THESIS

# A NONINVASIVE METHOD USING AUDITORY PREDATOR CALLS AND HAIR SNARES TO DETECT AND GENETICALLY SAMPLE COUGARS (*Puma concolor*)

Submitted by

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# ABSTRACT

## A NONINVASIVE METHOD USING AUDITORY PREDATOR CALLS AND HAIR SNARES TO DETECT AND GENETICALLY SAMPLE COUGARS (*Puma concolor*)

A noninvasive method that will sample all individuals in a population over multiple occasions is a useful tool in assessing population demographics with little disturbance to the target animals; however, finding such a method for large carnivores, such as cougars, is challenging due to their elusive nature and large home-range sizes. Current methods to sample cougars usually involve a physical capture component, but obtaining reliable estimates can be difficult and cost prohibitive when using capture as the sole sampling method. Because cougars leave sign, and exhibit behaviors like territoriality and curiosity, a noninvasive-genetic-sampling (NGS) method may be a plausible alternative. Hair contains DNA, which can be genetically analyzed to yield the individual identification necessary for population assessments and can be obtained without handling the animal. I tested NGS techniques using attractants, specifically scent lures and auditory calls, and hair snares to sample cougars at lure sites on the Front Range, Colorado during February – April, 2012 and November, 2012 – April, 2013. First, I established 16 – 20 sites over four ≈ 30-day sampling periods. At sites with auditory calls, photographs documented 40 visits by ≥ 13 individual cougars, and I obtained 14 hair samples. Only two hair samples were collected using scented scratch pads and no samples were acquired via a novel hair snare. Because my initial results indicated calls were more effective attractants than scents, I narrowed my focus to the cubby hair-snare design and increased my effort by establishing 148 lure sites over three or four sampling periods in two study areas: the Front Range (FR; 1,270 km$^2$) and the Uncompahgre Plateau (UP; 540 km$^2$). Each site was active an average of 28.5 days

(4,214 sampling nights). On the FR, I observed 98 detections by 13 independent marked cougars, two sibling groups, and ≥ 16 unique unmarked animals. On the UP, I documented 18 detections by seven independent marked cougars. Collectively, 14 of the 20 marked cougars detected were observed multiple times. I used the GPS location data of 27 previously marked cougars to determine availability and estimate detection probabilities. The probability of detecting via camera an independent marked cougar at least once during the study with no assumption of closure (superpopulation) was 0.65 ± 0.11 (FR) and 0.64 ± 0.15 (UP). I collected 59 hair samples. Thirty-two were genotyped at ≥ 8 loci identifying 26 unique cougars. I conclude that auditory calls and hair snares may be an effective way to collect the various biological data that are needed to inform management decisions.

# ACKNOWLEDGEMENTS

I sat in the dark in the middle of the woods, dart gun in hand, 10 meters away from a mountain lion in a snare. My first encounter with a lion was awe-inspiring and humbling. Every encounter thereafter was just as profound. I am extremely fortunate to have had the chance to work with such amazing animals and to further my education amongst bright and creative individuals at the forefront of wildlife research. I owe a debt of gratitude for the invaluable opportunity that I was offered.

I am proud to say that I am part of the Colorado Cooperative Fish and Wildlife Research Unit, a member of the Graduate Degree Program in Ecology, and a student in the Department of Fish, Wildlife, and Conservation Biology (FWCB) at Colorado State University (CSU). The faculty and student body are second to none, and I will forever be grateful for their dedication to teaching and for the encouragement and enthusiasm of my classmates.

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# PREFACE

In many states, cougars (*Puma concolor*) are a harvested species whose populations are affected by increasing cougar-human conflict in residential and recreational areas. Developers are expanding into previously undisturbed cougar habitat; pet loss complaints and depredation claims continue; and each year, municipalities acquire more land to be made available to the public. All of these pressures on cougar populations make responsible management imperative. Yet, obtaining a reliable estimate of population size is challenging.

To set harvest quotas, evaluate management practices, and understand the dynamics of predator-prey systems, reliable methods of obtaining population demographic estimates are needed. However, answering occupancy and abundance questions regarding carnivores is challenging (Kéry et al. 2011). In general, carnivores are elusive and occupy large home ranges that often vary in size across the population (Anderson et al. 2004). As a result, it can be very difficult and expensive to obtain a representative sample that is large enough to produce a reliable estimate (Ruell et al. 2009). Despite the cost, it is essential that managers have accurate population estimates that can support management decisions (Dreher et al. 2007, Immell and Anthony 2008).

A common way to estimate abundance in wildlife studies is to use raw count data as an index and assume the count ($C$) is proportional to the true population size ($N$). This assumption is seldom verified and often false. The actual association between $C$ and $N$ is rarely known (White 2005). Conceptually, the count is a product of population size and a capture, or detection, probability $p$ ($C \approx Np$). Thus, when a count is adjusted by a reliable estimate of $\hat{p}$ ($\hat{N} = C/\hat{p}$), bias is reduced (Anderson 2001). However, detection parameters can be difficult to

estimate as they may vary by observer, habitat type, or species-specific variables, such as age, sex, or individual (Anderson 2001). Obtaining the sample size needed to estimate all potential sources of variation may be cost-prohibitive or impossible, especially with rare and elusive species or those that occur at low densities, such as cougars (MacKenzie et al. 2005). If a detection probability can be incorporated into the count data, an accurate and precise abundance estimate can be derived via a single count, or occasion, making an index an effective tool for monitoring wildlife populations when resources are limited.

When animals can be sampled over multiple occasions, closed capture-recapture (C–R) models are widely used mainly because C–R models are versatile and with a reasonable sample size have been shown to be robust when assumptions, such as closure and equal probability of capture, are violated (Burnham and Overton 1978, Otis et al. 1978, Huggins 1989, Stanley and Burnham 1998, Kendall 1999, Pledger 2000). Fundamentally, closed C–R models estimate parameters, such as detection, abundance, and state transition rates, via encounter histories, requiring individuals to be individually identifiable and repeatedly observable (see Williams et al. 2002). Identifying animals by observation alone is often impossible so a band or a tag is applied and