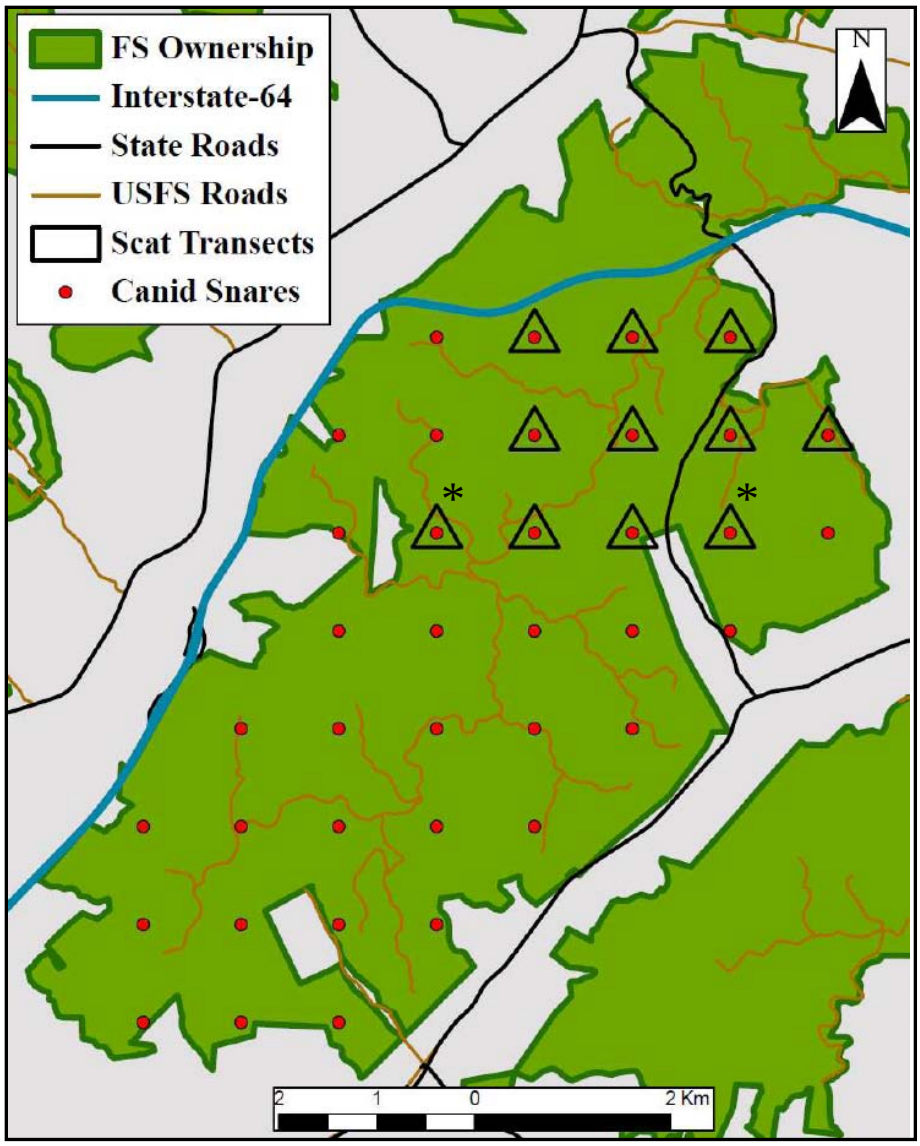

Figure 3.4: Scat detection dog transects fully and partially completed at the Big Perry study site of the Daniel Boone National Forest, Kentucky. A (*) indicates a partially completed transect.

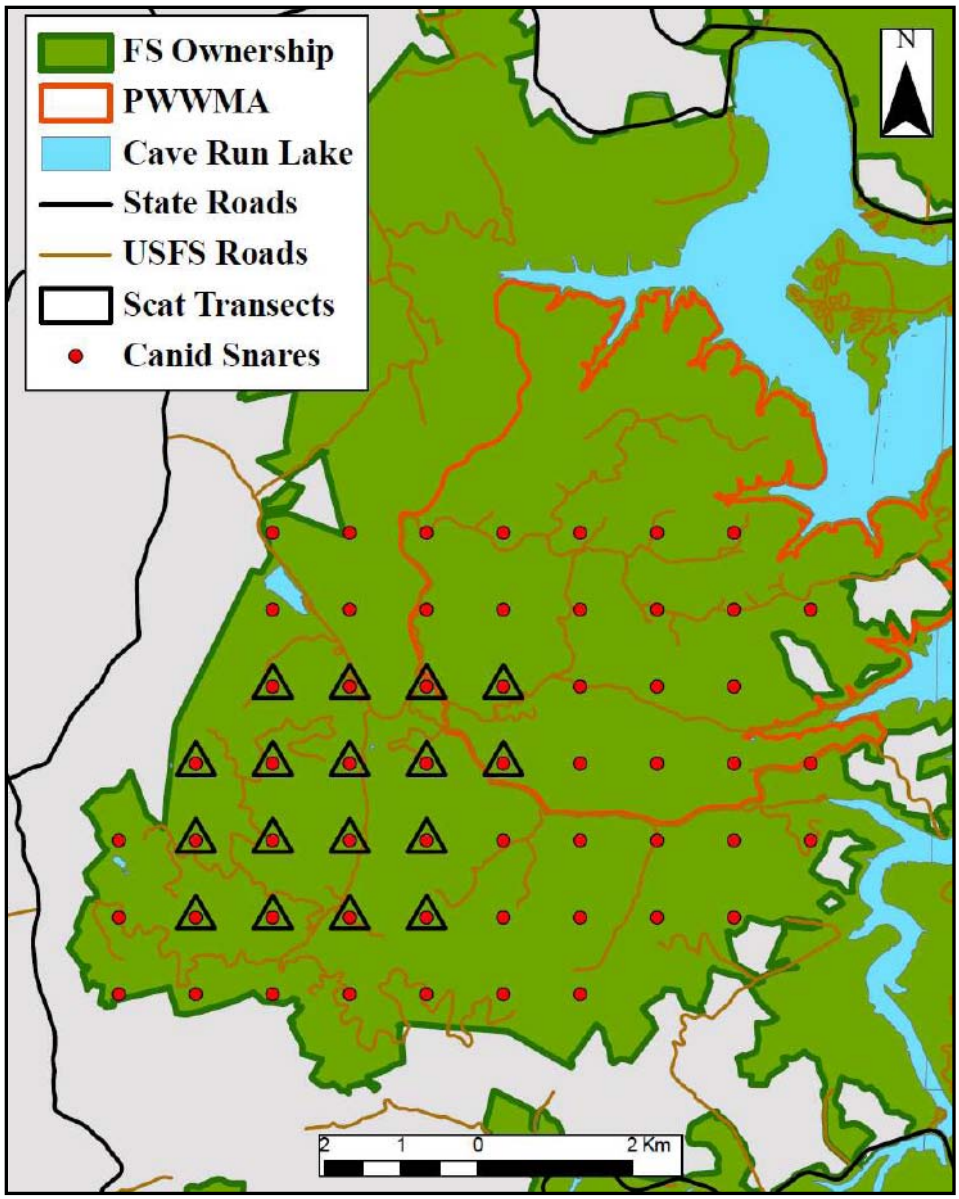

Figure 3.5: Scat detection dog transects completed at the Pioneer Weapons study site of the Daniel Boone National Forest, Kentucky.

Figure 3.6: Mesocarnivore hair snare consisting of a carpeted rub pad and nail design. The inset illustrates the copper connector wire attached to the nail that enhances hair capture from target animals.

Figure 3.7: Mesocarnivore rub pad and nail hair snare targeting felids.

Figure 3.8: Ground-based rub pad and nail hair snare targeting canids.

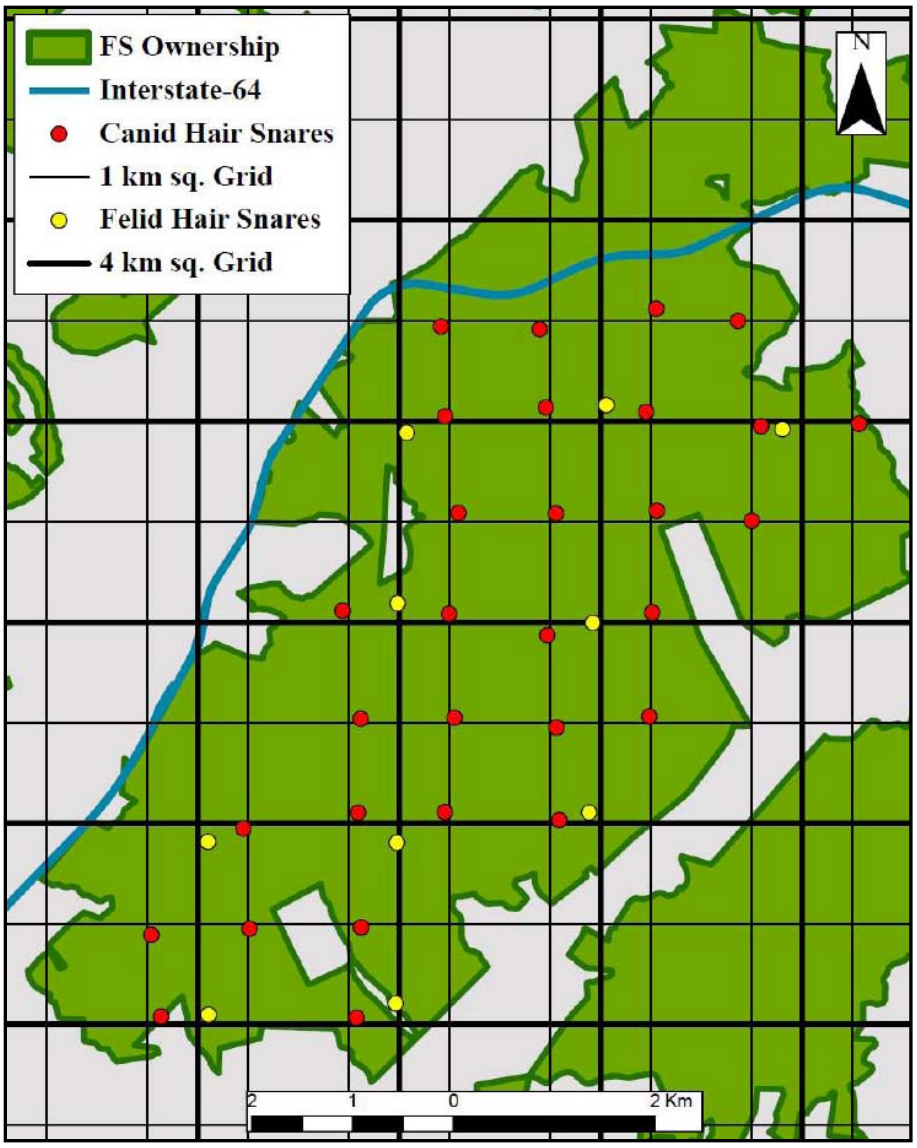

Figure 3.9: Locations of all felid and canid hair snares deployed at the Big Perry study site on the Daniel Boone National Forest, Kentucky.

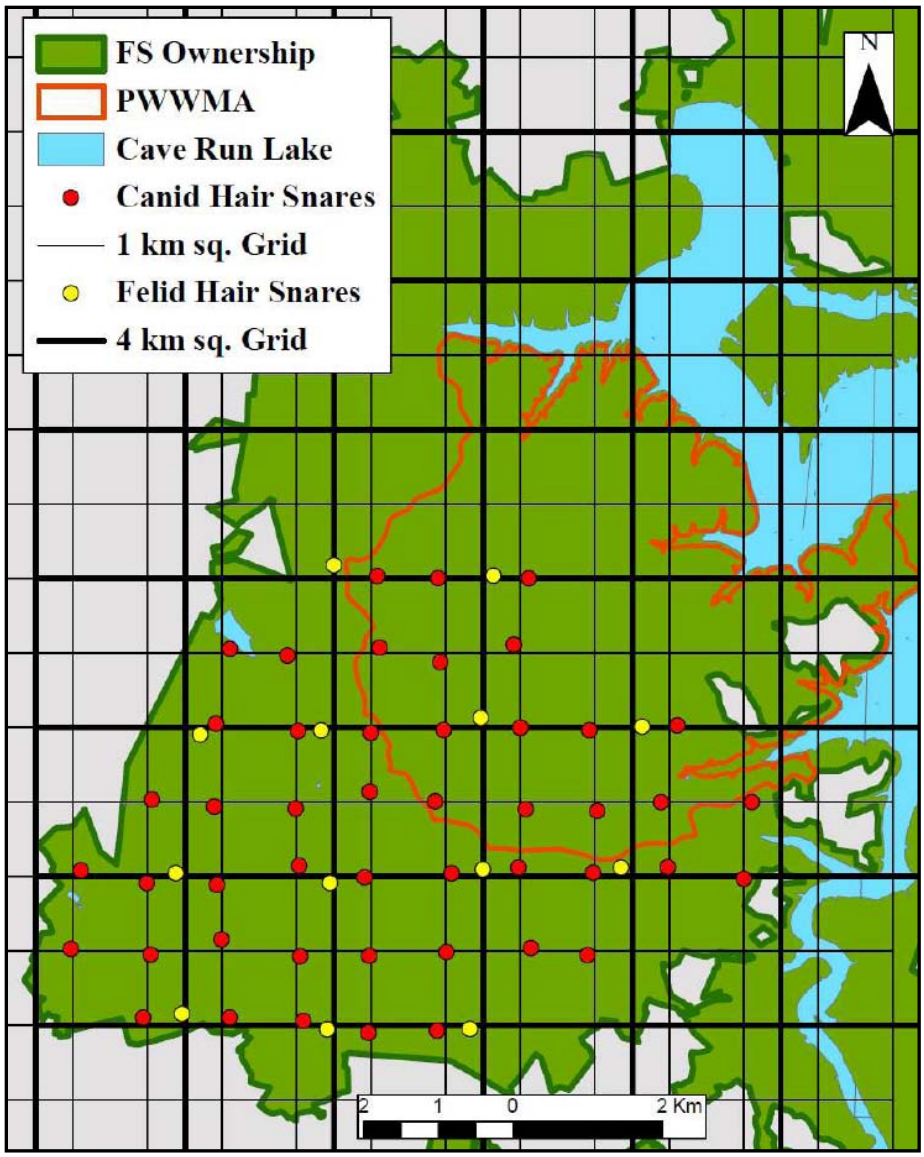

Figure 3.10: Locations of all felid and canid hair snares deployed at the Pioneer Weapons study site on the Daniel Boone National Forest, Kentucky.

CHAPTER 4: RESULTS and DISCUSSION

Results

Scat Detection Dogs

Detection dogs successfully located a total of 261 scats between both study sites. Detection rates varied between dogs, z = 2.079 (Table 4.2). Kasey located a total of 161 scats, of which 133 were identified to species at a 76.7% (102/133) target success rate. Nitro located a total of 100 scats, of which 60 were identified to species at a 63.3% (38/60) target success rate. Detection rates also varied between study sites, z = 3.164 (Table 4.3). At the Big Perry site a total of 89 scats were collected, Kasey and Nitro detected 67 and 22 scats, respectively. At the Pioneer Weapons site a total of 172 scats were collected, Kasey and Nitro detected 94 and 78 scats, respectively. To allow for direct comparison, the total number of scats per km was calculated by site and by detection dog (Table 4.4), At the Big Perry site, a total of 16.75 km was surveyed resulting in a detection rate of 5.13 scats/km. At the Pioneer Weapons site, a total of 25.5 km was surveyed resulting in a detection rate of 6.63 scats/km. Kasey surveyed 24.75 km of transect and detected a total of 6.51 scats/km. Nitro surveyed 17.5 km of transect and detected a total of 5.37 scats/km. Additionally, dry and wet samples were successfully identified at 79.2% and 71.6% respectively. However, a Chi-square test (data not shown) revealed the difference in identification success between dry and wet samples was not statistically significant at a 95% confidence level.

During 20 days of scat detection surveys a total of 98.5 hours were logged with an average effort of ~4.9 hrs/day. Scat survey effort was based on one observer, acting as

orienteer, because the handler was included in the detection dog contract and required to accompany dogs on all surveys. After accounting for collection materials, labor, and the detection dog contract, scat surveys resulted in a cost of $47/sample and $87/identified target sample (Table 4.1).

In addition, 7 scats were collected opportunistically without any indication from either dog. All 7 opportunistic scats were identified to species at a 71.4% (5/7) target success rate.

Hair Snares

During the 5 sampling occasions, a total of 7 hair samples were collected in 3500 trap nights, between both study sites; 2 hair samples were collected from one canid snare and one felid snare at Big Perry site in 1400 trap nights, and 5 hair samples were collected from 4 canid snares and one felid snare at the Pioneer Weapons in 2100 trap nights. No snare was hit more than once at both sites and an average of 1.7 hairs were collected per hit. The number of hairs per hit ranged from 1 to 4, with the majority of hits resulting in 1 hair.

All snares were deployed and checked over 30 working days within seven weeks. A total of 192.5 hours were logged disproportionately between 2 observers, with an average effort of ~6.4 hrs/day. Canid snare cost was ~ $0.94/snare and felid snare cost was ~ $0.52/snare. After accounting for all labor, materials, and transportation the hair snare surveys resulted in a cost of $397/sample (Table 4.1).

DNA Analysis

The portion of mtDNA cytochrome *b* gene was successfully amplified in 81.3% (218/268) of DNA extracted from samples. Restriction enzyme digest was performed on all 218 mtDNA amplification products resulting in unambiguous species identification for 111 (50.9%) samples. In addition, 72.5% (158/218) of mtDNA amplification products were sequenced resulting in unambiguous species identification for 76.6% (121/158) of sequenced mtDNA. To resolve ambiguous species identifications from the restriction enzyme method, 108 unidentified samples were sequenced. Sequencing resulted in species identification for 86 (79.6%) of these samples. To test the accuracy of restriction enzyme identifications, 35 samples identified as target species were sequenced resulting in 28 (80%) matching identifications.

Target and non-target species totals varied between study sites. Detection dogs located 60 target scats and 10 non-target scats at BP then 80 target scats and 44 non-target scats at PW. Target and non-target totals also varied between detection dogs (Figure 4.1). A total of 145 target samples were identified: bobcat (43), gray fox (29), and coyote (73). A total of 56 non-target samples were identified from six different species: raccoon, *Procyon lotor* (31), domestic dog, *Canis lupus familiaris* (15), striped skunk, *Mephitis mephitis* (6), domestic cattle, *Bos tarus* (2), domestic cat, *Felis silvestris catus* (1), and white-tailed deer, *Odocoileus virginianus* (1). A total of 17 samples remained unidentified after restriction enzyme and sequencing methods.

Individual Identification

Gray fox DNA microsatellite amplification was largely unsuccessful. All 29 gray fox samples were tested at 5 loci resulting in 10.3% amplification success. Ten gray fox samples were subsequently sent to the United States Forest Service (USFS) Rocky Mountain Research Station (RMRS) Wildlife Genetics Lab in Missoula, Montana to determine whether the low success rate was a result of poor DNA quality/quantity or suboptimal PCR conditions. The USFS RMRS Wildlife Genetics Lab successfully amplified and scored 30% of our gray fox samples and concluded that their limited amplification success was a product of low DNA quantity and/or poor DNA quality.

All 43 bobcat DNA samples were tested at ≤ 4 microsatellite loci. Based on the results, 16 samples were culled after failing to amplify at any locus. The remaining 27 bobcat samples were replicated 2 to 5 times at 4 loci, resulting in a consensus score for 66.7% (72/108) of possible samples/locus (Table 4.5). Observed allelic frequencies and heterozygosity rates for bobcat loci are listed in Table 4.6. Variation in consensus microsatellite scores prevented accurate individual identification among samples. Therefore, due to the incomplete dataset, an estimate of population size was not possible. Calculations of genotyping error rates using the program GIMLET were not possible for individual loci or samples due to an insufficient number of replicates for 56% of samples/locus.

Discussion

The two non-invasive mesocarnivore sampling methods I tested showed a clear difference in target species detection success. Scat detection dogs provided considerably more samples than hair snares within a smaller survey area. Additionally, scat dog surveys were conducted over a shorter timeframe and required less effort per day than hair snare surveys.

In general, the success of a hair snare survey is heavily dependent on the target species' behavior. Baited hair snares rely on a scent lure to induce a behavioral response or to guide animal movements across the snaring device (Kendall and McKelvey 2008). Hair snare surveys that use a lure to guide animal movements have proven quite successful for ursids (Boulanger et al. 2006; Dreher et al. 2007) and mustelids (Mowat and Paetkau 2002; Belant 2003; Zielinski et al. 2006; Mulders et al. 2007). However, the snare type tested by in research (i.e., rub pads) required a behavioral response to collect hair samples. I decided to test rub pads because they have been used successfully to sample each of my target species. While rub pads are typically used to survey felids (McDaniel et al. 2000; Shinn 2002; Weaver et al. 2005; Harrison 2006a; Downey et al. 2007; Ruell and Crooks 2007), significant by-catch samples have been collected from gray fox and coyote (Harrison 2006a; Downey et al. 2007; Ruell and Crooks 2007). Therefore, to sample my three target species simultaneously, rub pad hair snares provided a feasible option.

Lure type is critical to the success of rub pad surveys. The lure(s) must be capable of attracting a target species and inducing the appropriate behavioral response. I

chose lures which have proven effective at meeting these requirements for my target species based on previous research (Schlexer 2008) and their use in recreational fur trapping. In addition, I accounted for seasonal variation when choosing lures. Hair snare surveys were conducted during the breeding seasons of target species (Gese and Bekoff 2004; Fuller and Cypher 2004, Hansen 2007). Therefore, gland lures were used, in addition to beaver castoreum, to induce a behavioral response from canid target species. Because I surveyed areas which are open to public trapping of targeted species, and given the importance of lure type to rub pad hair snares, it is possible that sampling bias was responsible for the failure of my hair snares to adequately detect target species. Exploited species have been shown to exhibit trap avoidance and can be suspicious of human scents (Schlexer 2008). Hair snares used in this study were stored outdoors prior to the survey but human scents were inevitably introduced during deployment. Furthermore, most of my hair snare lures are frequently used by fur trappers. Future hair snare surveys targeting furbearers in Kentucky should consider testing a baited snare type that guides an animal's movements rather than attempting to elicit a behavioral response. However, these types of baited hair snares introduce a different form of sampling bias which must be accounted for (Kendall and McKelvey 2008).

Detection dogs can be trained to locate just about anything with a scent (Dr. Todd Steury, Ecodogs, pers. comm.) such as live animals (Reindl-Thompson et al. 2006; Nussear et al. 2008), carcasses (Homan et al. 2001; Paula et al 2011), den sites (Lydersen and Gjertz 1986), invasive plants (Goodwin et al. 2010), ungulate antler sheds (Dr. Todd Steury, Ecodogs, pers. comm.), root fungus infecting pine trees (Dr. Todd Steury, Ecodogs, pers. comm.), and scat from a variety of species (Beckmann 2006; Rolland et

al. 2006; Smith et al. 2006; Dematteo et al. 2009). Furthermore, scat detection dogs have consistently outperformed other survey methods in sampling yield and accuracy during direct comparison studies (Wasser et al. 2004; Harrison 2006b; Long et al. 2007b). This trend held true for my research as scat dogs greatly outperformed rub pad hair snares in total sampling yield. However, there were several inconsistencies related to detection rates per site and per dog that should be addressed.

The total number of scats collected differed between study sites (Table 4.3). Approximately 66% of scats located by detection dogs were collected from the second study site (PW). Furthermore, an additional 1.5 scats were collected per km at the PW site (Table 4.4). However, these figures are not likely to reflect a true difference in relative abundance between target or non-target species. While each study site was sampled for 10 days, 7 additional transects (which translated into an additional 8.75 km) were surveyed at the PW study site. Weather conditions can, in part, explain the number of transects surveyed per site. During the first week of sampling at BP, full and partial transects were surveyed despite snow cover of ≤ 3.8 cm. Detection dogs have been successful at locating source odors in arctic climates (Lydersen and Gjertz 1986) and both detection dogs were able to find scats under snow at BP. However, these adverse conditions occurred early in the study, when the dogs were acclimating to a new field environment. As a result, several transects were aborted due to either stress behavior exhibited by dogs or safety concerns.

The total number of scats detected and the percent target success from samples identified to species differed between detection dogs (Table 4.2). Both detection dogs were originally trained for explosives detection through the Animal Health and

Performance Program within the College of Veterinary Medicine, Auburn University (AU). Later, the dogs were transferred to the Ecodogs program at AU for wildlife detection work. Kasey received scat detection training for ~ 1 year and Nitro received scat detection training for < 1.5 months prior to my research. In addition, Nitro required odor reinforcement training during surveys at the BP site. The handler used aid placement and field imprinting techniques to build his confidence. As a result, the total number of scats located by Nitro increased ~ 3.5 fold at the PW site. Ecodogs now recommends detection dogs be trained for a minimum of 3 months before contract field work (Dr. Todd Steury, Ecodogs, pers. comm.).

Despite his brief training period and low scat total, Nitro achieved a relatively high target success rate at the BP site. However, target success decreased for both dogs at the second site. After discussing the detection data with Dr. Todd Steury and Lucas Epperson of Ecodogs, several conclusions, with implications to future detection dog research, were reached. Non-target detection frequencies for Kasey and Nitro suggest both dogs treated raccoon as target species. However, it is rare for two detection dogs to repeatedly mistake a non-target odor for a target odor during a given study (Lucas Epperson, Ecodogs, pers. comm.). Therefore, it is possible that one or more training aid scats I sent to Ecodogs for target imprinting were actually from raccoon given the source of some of this material came from local trappers and KDFWR. While many of these scats were extracted directly from recently harvested individuals, the rest were collected at trap sites. Given the scat morphology and dietary similarities between raccoon and gray fox, visual misidentification is conceivable (Chame 2003). Of the 7 scats I collected opportunistically during field surveys, two were later identified with molecular methods

as raccoon, thus supporting the possibility of field misidentification. This highlights the importance of accurately identifying training aids before using them to imprint detection dogs. Species identification methods, such as mtDNA sequencing should be strongly considered to ensure higher detection accuracy in subsequent field surveys and reduce the cost of non-target laboratory analyses. In addition, by accidentally imprinting a detection dog on non-target odors, the dog will be compromised for any future detection work.

Another possible explanation for the frequency of raccoon detections is the same explanation for the high number of coyote scats found by Kasey. Kasey was trained to detect gray fox and bobcat scats, but she located more scats from coyote than any other species. After reviewing the species identification data, it was revealed that the first scat located by Kasey, which was also the first scat of the study, was from coyote. We visually inspected this scat and agreed it was from gray fox before rewarding Kasey. Coyote scat length, diameter, and morphology can completely overlap those of gray fox and bobcat (Danner and Dodd 1982; Heinemeyer et al. 2008). Therefore, we believe by rewarding Kasey for coyote scat she was inadvertently trained to locate them, which she did with great efficiency. A clever, experienced dog, such as Kasey, can be rapidly imprinted on new odors (Lucas Epperson, Ecodogs, pers. comm.). Given the high abundance of coyote in my study areas, this new target odor provided Kasey with numerous opportunities for a reward, the underling driver for detection dogs. While this was not a problem for my research, as coyote was a target species, our misidentification has added coyote to Kasey's repertoire for any future studies.

The potential for visual misidentification brings up an important design consideration for detection dog research, especially for multiple species surveys. A priori

knowledge of both target and non-target species sympatric to a given study area is critical for scat detection dog accuracy. This knowledge will allow for strategic target odor training or the appropriate selection of detection dogs based on their repertoires. Training or selecting dogs which have been trained to locate scats with easily discernible morphologies can prevent visual misidentifications and ensure detection accuracy. However, for studies such as mine, where three target species and at least two non-target species (i.e., raccoon and striped skunk) all have similar scat morphologies, strategic training may be more of a challenge. While detection dogs have proven an extremely efficient sampling method, the development of an instantaneous in-field species identification assay for scat samples would nullify the limitations of strategic training and allow for the full potential of detection dogs to be realized.

Visual misidentification may have been responsible for reinforcing non-target detection by Kasey, but it does not explain why she originally indicated at the first coyote scat. Weather conditions during the first transect may have contributed to her non-target detection. Heavy precipitation was recorded the night before her first transect and moderate precipitation occurred during the survey. Rain events have been shown to decrease scat detectability (Hunter 2011; Reed 2011), however little research has been conducted on the potential for precipitation to alter scat odor. It is possible that rain events can degrade the species specific odors of scat leaving behind the scent of dietary components. Given the dietary similarity among gray fox, bobcat, and coyote (Fedriani et al. 2000; Neale and Sacks 2001) and the weather conditions during her survey, Kasey may have detected some dietary component associated with a target odor. Additionally, it is possible that this particular coyote scat contained the remains of a gray fox or bobcat

(Fedriani et al. 2000; Farias et al. 2005). This dietary issue related to degraded scats has occurred with detection dogs trained to locate black bear scat in Florida (Lucas Epperson, Ecodogs, pers. comm.). In this case, detection dogs were indicating at coyote scats which contained a large proportion of digested berries, a common component in black bear scats within the study area. However, because the scat morphologies of black bear and coyote are distinct, the dogs were not rewarded for these finds and therefore not inadvertently trained for non-target scats.

Scat degradation can account for the few unexpected non-target finds (i.e., cow and white-tailed deer) recorded by Kasey and Nitro. Detection dogs can locate degraded scats that have been exposed to field conditions for over 3 months (Hunter 2011). During my field surveys, Kasey and Nitro found many scats which were severely degraded and indicated at locations where no scats were recovered 39 and 34 times respectively. On these occasions, the leaf litter was searched thoroughly but scats were likely degraded beyond visual detection.

Molecular methods to identify species from noninvasively collected samples typically involve amplifying a short mtDNA fragment, then either digestion with restriction enzymes (Paxinos et al. 1997; Mills et al. 2000a; Bidlack et al. 2007) or direct sequencing (Farrell et al. 2000). While restriction enzyme digest is cheaper (~ $800 for this study) than direct sequencing (~ $1700 for this study), restriction enzyme digest patterns can be ambiguous, resulting in low species identification rates or the need for replication. Regardless of study-specific success rates, mtDNA analyses are favored over those of nuclear DNA to identify species (Schwartz and Monfort 2008). Mitochondrial DNA codes for proteins essential to cellular energy production and, therefore, is essential

to individual cell survival (Shadel and Clayton 1997). Because the role of mtDNA is critical to survival, mtDNA sequences remain conserved through low mutation rates. In addition to highly conserved sequences, mtDNA exhibits a significant degree of variation among species (Shadel and Clayton 1997). Furthermore, multiple copies of mtDNA (typically 10^3-10^4 in vertebrates) are present per somatic cell, compared to 1 diploid copy of nuclear DNA (Shadel and Clayton 1997). These characteristics make mtDNA ideal for species identification and its frequency per cell allows for easier amplification.

To identify species from scat samples, I amplified a short section of mtDNA using previously developed primers designed to amplify a region of the cytochrome *b* gene from 7 species (Bidlack et al. 2007). However, I found these primers were capable of amplifying the region of cytochrome *b* from species not listed in the Bidlack et al. (2007) protocol, such as domestic dog, domestic cat, domestic cattle, and white-tailed deer. Furthermore, amplicons from these unlisted species contained restriction sites specific to restriction enzymes used in Bidlack et al. (2007), resulting in digest patterns similar to those of listed species. In particular, I found domestic dog samples would cut in a pattern similar to bobcat under the first double-digest protocol and a pattern similar to red fox under the second digest protocol. If I had only used the Bidlack et al. (2007) species identification protocol, all domestic dog samples would have been misidentified.

While I used restriction enzymes and sequencing to identify species, my tests were designed to identify as many samples as possible and not to directly compare these methods. However, I compared which method successfully identified bobcat samples that were later confirmed by microsatellite amplification of ≥ 1 locus. Of the 43 samples identified as bobcat, 31 were both confirmed by microsatellites and sequenced (all

samples were subjected to the restriction enzyme protocol). Approximately 80% of these samples were accurately identified by sequencing. Therefore, sequencing provided a significantly more accurate method for bobcat species identification.

Many factors act to degrade DNA and pose problems for noninvasively collected samples which are freely exposed to environmental conditions (Taberlet et al. 1996; Taberlet and Luikart 1999; Schwartz and Monfort 2008). As a result, mtDNA amplification success from noninvasive samples varies widely in the literature, ranging from 8% to 100% (Broquet et al. 2007). Exposure to water has been identified as a major source of DNA degradation through the process of hydrolysis (Regnaut et al. 2006). To test the effects of water on my samples I recorded the condition of all scats at the time of collection as either dry or wet and compared this to total species identification success (including target and non-target identifications from both restriction enzyme and sequencing methods). In contrast to the literature; my results suggest there was not a statistically significant difference in species identification success between dry and wet samples.

Microsatellite amplification success can be affected by all of the same DNA degrading factors which influence mtDNA success. Furthermore, these factors can be enhanced by the low concentration of nuclear DNA present in noninvasive samples (Taberlet et al. 1996). DNA extracted from scat presents a unique challenge to microsatellite amplification in the form of PCR inhibitors such as bile salts and secondary compounds from digestion (Ball et al. 2007; Marrero et al. 2009). Sample dilution can counteract PCR inhibitors but it also decreases DNA concentration. Other factors, like microsatellite repeat motif and allele length have been shown to influence amplification

success (Broquet et al. 2007). Methods for noninvasive sample storage and DNA extraction vary and even contradict in the literature, resulting in random success rates (Schwartz and Monfort 2008). Microsatellite success rates range from 42% to 99.6% in the literature (Broquet et al. 2007) however, this range does not likely reflect the true variation in success as insufficient results are rarely published. In addition, the influence of environmental exposure is difficult to quantify for noninvasive samples. Consequently, optimal protocols for sample storage, extraction, and amplification vary, with little standardization among studies, making direct comparisons difficult. For these reasons, identifying the precise cause(s) for low microsatellite amplification success can be extremely challenging.

Based on the gray fox microsatellite results from the USFS RMRS Wildlife Genetics Lab and my relative success with bobcat sample amplification, I believe my PCR protocol was near optimal. To account for PCR inhibitors, I tested several template dilutions for each bobcat sample. Amplification success varied among dilutions, but remained relatively consistent among samples (data not shown). For example, sample *A* amplified at ≤ 4 loci using either 1:2 or 1:5 template dilution and sample *B* amplified at ≤ 4 loci using an undiluted template. This provides evidence for the presence of PCR inhibitors at varying concentrations among bobcat samples. Microsatellite allele length for my bobcat samples ranged from 131 to 171 bases among loci (Table 4.6). A review of amplification success suggests shorter alleles (100-200 b) are more likely to amplify (Broquet et al. 2007).

After accounting for the laboratory methods and microsatellite characteristics presented above, I believe my inability to generate an adequate number of consensus

scores for estimating population size was primarily a result of poor DNA quality from environmental exposure. Historical precipitation data from the Kentucky Mesonet weather station in Morehead, Kentucky recorded 10 days of rain totaling 11.71 cm during my scat surveys (kymesonet.org). However, scat detection dogs have been shown to locate scats > 3 months old (Hunter 2011). Therefore, historical data from the 3 month period prior to my surveys recorded 50 days of precipitation totaling 22.17 cm. Temperature and temperature change also act to degrade DNA. Specifically, ≥ 4 freeze-thaw cycles has been shown to degrade DNA and affect amplification success (Lahiri and Schnabel 1993). During my surveys, 12 freeze-thaw cycles were recorded by the Kentucky Mesonet weather station and 54 cycles during the 3 months prior. Future noninvasive genetic surveys in Kentucky utilizing scat as a DNA source should consider the number of precipitation events and freeze-thaw cycles during the sampling timeframe.

Study design can be used to minimize the potential for analyzing low quality DNA samples. By clearing transects of target scats prior to sampling via a CMR design, scats collected during each occasion are much more likely to be fresh and therefore contain higher quality DNA. If a general search design is preferred, such as that of Kohn et al. (1999), scats can be culled based on age. However, certain factors should be considered when a general search design is employed. The species of interest should be relatively abundant within the study area to allow for an adequate number of samples post cull. In addition, scat age can be difficult to quantify due to a variety of environmental factors (Tsaparis et al. 2009; Hunter 2011). Therefore, local scat degradation rates should be assessed and classified for the species of interest to guide study sample culling.

Finally, obtaining a cost-effective, consistent, and timely final product are important goals of any molecular analyses. As I, a rookie molecular biologist, and others have discovered, working with microsatellite DNA poses a suite of unique challenges often not encountered using other types of DNA. Lab experience with microsatellites therefore is one of the most important factors in obtaining successful laboratory results. Although typically more expensive on the front end than in-house DNA processing, professional wildlife genetics laboratories like the USFS RMRS Wildlife Genetics Lab in Missoula, MT and Wildlife Genetics International in Nelson, BC have considerable experience and optimized laboratory protocols specifically for analyzing noninvasively collected samples, and therefore are likely to produce a more consistent product. Given these established molecular laboratories, wildlife researchers that seldom have the need for frequent processing of large quantities of DNA may be better off outsourcing this type of molecular work unless there is an economic or scientific need or desire to establish a noninvasive genetics laboratory.

Current methods of furbearer management in Kentucky are common among state agencies in North America and appear to be adequate for predicting harvest rates to establish bag limits and season timing/duration (Wolfe and Chapman 1987; Roberts and Crimmins 2010). Furthermore, wildlife management agencies set these management parameters conservatively to account for errors inevitable to anecdotal data sources. Furbearers such as gray fox, bobcat, and especially coyote are resilient and their populations can typically rebound from regulated harvest (Palomares et al. 1995; Henke and Bryant 1999). However, population trends based on harvest rates are subject to various sources of bias (Gese 2001). To correlate harvest rates to actual population trends

and to establish the causes for these trends, methods which offer greater accuracy and precision are required. My research provides a field test of methods which are capable of surveying multiple furbearer species simultaneously and, under the appropriate study design, can estimate population abundance. In addition, these methods can be repeated over time for population monitoring and provide fundamental data for establishing the causes of population trends.

Table 4.1: Labor and cost analysis between detection dog and hair snare survey methods.

Time	Detection Dogs	Hair Snares
Working Days	20	30
Total Hours	98.5	192.5
Hours/Day	4.9	6.4
Cost		
Labor [a]	739	1,669 [b]
Materials	383	651
Transportation	0	459
Contract	11,100 [c]	0
Total Cost	12,222	2,779
Cost/Sample (target)	87	N/A
Cost/Sample (total)	47	397
Samples/Dollar (total)	0.02	0.003

[a]: Fixed labor cost of $7.50/hr

[b]: Includes snare construction labor time (30 hrs)

[c]: A flat fee of $500 was charged for training both dogs to locate gray fox and bobcat scats. A special training fee of $2,300 was charged to train Nitro for coyote scats.

Table 4.2: Number of scats detected by dogs during 20 sampling days in the northeastern DBNF, Kentucky, 2011.

Dog	Total scats	Species ID [a]	Target [b]	Non-target [c]	% Success [d] *
Kasey	161	133	102	31	77
Nitro	100	60	38	22	63

[a]: Number of scats successfully identified to species by mtDNA analysis
[b]: Total number of scats identified as target species
[c]: Total number of scats identified as non-target species
[d]: Percent success for target species scat detections
*: Statistically significant difference ($p < 0.05$)

Table 4.3: Detection rate totals between detection dogs and study sites during 20 sampling days in the northeastern DBNF, Kentucky, 2011.

Site	Dog	Total Scats	Target [a]	Non-target [b]	% Success [c] *
BP	Kasey	67	47 [d]	7	87
	Nitro	22	13	3	81
	Total	89	60	10	86
PW	Kasey	94	55 [d]	24	70
	Nitro	78	25	20	56
	Total	172	80	44	65

[a]: Total number of scats identified as target species
[b]: Total number of scats identified as non-target species
[c]: Percent success for target species scat detections
[d]: Includes coyote samples
*: Statistically significant difference between site totals ($p < 0.05$)

Table 4.4: Scats located per km between detection dogs and study sites.

Site	Kasey	Nitro	Total [a]
BP	5.96	3.45	5.13
PW	6.96	6.25	6.63
Total [b]	6.51	5.37	

[a]: Total scats/km by study site

[b]: Total scats/km by detection dog

Table 4.5: Consensus bobcat microsatellite scores generated by the program GIMLET from DNA extracted from scat samples collected in northeastern Kentucky, 2011.

Site	Sample	Microsatellite Locus							
		FCA-26		FCA-45		FCA-43		FCA-57	
BP	BP15	144	146	0		0		146	154
	BP51	146	150	0		131	133	144	146
	BP57	144		0		133	135	146	
	BP60	0		165	167	0		144	146
	BP67	144	150	163	165	0		0	
	BP69	0		163	165	0		0	
	BP88	142	144	0		0		144	146
PW	PW18	142	146	165		131	135	142	146
	PW21	0		0		131	135	0	
	PW22	0		163	165	0		0	
	PW26	142	150	165		133	135	144	146
	PW27	0		165	171	0		0	
	PW34	146	148	165	167	135		146	
	PW48	0		167		133	135	144	146
	PW55	144		165		0		142	146
	PW62	146	150	165	167	133	135	146	
	PW75	146	148	165	167	135		146	
	PW78	0		165	167	0		144	146
	PW80	144	146	0		135		146	
	PW85	144	146	165	167	135		146	148
	PW91	0		165	167	133	135	144	146
	PW124	146	150	163	165	135		146	
	PW141	0		165	167	133	135	142	146
	PW146	0		165	167	0		144	146
	PW160	144	146	0		0		146	
	PW165	0		0		133	135	0	
	PW171	148		165		133		142	146

0: No consensus microsatellite score

Table 4.6: Bobcat microsatellite allele rates and frequencies from consensus scores.

Locus	Htz [a]	Hmz [b]	Allele [c]	Frequency [d]
FCA 26	0.81	0.19	142	0.107
			144	0.286
			146	0.357
			148	0.107
			150	0.179
FCA 45	0.74	0.26	163	0.121
			165	0.545
			167	0.303
			171	0.030
FCA 43	0.63	0.38	131	0.115
			133	0.346
			135	0.538
FCA 57	0.67	0.33	142	0.114
			144	0.229
			146	0.600
			148	0.029
			154	0.029

[a]: Heterozygosity rate
[b]: Homozygosity rate
[c]: Allele score in bases
[d]: Observed allele frequencies

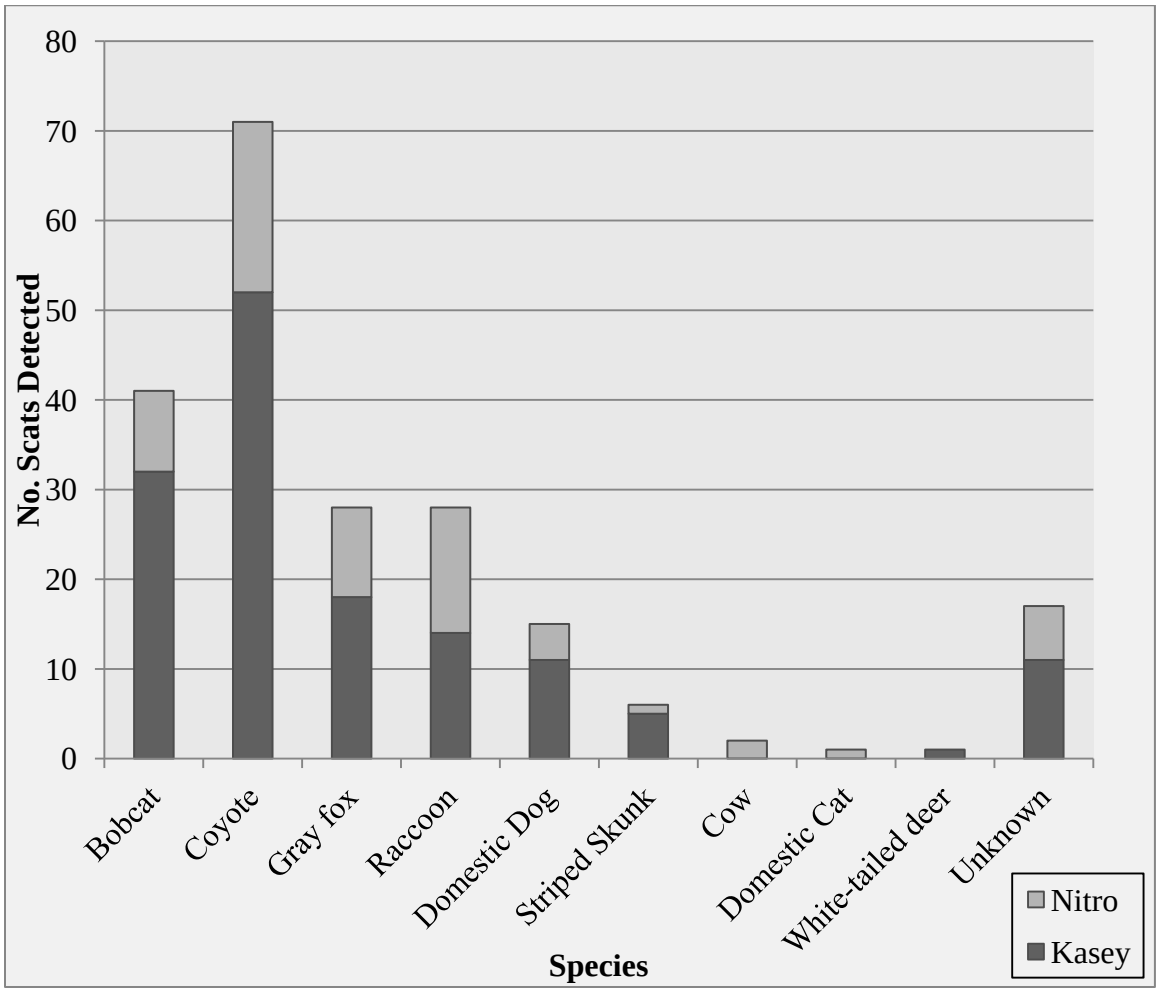

Figure 4.1: Total number of species identified scats detected by Kasey and Nitro during 20 sampling days in the northeastern DBNF, Kentucky, 2011.

CHAPTER 5: MANAGEMENT RECOMMENDATIONS

Noninvasive genetic methods provide a powerful tool for wildlife managers to estimate abundance and monitor population trends. By adopting microsatellite techniques for individual identification, population estimates and monitoring, wildlife agencies can construct genetic databases that are useful for assessment or long-term monitoring of population parameters beyond those of traditional field sampling so as to provide a genetic basis for management. However, noninvasive field surveys must be consistently implemented and can be problematic due to site and species-specific variability, and molecular identification success that can be influenced by a variety of environmental and laboratory factors. A localized pilot study is essential to assess the feasibility of individual methods and design a field study which can accurately guide management decisions. My research will hopefully serve as an informative pilot study for KDFWR in their decision to incorporate noninvasive genetic methods in their wildlife monitoring programs.

Prior experience with molecular identification techniques from noninvasively collected samples can significantly increase genotyping accuracy, particularly when working with microsatellite DNA that can prove problematic. The decision to conduct in-house processing of noninvasive sources of DNA from biological samples should be carefully evaluated in the context of in-house lab experience, cost, product quality and consistency, and time to end product. While professional DNA processing labs may be more expensive on a per sample basis, the cost may be justified depending on the goals of the project, and costs can be reduced by identifying samples to species using molecular methods in order to submit the minimum number of samples for professional analysis.

Scat from wildlife offers a diversity of biological data relevant to management. This invaluable data source can provide basic assessments such as diet, space use, and distribution or advanced analyses of abundance, demography, stress, and genetic diversity. However, traditional methods of scat collection are subject to sampling bias and often result in low yield. I recommend the use of detection dogs for effectively acquiring scat to meet any of the objectives listed above for many terrestrial mammal species in Kentucky. In addition, if molecular identification is required I recommend developing a survey design, according to study objectives, which culls degraded scat samples.

Despite its efficiency, contract detection dog work is expensive in relation to other noninvasive survey methods. This cost is compounded for multiple-species surveys and regular assessments to establish population trends. If the state intends to regularly utilize detection dogs for population monitoring, I recommend strategically training two or more rescue dogs using, in part, the techniques described by Smith et al. (2003). Detection dogs can be trained for multiple species in 3-5 months although each dog is unique and the training timeframe can vary. Careful consideration should be given with regards to dog and handler/trainer selection, as these components are critical to survey success.

LITERATURE CITED

Allaine, D., D. Pontier, J. M. Gaillard, J. D. Lebreton, J. Trouvilliez, and J. Colbert. 1987. The relationship between fecundity and adult body weight in homeotherms. Oecologia 73:478-480.

Arif, I. A. and H. A. Khan. 2009. Molecular markers for biodiversity analysis of wildlife animals: a brief review. Animal Biodiversity and Conservation 32:9-17.

Babb, J. G. and M. L. Kennedy. 1989. An estimate of minimum density for coyotes in western Tennessee. Journal of Wildlife Management 53:186-188.

Bailey, T. N. 1974. Social organization in a bobcat population. Journal of Wildlife Management 38:435-446.

Balestrieri, A., L. Remonti, A. C. Frantz, E. Capelli, M. Zenato, E. E. Dettori, F. Guidali, and C. Prigioni. 2010. Efficacy of passive hair-traps for the genetic sampling of a low-density badger population. Hystrix It. J. Mamm. 21:137-146.

Ball, M. C., R. Pither, M. Manseau, J. Clark, S. D. Petersen, S. Kingston, N. Morrill, and P. Wilson. 2007. Characterization of target nuclear DNA from faeces reduces technical issues associated with the assumptions of low-quality and quantity template. Conservation Genetics 8:577-586.

Barton, B. T. 2005. Cascading effects of predator removal on the ecology of sea turtle nesting beaches. Thesis, University of Central Florida, Orlando, Florida.

Beckmann, J. P. 2006. Carnivore conservation and search dogs: the value of a novel, non-invasive technique in the Greater Yellowstone Ecosystem. Pages 20-26 in A. W. Biel (ed.). Greater yellowstone public lands: a century of discovery, hard lessons, and bright prospects. 8th Biennial Scientific Conf. on the Greater Yellowstone Ecosystem.

Beier, L. R., S. B. Lewis, R. W. Flynn, G. Pendleton, and T. V. Schumacher. 2005. A single-catch snare to collect brown bear hair for genetic mark-recapture studies. Wildlife Society Bulletin 33:766-773.

Belant, J. L. 2003. A hairsnare for forest carnivores. Wildlife Society Bulletin 31:482-485.

Berger, K. M., E. M. Gese, and J. Berger. 2008. Indirect effects and traditional trophic cascades: a test involving wolves, coyotes, and pronghorn. Ecology 89:818-828.

Bhagavatula, J. and L. Singh. 2006. Genotyping faecal samples of Bengal tiger (*Pathera tigris tigris*) for population estimation: a pilot study. BMC Genetics 7:48.

Bidlack, A. L., S. E. Reed, P. J. Palsboll, and W. M. Getz. 2007. Characterization of a western North American carnivore community using PCR-RFLP of cytochrome *b* obtained from fecal samples. Conservation Genetics 8:1511-1513.

Bininda-Emonds, O. R. P., J. L. Gittleman, and A. Purvis. 1999. Building large trees by combining Phylogenetic information: a complete phylogeny of the extant Carnivora (Mammalia). Biol. Rev. 74:143-175.

Boulanger, J. M., K. C. Kendall, J. B. Stetz, D. A. Roon, L. P. Waits, and D. Paetkau. 2008. Multiple data sources improve DNA-based mark-recapture population estimates of grizzly bears. Ecological Applications 18:577-589.

Boulanger, J., M. Proctor, S. Himmer, G. Stenhouse, D. Paetkau, and J. Cranston. 2006. An empirical test of DNA mark-recapture sampling strategies for grizzly bears. Ursus 17:149-158.

Boyko, A. R. 2011. The domestic dog: man's best friend in the genomic era. Genome Biology 12:216-226.

Brashares, J. S., L. R. Prugh, C. J. Stoner, and C. W. Epps. 2010. Ecological and conservation implications of mesopredator release *in* J. Terborgh and J. A. Estes (eds.). Trophic cascades. Island Press, Washington DC, USA.

Braun, E. L. 1950. Deciduous forests of eastern North America. McGraw-Hill Book Company, New York, New York.

Bremner-Harrison, S., S. W. R. Harrison, B. L. Cypher, J. D. Murdoch, J. Maldonado, and S. K. Darden. 2006. Development of a single-sample noninvasive hair snare. Wildlife Society Bulletin 34:456-461.

Broquet, T., N. Menard, and E. Petit. 2007. Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. Conservation Genetics 8:249-260.

Browne, C., K. Stafford, and R. Fordham. 2006. The use of scent-detection dogs. Irish Veterinary Journal 59:97-104.

Bullard, R. W., F. J. Turkowski, and S. R. Kilburn. 1983. Responses of free-ranging coyotes to lures and their modifications. Journal of Chemical Ecology 9:877-888.

Buskirk, S. W. 1999. Mesocarnivores of yellowstone. Pages 167-187 *in* Clark, T. W., Peyton Curlee, A., Minta, S. C., and Kareiva, P. M. eds. Carnivores in ecosystems: the yellowstone experience. Yale University Press, London.

Cablk, M. E., J. C. Sagebiel, J. S. Heaton, and C. Valentin. 2008. Olfaction-based detection distance: a quantitative analysis of how far away dogs recognize tortoise odor and follow in to source. Sensors 8:2208-2222.

Carbone, C., A. Teacher, and J. M. Rowcliffe. 2007. The costs of carnivory. PLoS Biology 5:363-368.

Carbone, C., G. Cowlishaw, N. J. B. Isaac, and J. M. Rowcliffe. 2005. How far do animals go? Determinants of day range in mammals. The American Naturalist 165:290-297.

Carbone, C., G. M. Mace, S. C. Roberts, and D. W. Macdonald. 1999. Energetic constraints on the diet of terrestrial carnivores. Nature 402:286-288.

Carlton, J. T. and J. Hodder. 2003. Maritime mammals: terrestrial mammals as consumers in marine intertidal communities. Mar. Ecol. Prog. Ser. 256:271-286.

Case, T. J. and M. E. Gilpin. 1974. Interference competition and niche theory. Proc. Nat. Acad. Sci. 71:3073-3077.

Chamberlain, M. J. and B. D. Leopold. 2005. Overlap in space use among bobcats (*Lynx rufus*), coyotes (*Canis latrans*), and gray foxes (*Urocyon cinereoargenteus*). American Midland Naturalist 153:171-179.

Chame, M. 2003. Terrestrial mammal feces: a morphometric summary and description. Mem. Inst. Oswaldo Cruz 98:71-94.

Clement, J. L., R. Hagege, A. Le Pareux, J. Connet, and G. Gastaldi. 1981. New concepts about hair identification revealed by electron microscope studies. Journal of Forensic Sciences 26:447-458.

Cochran, W. W. and R. D. Lord Jr. 1963. A radio-tracking system for wild animals. Journal of Wildlife Management 27:9-24.

Connelly, J. W., J. H. Gammonley, and J. M. Peek. 2005. Harvest management. Pages 658-690 *in* Braun, C. E. (ed.). Techniques for wildlife investigations and management 6th edition. The Wildlife Society, Baltimore, Maryland, USA.

Conner, M. C., R. F. Labisky, and D. R. Progulske Jr. 1983. Scent-station indices as measures of population abundance for bobcats, raccoons, and gray foxes. Wildlife Society Bulletin 11:146-152.

Constible, J. M., M. J. Chamberlain, and B. D. Leopold. 2006. Relationships between landscape pattern and space use of three mammalian carnivores in central Mississippi. American Midland Naturalist 155:352-362.

Croll, D. A., J. L. Maron, J. A. Estes, E. M. Danner, and G. V. Byrd. 2005. Introduced predators transform subarctic islands form grassland to tundra. Science 307:1959-1961.

Crooks, K. R. 2002. Relative sensitivities of mammalian carnivores to habitat fragments. Conservation Biology 16:488-502.

Crooks, K. R. and M. E. Soule. 1999. Mesopredator release and avifaunal extinctions in a fragmented system. Nature 400:563-566.

Cunningham, S. C., L. Kirkendall, and W. Ballard. 2006. Gray fox and coyote abundance and diet responses after a wildfire in central Arizona. Western North American Naturalist 66:169-180.

Currie, D. J. 1993. What shape is the relationship between body size and population density? Oikos 66:353-358.

Cutler, T. L. and D. E. Swann. 1999. Using remote photography in wildlife ecology: a review. Wildlife Society Bulletin 27:571-581.

Cypher, B.L., T. K. Fuller and R. List. 2008. Urocyon cinereoargenteus *in* IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. <www.iucnredlist.org>. Downloaded on 20 May 2012.

Danner, D. A. and N. Dodd. 1982. Comparison of coyote and gray fox scat diameters. Journal of Wildlife Management 46:240-241.

Dematteo, K. E., M. A. Rinas, M. M. Sede, B. Davenport, C. F. Arguelles, K. Lovett, and P. G. Parker. 2009. Detection dogs: an effective technique for bush dog surveys. Journal of Wildlife Management 73:1436-1440.

De Oliveira, M. L., D. Norris, J. F. M. Ramirez, P. H. de F. Peres, M. Galetti, and J. M.B. Duarte. 2012. Dogs can detect scat samples more efficiently than humans: an experiment in a continuous atlantic forest remnant. Zoologia 29:183-186.

DePue, J. E. and M. Ben-David. 2007. Hair sampling techniques for river otters. Journal of Wildlife Management 71:671-674.

Downey, P. J., E. C. Hellgren, A. Caso, S. Carvajal, and K. Frangioso. 2007. Hair snares for noninvasive sampling of felids in North America: do gray foxes affect success? Journal of Wildlife Management 71:2090-2094.

Dreher, B. P., S. R. Winterstein, K. T. Scribner, P. M. Lukacs, D. R. Etter, G. J. M. Rosa, V. A. Lopez, S. Libants, and K. B. Filcek. 2007. Noninvasive estimation of black bear abundance incorporating genotyping errors and harvested bears. Journal of Wildlife Management 71:2684-2693.

Drummond, A. J., B. Ashton, S. Buxton, M. Cheung, A. Cooper, J. Heled, M. Kearse, R. Moir, S. Stones-Havas, S. Sturrock, T. Thierer, and A. Wilson. 2010. Geneious v5.5.6. Available from http://www.geneious.com.

Duggan, J. M., E. J. Heske, R. L. Schooley, A. Hurt, and A. Whitelaw. 2011. Comparing detection dogs and livetrapping surveys for a cryptic rodent. Journal of Wildlife Management 75:1209-1217.

Eastridge, R. 2000. Experimental repatriation of black bear to the Big South Fork area of Kentucky and Tennessee. Thesis, University of Tennessee, Knoxville, Tennessee.

Elmhagen, B. and S. P. Rushton. 2007. Trophic control of mesopredators in terrestrial ecosystems: top-down or bottom-up? Ecology Letters 10:197-206.

Farias, V., T. K. Fuller, R. K. Wayne, and R. M. Sauvajot. 2005. Survival and cause-specific mortality of gray foxes (*Urocyon cinereoargenteus*) in southern California. J. Zool., London 266:249-254.

Farrell, L. E., J. Roman, and M. E. Sunquist. 2000. Dietary separation of sympatric carnivores identified by molecular analysis of scats. Molecular Ecology 9:1583-1590.

Fedriani, J. M., T. K. Fuller, R. M. Sauvajot, and E. C. York. 2000. Competition and intraguild predation among three sympatric carnivores. Oecologia 125:258-270.

Fernando, P., M. E. Pfrender, S. E. Encalada, and R. Lande. 2000. Mitochondrial DNA variation, phylogeography and population structure of the Asian elephant. Heredity 84:362-372.

Fisher, J. T. 2004. Alberta wolverine experimental monitoring project: 2003-2004 annual report. Alberta Research Council Inc., Vegreville, Alberta, Canada.

Foley, C. A. H., S. Papageorge, and S. K. Wasser. 2001. Noninvasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants. Conservation Biology 15:1134-1142.

Follmann, E. H. 1973. Comparative ecology and behavior of red and gray foxes. Dissertation, Southern Illinois University, Carbondale, Illinois, USA.

Foresman, K. R. and D. E. Pearson. 1998. Comparison of proposed survey procedures for detection of forest carnivores. Journal of Wildlife Management 62:1217-1226.

Frantz, A. C., M. Schaul, L. C. Pope, F. Fack, L. Schley, C. P. Muller, and T. J. Roper. 2004. Estimating population size by genotyping remotely plucked hair: the Eurasian badger. Journal of Applied Ecology 41:985-995.

Fritzell, E. K. and K. J. Haroldson. 1982. *Urocyon cinereoargenteus*. American Society of Mammalogists 189:1-8.

Fuller, M. R., J. J. Millspaugh, K. E. Church, and R. E. Kenward. 2005. Wildlife radiotelemetry. Pages 377-417 *in* Braun, C. E. (ed.). Techniques for wildlife investigations and management 6th edition. The Wildlife Society, Baltimore, Maryland, USA.

Fuller, T. K. and B. L. Cypher. 2004. Gray fox. Pages 92-97 *in* C. Sillero-Zubiri, M. Hoffman, and D. W. Macdonald (eds.). Canids: foxes, wolves, jackals, and dogs. status survey and conservation action plan. IUCN/SSC Canid Specialist Group. Gland, Switzerland and Cambridge, UK.

Graphodatsky, A. S., P. L. Perelman, N. V. Sokolovskaya, V. R. Beklemisheva, N. A. Serdukova, G. Dobigny, S. J. O'Brien, M. A. Ferguson-Smith, and F. Yang. 2008. Phylogenomics of the dog and fox family (Canidae, Carnivora) revealed by chromosome painting. Chromosome Research 16:129-143.

Gese, E. M. 2001. Monitoring of terrestrial carnivore populations. Pages 372-396 *in* Gittleman, J. L., S. M. Funk, D. W. Macdonald, and R. K. Wayne (eds.). Carnivore conservation. Cambridge University Press, UK.

Gese, E. M. and M. Bekoff. 2004. Coyote. Pages 81-87 *in* C. Sillero-Zubiri, M. Hoffman, and D. W. Macdonald (eds.). Canids: foxes, wolves, jackals, and dogs. status survey and conservation action plan. IUCN/SSC Canid Specialist Group. Gland, Switzerland and Cambridge, UK.

Gese, E.M., M. Bekoff, W. Andelt, L. Carbyn, and F. Knowlton. 2008. Canis latrans *in* IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. <www.iucnredlist.org>. Downloaded on 20 May 2012.

Gese, E. M., O. J. Rongstad, and W. R. Mytton. 1989. Population dynamics of coyotes in southeastern Colorado. Journal of Wildlife Management 53:174-181.

Gese, E. M. and S. Grothe. 1995. Analysis of coyote predation on deer and elk during winter in Yellowstone National Park, Wyoming. American Midland Naturalist 133:36-43.

Gittleman, J. L. and A. Purvis. 1998. Body size and species-richness in carnivores and primates. Proc. R. Soc. Lond. B 265:113-119.

Gobush, K. S., B. M. Mutayoba, and S. K. Wasser. 2008. Long-term impacts of poaching on relatedness, stress physiology, and reproductive output of adult female African elephants. Conservation Biology 22:1590-1599.

Gompper, M. E. 2002. Top carnivores in the suburbs? Ecological and conservation issues raised by colonization of north-eastern North America by coyotes. BioScience 52:185-190.

Gompper, M. E., R. W. Kays, J. C. Ray, S. D. Lapoint, D. A. Bogan, and J. R. Cryan. 2006. A comparison of noninvasive techniques to survey carnivore communities in northeastern North America. Wildlife Society Bulletin 34:1142-1151.

Goodwin, K. M., R. E. Engel, and D. K. Weaver. 2010. Trained dogs outperform human surveyors in the detection of rare spotted knapweed (*Centaurea stoebe*). Invasive Plant Science and Management 3:113-121.

Green, G. I. and D. J. Mattson. 2003. Tree rubbing by Yellostone grizzly bears *Ursus arctos*. Wildlife Biology 9:1-9.

Guichoux, E., L. Lagache, S. Wagner, P. Chaumeil, P. Leger, O. Lepais, C. Lepoittevin, T. Malausa, E. Revardel, F. Salin, and R. J. Petit. 2011. Current trends in microsatellite genotyping. Molecular Ecology Resources 11:591-611.

Hackett IV, H. M. 2008. Occupancy modeling of forest carnivores in Missouri. Dissertation, University of Missouri, Columbia, Missouri.

Hairston, N.G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure, population control, and competition. The American Naturalist. 94:421-425.

Hansen, K. 2007. Bobcat: master of survival. Oxford University Press, New York, New York, USA.

Harrison, R. L. 2006a. A comparison of survey methods for detecting bobcats. Wildlife Society Bulletin 34:548-552.

Harrison, R. L. 2006b. A comparison of detector dogs, hair snares, cameras, and scent stations for detection of bobcats. CAT News 44:20-22.

Hast, J. T. 2010.Genetic diversity, structure, and recolonization patterns of Kentucky black bears. Thesis, University of Kentucky, Lexington, Kentucky.

Hayward, L. S., A. E. Bowles, J. C. Ha, and S. K. Wasser. 2011. Impacts of acute and long-term vehicle exposure on physiology and reproductive success of the northern spotted owl. Ecosphere 2:1-20.

Heilbrun, R. D., N. J. Silvy, M. E. Tewes, and M. J. Peterson. 2003. Using automatically triggered cameras to individually identify bobcats. Wildlife Society Bulletin 31:748-755.

Heilbrun, R. D., N. J. Silvy, M. J. Peterson, and M. E. Tewes. 2006. Estimating bobcat abundance using automatically triggered cameras. Wildlife Society Bulletin 34:69-73.

Heinemeyer, K. S., T. J. Ulizio, and R. L. Harrison. 2008. Natural sign: tracks and scats. Pages 45-74 *in* Long, R. A., P. MacKay, W. J. Zielinski, and J. C. Ray (eds.). Noninvasive survey methods for carnivores. Island Press, Washington DC, USA.

Henke, S. E. and F. C. Bryant. 1999. Effects of coyote removal on the faunal community in western Texas. The Journal of Wildlife Management 63:1066-1081.

Hensley, M. S. and J. E. Fisher. 1975. Effects of intensive gray fox control on population dynamics of rodents and sympatric carnivores. Southeastern Assoc. Game and Fish Comm. Ann. Conf. Proc. 29:694-705.

Hill, E. P., P. W. Sumner, and J. B. Wooding. 1987. Human influences on range expansion of coyotes in the southeast. Wildlife Society Bulletin 15:521-524.

Hill, J. D. 2005. Kentucky weather. The University Press of Kentucky, Lexington, Kentucky.

Hilton, H. and N. P. Kutscha. 1978. Distinguishing characteristics of the hairs of eastern coyote, domestic dog, red fox, and bobcat in Maine. American Midland Naturalist 100:223-227.

Homan, H. J., G. Linz, and B. D. Peer. 2001. Dogs increase recovery of passerine carcasses in dense vegetation. Wildlife Society Bulletin 29:292-296.

Huck, M., A. C. Frantz, D. A. Dawson, T. Burke, and T. J. Roper. 2008. Low genetic variability, female-biased dispersal and high movement rates in an urban population of Eurasian badgers *Meles meles*. Journal of Animal Ecology 77:905-915.

Hunter, S. A. 2011. Resource portioning between generalist competitors & Factors affecting the detectability of scat by dogs. Thesis, Auburn University, Auburn, Alabama.

Hutchings, M. R. and P. C. L. White. 2000. Mustelid scent-marking in managed ecosystems: implications for population management. Mammal Review 30:157-169.

Imam, T. S. and N. Abdullahi. 2011. Review on the applications of ecosystem models in biodiversity analyses. Bayero Journal of Pure and Applied Sciences 4:98-104.

Jeffreys, A. J., V. Wilson, and S. L. Thein. 1985a. Hypervariable "minisatellite" regions in human DNA. Nature 314:67-73.

Jeffreys, A. J., V. Wilson, and S. L. Thein. 1985b. Individual-specific fingerprints of human DNA. Nature 316:76-79.

Johnson, W. E., E. Eizirk, J. Pecon-Slattery, W. J. Murphy, A. Antunes, E. Teeling, and S. J. O'Brien. 2006. The late Miocene radiation of modern Felidae: a genetic assessment. Science 311:73-77.

Jones, K. L., D. H. Van Vuren, and K. R. Crooks. 2008. Sudden increase in a rare endemic carnivore: ecology of the island spotted skunk. Journal of Mammalogy 89:75-86.

Karamanlidis, A. A., D. Youlatos, S. Sgardelis, and Z. Scouras. 2007. Using sign at power poles to document presence of bears in Greece. Ursus 18:54-61.

Karmacharya, D. B., K. Thapa, R. Shrestha, M. Dhakal, and J. E. Janecka. 2011. Noninvasive genetic population survey of snow leopards (*Panthera uncia*) in Kangchenjunga conservation area, Shey Phoksundo National Park and surrounding buffer zones of Nepal. BMC Research Notes 4:516-524.

Kays, R. W. and K. M. Smith. 2008. Remote cameras. Pages 110-140 *in* Long, R. A., P. MacKay, W. J. Zielinski, and J. C. Ray (eds.). Noninvasive survey methods for carnivores. Island Press, Washington DC, USA.

Kelly, B. T., A. Beyer, and M. K. Phillips. 2004. Red wolf. Pages 87-92 *in* C. Sillero-Zubiri, D. Randall, and M. Hoffman (eds.). Canids: foxes, wolves, jackals, and dogs. status survey and conservation action plan. IUCN/SSC Canid Specialist Group. Gland, Switzerland and Cambridge, UK.

Kelly, M., A. Caso, and C. Lopez Gonzalez. 2008. Lynx rufus *in* IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. <www.iucnredlist.org>. Downloaded on 20 May 2012.

Kendall, K. C., J. B. Stetz, D. A. Roon, L. P. Waits, J. B. Boulanger, and D. Paetkau. 2008. Grizzly bear density in Glacier National Park, Montana. Journal of Wildlife Management 72:1693-1705.

Kendall, K. C. and K. S. McKelvey. 2008. Hair collection. Pages 141-182 *in* Long, R. A., P. MacKay, W. J. Zielinski, and J. C. Ray (eds.). Noninvasive survey methods for carnivores. Island Press, Washington DC, USA.

Kerley, L. L. 2010. Using dogs for tiger conservation and research. Integrative Zoology 5:390-395.

Kerley, L. L. and G. P. Salkina. 2007. Using scent-matching dogs to identify individual amur tigers from scats. Journal of Wildlife Management 71:1349-1356.

Kleiman, D. G. and J. F. Eisenberg. 1973. Comparisons of canid and felid social systems from on evolutionary perspective. Animal Behavior 21:637-659.

Kohn, M. H., E. C. York, D. A. Kamradt, G. Haught, R. M. Sauvajot, and R. K. Wayne. 1999. Estimating population size by genotyping faeces. Proc. Biol. Sci. 266:657-663.

Kyle, C. J. and C. Strobeck. 2001. Genetic structure of North American wolverine (*Gulo gulo*) populations. Molecular Ecology 10:337-347.

Lahiri, D. K. and B. Schnabel. 1993. DNA isolation by a rapid method from human blood samples: effects of $MgCl_2$, EDTA, storage time, and temperature on DNA yield and quality. Biochemical Genetics 31:321-328.

Laliberte, A. S. and W. J. Ripple. 2004. Range contractions of North American carnivores and ungulates. BioScience 54:123-138.

Lancia, R. A., W. L. Kendall, K. H. Pollock, and J. D. Nichols. 2005. Estimating the number of animals in wildlife populations. Pages 106-153 *in* Braun, C. E. (ed.). Techniques for wildlife investigations and management 6th edition. The Wildlife Society, Baltimore, Maryland, USA.

Larrucea, E. S., G. Serra, M. M. Jaeger, and R. H. Barrett. 2007a. Censusing bobcats using remote cameras. Western North American Naturalist 67:538-548.

Larrucea, E. S., P. F. Brussard, M. M. Jaeger, and R. H. Barrett. 2007b. Cameras, coyotes, and the assumption of equal detectability. Journal of Wildlife Management 71:1682-1689.

LeMunyan, C. D., W. White, E. Nyberg, and J. J. Christian. 1959. Design of a miniature radio transmitter for use in animal studies. Journal of Wildlife Management 23:107-110.

Leonard, J. A., C. Vila, and R. K. Wayne. 2005. Legacy lost: genetic variability and population size of extirpated US grey wolves (*Canis lupus*). Molecular Ecology 14:9-17.

Leopold, A. 1933. Game management. Charles Scribner's Sons, New York, USA.

Lincoln, F. C. 1930. Calculating waterfowl abundance on the basis of banding returns. U.S. Department of Agriculture, Circular Number 118.

Lindstedt, S. L., B. J. Miller, and S. W. Buskirk. 1986. Home range, time, and body size in mammals. Ecology 67:413-418.

Litvaitis, J. A. and D. J. Harrison. 1989. Bobcat-coyote niche relationships during a period of coyote population increase. Can. J. Zool. 67:1180-1188.

Long, R. A., T. M. Donovan, P. Mackay, W. J. Zielinski, and J. S. Buzas. 2007a. Effectiveness of scat detection dogs for detecting forest carnivores. Journal of Wildlife Management 71:2007-2017.

Long, R. A., T. M. Donovan, P. Mackay, W. J. Zielinski, and J. S. Buzas. 2007b. Comparing scat detection dogs, cameras, and hair snares for surveying carnivores. Journal of Wildlife Management 71:2018-2025.

Long, R. A. and W. J. Zielinski. 2008. Designing effective noninvasive carnivore surveys. Pages 8-44 *in* Long, R. A., P. MacKay, W. J. Zielinski, and J. C. Ray (eds.). Noninvasive survey methods for carnivores. Island Press, Washington DC, USA.

Lovell, C. D., B. D. Leopold, and C. C. Shropshire. 1998. Trends in Mississippi predator populations, 1980-1995. Wildlife Society Bulletin 26:552-556.

Lucchini, V., E. Fabbri, F. Marucco, S. Ricci, L. Boitani, and E. Randi. 2002. Noninvasive molecular tracking of colonizing wolf (*Canis lupus*) packs in the western Italian Alps. Molecular Ecology 11:857-868.

Lydersen, C. and I. Gjertz. 1986. Studies of the ringed seal (*Phoca hispida* Schreber 1775) in its breeding habitat in Kongsfjorden, Svalbard. Polar Research 4:57-63.

Macdonald, D. W., A. J. Loveridge, and K. Nowell. 2010. Dramatis personae: an introduction to the wild felids. Pages 3-58 *in* Macdonald, D. W. and A. J. Loveridge (eds.). Biology and conservation of wild felids. Oxford University Press, New York, USA.

MacKay, P., D. A. Smith, R. A. Long, and M. Parker. 2008b. Scat detection dogs. Pages 183-222 *in* Long, R. A., P. MacKay, W. J. Zielinski, and J. C. Ray (eds.). Noninvasive survey methods for carnivores. Island Press, Washington DC, USA.

MacKay, P., W. J. Zielinski, R. A. Long, and J. C. Ray. 2008a. Noninvasive research and carnivore conservation. Pages 1-7 *in* Long, R. A., P. MacKay, W. J. Zielinski, and J. C. Ray (eds.). Noninvasive survey methods for carnivores. Island Press, Washington DC, USA.

Maron, J. L., J. A Estes, D. A. Croll, E. M. Danner, S. C. Elmendorf, and S. L. Buckelew. 2006. An introduced predator alters Aleutian Island plant communities by thwarting nutrient subsidies. Ecological Monographs 76:3-24.

Marrero, P., R. Fregel, V. M. Cabrera, and M. Nogales. 2009. Extraction of high-quality host DNA from feces and regurgitated seeds: a useful tool for vertebrate ecological studies. Biological Resources 42:147-151.

Mayer, W. V. 1952. The hair of California mammals with keys to the dorsal guard hairs of California mammals. American Midland Naturalist 48:480-512.

McDaniel, G. W., K. S. McKelvey, J. R. Squires, and L. F. Ruggiero. 2000. Efficacy of lures and hair snares to detect lynx. Wildlife Society Bulletin 28:119-123.

McDonald, P. T., C. K. Nielsen, T. J. Oyana, and W. Sun. 2008. Modelling habitat overlap among sympatric mesocarnivores in southern Illinois, USA. Ecological Modelling 215:267-286.

McKelvey, K. S. and M. K. Schwartz. 2005. DROPOUT: a program to identify problem loci and samples for noninvasive genetic samples in a capture-mark-recapture framework. Molecular Ecology Notes 5:716-718.

Meadows, R. 2002. Scat-sniffing dogs. Zoogoer 31:5.

Mellen, J. D. 1993. A comparative analysis of scent-marking, social and reproductive behavior in 20 species of small cats (*Felis*). American Zoologist 33:151-166.

Menotti-Raymond, M., V. A. David, L. A. Lyons, A. A. Schaffer, J. F. Tomlin, M. K. Hutton, and S. J. O'Brien. 1999. A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). Genomics 57:9-23.

Miller, C. R., P. Joyce, and L. P. Waits. 2002. Assessing allelic dropout and genotype reliability using maximum likelihood. Genetics 160:357-366.

Mills, L. S., K. L. Pilgrim, M. K. Schwartz, and K. McKelvey. 2000a. Identifying lynx and other North American felids based on mtDNA analysis. Conservation Genetics 1:285-288.

Mills, L. S., J. J. Citta, K. P. Lair, M. K. Schwartz, and D. A. Tallmon. 2000b. Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. Ecological Applications 10:283-294.

Mondol, S., R. Navya, V. Athreya, K. Sunagar, V. M. Selvaraj, and U. Ramakrishnan. 2009. A panel of microsatellites to individually identify leopards and its application to leopard monitoring in human dominated landscapes. BMC Genetics 10:79.

Morin, P. A., K. E. Chambers, C. Boesch, and L. Vigilant. 2001. Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (*Pan troglodytes vernus*). Molecular Ecology 10:1835-1844.

Moruzzi, T. L., T. K. Fuller, R. M. DeGraaf, R. T. Brooks, and W. Li. 2002. Assessing remotely triggered cameras for surveying carnivore distribution. Wildlife Society Bulletin 30:380-386.

Mowat, G. and D. Paetkau. 2002. Estimating marten *Martes americana* population size using hair capture and genetic tagging. Wildlife Biology 8:201-209.

Mulders, R., J. Boulanger, and D. Paetkau. 2007. Estimation of population size for wolverines *Gulo gulo* at Daring Lake, Northwest Territories, using DNA based mark-recapture methods. Wildlife Biology 13:38-51.

Mullis, K., F. Faloona, S. Scharf, R. Saiki, G. Horn, and H. Erlich. 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symposia on Quantitative Biology 51:263-273.

Murata, K. and R. Masuda. 1996. Gender determination of the Linne's two-toed sloth (*Choloepus didactylus*) using SRY amplified from hair. J. Vet. Med. Sci. 58:1157-1159.

Murphy, M. A., K. C. Kendall, A. Robinson, and L. P. Waits. 2007. The impact of time and field conditions on brown bear (*Ursus arctos*) faecal DNA amplification. Conservation Genetics 8:1219-1224.

Murphy, S. M. 2011. Status of a reintroduced black bear population in the Big South Fork area of Kentucky. Thesis, University of Kentucky, Lexington, Kentucky.

Murray, D. L., S. Boutin, and M. O'Donoghue. 1994. Winter habitat selection by lynx and coyotes in relation to snowshoe hare abundance. Can. J. Zool. 72:1444-1451.

Navidi, W., N. Arnheim, and M. S. Waterman. 1992. A multi-tubes approach for accurate genotyping of very small DNA samples by using PCR: statistical considerations. Am. J. Hum. Genet. 50:347-359.

Neale, J. C. C. and B. N. Sacks. 2001. Food habits and space use of gray foxes in relation to sympatric coyotes and bobcats. Can. J. Zool. 79:1794-1800.

Nussear, K. E., T. C. Esque, J. S. Heaton, M. E. Cablk, K. K. Drake, C. Valentin, J. L. Yee, and P. A. Medica. 2008. Are wildlife detector dogs of people better at finding desert tortoises (*Gopherus agassizii*)? Herpetological Conservation and Biology 3:103-115.

O'Connell Jr., A. F., N. W. Talancy, L. L. Bailey, J. R. Sauer, R. Cook, and A. T. Gilbert. Estimating site occupancy and detection probability parameters for meso- and large mammals in a coastal ecosystem. Journal of Wildlife Management 70:1625-1633.

Onorato, D., R. Desimone, C. White, and L. P. Waits. 2011. Genetic assessment of paternity and relatedness in a managed population of cougars. Journal of Wildlife Management 75:378-384.

Otis, D. L. and G. C. White. 1999. Autocorrelation of location estimates and the analysis of radiotracking data. Journal of Wildlife Management 63:1039-1044.

Owen-Smith, N. and M. G. L. Mills. 2008. Predator-prey size relationships in an African large-mammal food web. Journal of Animal Ecology 77:173-183.

Oyler-McCance, S. J. and P. L. Leberg. 2005. Conservation genetics in wildlife biology. Pages 632-657 *in* Braun, C. E. (ed.). Techniques for wildlife investigations and management 6th edition. The Wildlife Society, Baltimore, Maryland, USA.

Paetkau, D. 2003. An empirical exploration of data quality in DNA-based population inventories. Molecular Ecology 12:1375-1387.

Paloheimo, J. E. and D. Fraser. 1981. Estimation of harvest rate and vulnerability from age and sex data. Journal of Wildlife Management 45:948-958.

Palomares, F., P. Gaona, P. Ferreras, and M. Delibes. 1995. Positive effects on game species of top predators by controlling smaller predator populations: an example with lynx, mongooses, and rabbits. Conservation Biology 9:295-305.

Paula, J., M. C. Leal, M. J. Silva, R. Mascarenhas, H. Costa, and M. Mascarenhas. 2011. Dogs as a tool to improve bird-strike mortality estimates at wind farms. Journal for Nature Conservation 19:202-208.

Paxinos, E., C. Mcintosh, K. Ralls, and R. Fleischer. 1997. A noninvasive method for distinguishing among canid species: amplification and enzyme restriction of DNA from dung. Molecular Ecology 6:483-486.

Peters, R. H. and J. V. Raelson. 1984. Relations between individual size and mammalian population density. The American Naturalist 124:498-517.

Piggott, M. P. 2004. Effect of sample age and season of collection on the reliability of microsatellite genotyping of faecal DNA. Wildlife Research 31:485-493.

Pilgrim, K. L., K. S. McKelvey, A. E. Riddle, and M. K. Schwartz. 2005. Felid sex identification based on noninvasive genetic samples. Molecular Ecology Notes 5:60-61.

Polis, G. A. and R. D. Holt. 1992. Intraguild predation: the dynamics of complex trophic interactions. Elsevier Science Publishers Ltd., UK.

Pompanon, F., A. Bonin, E. Bellemain, and P. Taberlet. 2005. Genotyping errors: causes, consequences and solutions. Nature Reviews 6:847-859.

Prugh, L. R., C. J. Stoner, C. W. Epps, T. W. Bean, W. J. Ripple, A. S. Laliberte, J. S. Brashares. 2009. The rise of the mesopredator. BioScience 59:779-791.

Radloff, F. G. T. and J. T. Du Toit. 2004. Large predators and their prey in a southern African savanna: a predator's size determines its prey size range. Journal of Animal Ecology 73:410-423.

Ray, A. J. 1987. The fur trade in North America: an overview from a historical geographical perspective. Pages 21-30 *in* Novak, M., J. A. Baker, M. E. Obbard, and B. Malloch (eds.). Wild furbearer management and conservation in North America. The Ontario Trappers Association, Ontario, Canada.

Ray, J. C., K. H. Redford, R. S. Steneck, and J. Berger. 2005. Large carnivores and the conservation of biodiversity. Island Press, Washington DC, USA.

Ray, J. C. and W. J. Zielinski. 2008. Track stations. Pages 75-109 *in* Long, R. A., P. MacKay, W. J. Zielinski, and J. C. Ray (eds.). Noninvasive survey methods for carnivores. Island Press, Washington DC, USA.

Reed, S. E., A. L. Bidlack, A. Hurt, and W. M. Getz. 2011. Detection distance and environmental factors in conservation detection dog surveys. Journal of Wildlife Management 75:243-251.

Regnaut, S., F. S. Lucas, and L. Fumagalli. 2006. DNA degradation in avian faecal samples and feasibility of non-invasive genetic studies of threatened capercaillie populations. Conservation Genetics 7:449-453.

Reindl-Thompson, S. A., J. A. Shivik, A. Whitelaw, A. Hurt, and K. F. Higgins. 2006. Efficacy of scent dogs in detecting black-footed ferrets at a reintroduction site in South Dakota. Wildlife Society Bulletin 34:1435-1439.

Riley, S. P. D., R. M. Sauvajot, T. K. Fuller, E. C. York, D. A. Kamradt, C. Bromley, and R. K. Wayne. 2003. Effects of urbanization and habitat fragmentation on bobcats and coyotes in southern California. Conservation Biology 17:566-576.

Ripple, W. J. and R. L. Beschta. 2004. Wolves and the ecology of fear: can predation risk structure ecosystems? BioScience 54:755-766.

Roberts, N. M. and S. M. Crimmins. 2010. Bobcat population status and management in North America: evidence of large-scale population increase. Journal of Fish and Wildlife Management 1:169-174.

Roemer, G. W., C. J. Donlan, and F. Courchamp. 2002. Golden eagles, feral pigs, and insular carnivores: how exotic species turn native predators into prey. Proc. Nat. Acad. Sci. 99:791-796.

Roemer, G.W., M. E. Gompper, B. Van Valkenburgh. 2009. The ecological role of the mammalian mesocarnivore. BioScience 59:165-173.

Roemer, G. W., T. J. Coonan, D. K. Garcelon, J. Bascompte, and L. Laughrin. 2001. Feral pigs facilitate hyperpredation by golden eagles and indirectly cause the decline of the island fox. Animal Conservation 4:307-318.

Rolland, R. M., P. K. Hamilton, S. D. Kraus, B. Davenport, R. M. Gillett, and S. K. Wasser. 2006. Faecal sampling using detection dogs to study reproduction and health in North Atlantic right whales (*Eubalaena glacialis*). J. Cetacean Res. Management. 8:121-125.

Rolley, R. E. 1987. Bobcat. Pages 671-681 *in* Novak, M., J. A. Baker, M. E. Obbard, and B. Malloch (eds.). Wild furbearer management and conservation in North America. The Ontario Trappers Association, Ontario, Canada.

Roon, D. A., L. P. Waits, and K. C. Kendall. 2003. A quantitative evaluation of two methods for preserving hair samples. Molecular Ecology Notes 3:163-166.

Rose, M. D. and G. A. Polis. 1998. The distribution and abundance of coyotes: the effects of allochthonous food subsidies from the sea. Ecology 79:998-1007.

Rucker, R. A., M. L. Kennedy, G. A. Heidt, and M. J. Harvey. 1989. Population density, movements, and habitat use of bobcats in Arkansas. Southwestern Naturalist 34:101-108.

Ruell, E. W. and K. R. Crooks. 2007. Evaluation of noninvasive genetic sampling methods for felid and canid populations. Journal of Wildlife Management 71:1690-1694.

Ruell, E. W., S. P. D. Riley, M. R. Douglas, J. P. Pollinger, and K. R. Crooks. 2009. Estimating bobcat population sizes and densities in a fragmented urban landscape using noninvasive capture-recapture sampling. Journal of Mammalogy 90:129-135.

Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim. 1985. Enzymatic amplification of beta-globin benomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350-1354.

Saltz, D. 1994. Reporting error measures in radio locations by triangulation: a review. Journal of Wildlife Management 58:181-184.

Santini, A., V. Lucchini, E. Fabbri, and E. Randi. 2007. Ageing and environmental factors affect PCR success in wolf (*Canis lupus*) excrement DNA samples. Molecular Ecology Notes 7:955-961.

Sarmento, P., J. Cruz, C. Eira, and C. Fonseca. 2009. Evaluation of camera trapping for estimating red fox abundance. Journal of Wildlife Management 73:1207-1212.

Savidge, J. A., J. W. Stanford, R. N. Reed, G. R. Haddock, and A. A. Yackel Adams. 2011. Canine detection of free-ranging brown treesnakes on Guam. New Zealand Journal of Ecology 35:174-181.

Scheppers, T. L. J., T. J. Roper, A. C. Frantz, M. Schaul, E. Engel, P. Breyne, and L. Schley. 2007. Estimating social group size in Eurasian badgers *Meles meles* by genotyping remotely plucked single hairs. Wildlife Biology 13:195-207.

Schlexer, F. V. 2008. Attracting animals to detection devices. Pages 263-292 *in* Long, R. A., P. MacKay, W. J. Zielinski, and J. C. Ray (eds.). Noninvasive survey methods for carnivores. Island Press, Washington DC, USA.

Schmidt, K. and R. Kowalczyk. 2006. Using scent-marking stations to collect hair samples to monitor Eurasian lynx populations. Wildlife Society Bulletin 34:462-466.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18:233-234.

Schwartz, M. K., G. Luikart, and R. S. Waples. 2006. Genetic monitoring as a promising tool for conservation and management. Trends in Ecology and Evolution 22:25-33.

Schwartz, M. K. and S. L. Monfort. 2008. Genetic and endocrine tools for carnivore surveys. Pages 239-262 *in* Long, R. A., P. MacKay, W. J. Zielinski, and J. C. Ray (eds.). Noninvasive survey methods for carnivores. Island Press, Washington DC, USA.

Selkoe, K. A. and R. J. Toonen. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology Letters 9:615-629.

Sequin, E. S., M. M. Jaeger, P. F. Brussard, and R. H. Barrett. 2003. Wariness of coyotes to camera traps relative to social status and territory boundaries. USDA National Wildlife Research Center, Staff Publications. Paper 233:2015-2025.

Shadel, G. S. and D. A. Clayton. 1997. Mitochondrial DNA maintenance in vertebrates. Annu. Rev. Biochem. 66:409-435.

Shinn, K. J. 2002. Ocelot distribution in the Lower Rio Grande Valley National Wildlife Refuge. Thesis, University of Texas-Pan American, Edinburg, Texas.

Shipley, A. J. In press. Space use and habitat selection by bobcats (Lynx rufus) in southeastern Kentucky. Thesis, Eastern Kentucky University, Richmond, Kentucky.

Short, H. L. 1978. Analysis of cuticular scales on hairs using the scanning electron microscope. Journal of Mammalogy 59:261-268.

Silva, M. and J. A. Downing. 1995. The allometric scaling of density and body mass: a nonlinear relationship for terrestrial mammals. The American Naturalist 145:704-727.

Silvy, N. J., R. R. Lopez, and M. J. Peterson. 2005. Wildlife marking techniques. Pages 339-376 *in* Braun, C. E. (ed.). Techniques for wildlife investigations and management 6th edition. The Wildlife Society, Baltimore, Maryland, USA.

Sinclair, K. E., G. R. Hess, C. E. Moorman, and J. H. Mason. 2005. Mammalian nest predators respond to greenway width, landscape context and habitat structure. Landscape and Urban Planning 71:277-293.

Smallwood, K. S. and E. L. Fitzhugh. 1993. A rigorous technique for identifying individual mountain lions *Felis concolor* by their tracks. Biological Conservation 65:51-59.

Smith, D. A., K. Ralls, A. Hurt, B. Adams, M. Parker, B. Davenport, M. C. Smith, and J. E. Maldonado. 2003. Detection and accuracy rates of dogs trained to find scats of San Joaquin kit foxes (*Vulpes macrotis mutica*). Animal Conservation 6:339-346.

Smith, D. A., K. Ralls, B. L. Cypher, H. O. Clark Jr., P. A. Kelly, D. F. Williams, and J. E. Maldonado. 2006. Relative abundance of endangered San Joaquin kit foxes (*Vulpes macrotis mutica*) based on scat-detection dog surveys. Southwestern Naturalist 51:210-219.

Smith, D. A., K. Ralls, B. L. Cypher, and J. E. Maldonado. 2005. Assessment of scat-detection dog surveys to determine kit fox distribution. Wildlife Society Bulletin 33:897-904.

Soule, M. E., D. T. Bolger, A. C. Alberts, J. Wright, M. Sorice, and S. Hill. 1988. Reconstructed dynamics of rapid extinctions of chaparral-requiring birds in urban habitat islands. Conservation Biology 2:75-92.

Stake, M. M. and D. A. Cimprich. 2003. Using video to monitor predation at black-capped vireo nests. The Condor 105:348-357.

Stenglein, J. L., M. De Barba, D. E. Ausband, and L. P. Waits. 2010. Impacts of sampling location within a faeces on DNA quality in two carnivore species. Molecular Ecology Resources 10:109-114.

Stetz, J. B., K. C. Kendall, and C. Servheen. 2010. Evaluation of bear rub surveys to monitor grizzly bear population trends. Journal of Wildlife Management 74:860-870.

Swihart, R. K., N. A. Slade, and B. J. Bergstrom. 1988. Relating body size to the rate of home range use in mammals. Ecology 69:393-399.

Syrotuck, W. 1972. Scent and the scenting dog. Barkleigh Productions, Mechanicsburg, Pennsylvania, USA.

Taberlet, P. and G. Luikart. 1999. Non-invasive genetic sampling and individual identification. Biol. J. Linn. Soc. 68:41-55.

Taberlet, P., L. P. Waits, and G. Luikart. 1999. Noninvasive genetic sampling: look before you leap. TREE 14:323-327.

Taberlet, P., S. Griffin, B. Goossens, S. Questiau, V. Manceau, N. Escaravage, L. P. Waits, and J. Bouvet. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research 24:3189-3194.

Tannerfeldt, M., B. Elmhagen, and A. Angerbjorn. 2002. Exclusion by interference competition? The relationship between red and arctic foxes. Oecologia 132:213-220.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Reseach 17:6463-6471.

Temple, D. L., M. J. Chamberlain, and L. M. Conner. 2010. Spatial ecology, survival, and cause-specific mortality of gray foxes (*Urocyon cinereoargenteus*) in a longleaf pine ecosystem. American Midland Naturalist 163:413-422.

Tewes, M. E., J. M. Mock, and J. H. Young. 2002. Bobcat predation on quail, birds, and mesomammals. Pages 65-70 *in* S. J. DeMaso, W. P. Kuvlesky, Jr., F. Hernandez, and M. E. Berger (eds.). Quail V: Proceedings of the Fifth National Quail Symposium. Texas Parks and Wildlife Department, Austin, TX.

Thornton, D. H., M. E. Sunquist, and M. B. Main. 2004. Ecological separation within newly sympatric populations of coyotes and bobcats in south-central Florida. Journal of Mammalogy 85:973-982.

Trappe, G. R. 1978. Comparative behavioral ecology of the ringtail (*Bassariscus astutus*) and gray fox (*Urocyon cinereoargenteus*) in southwestern Utah. Carnivore 1:3-32.

Tsaparis, D., S. Katsanevakis, E. Ntolka, and A. Legakis. 2009. Estimating dung decay rates of roe deer (*Capreolus capreolus*) in different habitat types of a Mediterranean ecosystem: an information theory approach. Eur. J. Wildl. Res. 55:167-172.

Valiere, N. 2002. GIMLET: a computer program for analyzing genetic individual identification data. Molecular Ecology Notes 2:377-379.

Valiere, N., C. Bonenfant, C. Toigo, G. Luikart, J. M. Gaillard, and F. Klein. 2007. Importance of a pilot study for non-invasive genetic sampling: genotyping errors and population size estimation in red deer. Conservation Genetics 8:69-78.

Vance-Chalcraft, H. D., J. A. Rosenheim, J. R. Vonesh, C. W. Osenberg, and A. Sih. 2007. The influence of intraguild predation on prey suppression and prey release: a meta-analysis. Ecology 88:2689-2696.

Van Valkenburgh, B. 2007. Déjà vu: the evolution of feeding morphologies in the Carnivora. Integrative and Comparative Biology 47:147-163.

Vynne, C., J. R. Skalski, R. B. Machado, M. J. Groom. A. T. A. Jacomo, J. Marinho-Filho, M. B. Ramos Neto, C. Pomilla, L. Silveira, H. Smith, and S. K. Wasser. 2011. Effectiveness of scat-detection dogs in determining species presence in a tropical savanna landscape. Conservation Biology 25:154-162.

Waits, L. P., and D. Paetkau. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. Journal of Wildlife Management 69:1419-1433.

Waits, L. P., G. Luikart, and P. Taberlet. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology 10:249-256.

Wang, X., R. H. Tedford, B. Van Valkenburgh, and R. K. Wayne. 2004. Ancestry: evolutionary history, molecular systematic and evolutionary ecology of Canidae. Pages 39-54 *in* Macdonald, D. W. and C. Sillero-Zubiri (eds.). Biology and conservation of wild canids. Oxford University Press, New York, USA.

Wasser, S. K., B. Davenport, E. R. Ramage, K. E. Hunt, M. Parker, C. Clarke, and G. Stenhouse. 2004. Scat detection dogs in wildlife research and management: application to grizzly and black bears in the yellowhead ecosystem, Alberta, Canada. Can. J. Zool. 82:475-492.

Wasser, S. K., H. Smith, L. Madden, N. Marks, and C. Vynne. 2009. Scent-matching dogs determine number of unique individuals from scat. Journal of Wildlife Management 73:1233-1240.

Waters, J., S. O'Connor, K. J. Park, and D. Goulson. 2010. Testing a detection dog to locate bumblebee colonies and estimate nest density. <www.apidologie.org>. Downloaded on 15 May 2012.

Weaver, J. L., P. Wood, D. Paetkau, and L. L. Laack. 2005. Use of scented hair snares to detect ocelots. Wildlife Society Bulletin 33:1384-1391.

Weber, J. L. and P. E. May. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am. J. Hum. Genet. 44:388-396.

Wegge, P., C. P. Pokheral, and S. R. Jnawali. 2004. Effects of trapping effort and trap shyness on estimates of tiger abundance from camera trap studies. Animal Conservation 7:251-256.

Weston, J. L., N. A. Schable, and T. C. Glenn. 2004. Characterization of six microsatellite primers for the grey fox (*Urocyon cinereoargenteus*). Molecular Ecology Notes 4:503-505.

Wetton, J. H., R. E. Carter, D. T. Parkin, and D. Walters. 1987. Demographic study of a wild house sparrow population by DNA fingerprinting. Nature 327:147-149.

Whitaker, J., Frederick, R.B. and T.L. Edwards. 1987. Home-range size and overlap of eastern Kentucky bobcats. Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 41: 417-423.

Wolfe, M. L. and J. A. Chapman. 1987. Principles of furbearer management. Pages 101-112 *in* Novak, M., J. A. Baker, M. E. Obbard, and B. Malloch (eds.). Wild furbearer management and conservation in North America. The Ontario Trappers Association, Ontario, Canada.

Woodroffe, R. 2001. Strategies for carnivore conservation: lessons from contemporary extinctions. Pages 61-92 *in* Gittleman, J. L., S. M. Funk, D. W. Macdonald, and R. K. Wayne (eds.). Carnivore conservation. Cambridge University Press, UK.

Woods, J. G., D. Paetkau, D. Lewis, B. N. McLellan, M. Proctor, and C. Strobeck. 1999. Genetic tagging of free-ranging black and brown bears. Wildlife Society Bulletin 27:616-627.

Yearsley, E. F. and D. E. Samuel. 1980. Use of reclaimed surface mines by foxes in West Virginia. Journal of Wildlife Management 44:729-734.

York, E. C., T. L. Moruzzi, T. K. Fuller, J. F. Organ, R. M. Sauvajot, and R. M. DeGraaf. 2001. Description and evaluation of a remote camera and triggering system to monitor carnivores. Wildlife Society Bulletin 29:1228-1237.

Zane, L., L. Bargelloni, and T. Patarnello. 2002. Strategies for microsatellite isolation: a review. Molecular Ecology 11:1-16.

Zielinski, W. J., F. V. Schlexer, K. L. Pilgrim, and M. K. Schwartz. 2006. The efficacy of wire and glue hair snares in identifying mesocarnivores. Wildlife Society Bulletin 34:1152-1161.

Zielinski, W. J., R. L. Truex, F. V. Schlexer, L. A. Campbell, and C. Carroll. 2005. Historical and contemporary distributions of carnivores in forests of the Sierra Nevada, California, USA. Journal of Biogeography 32:1385-1407.

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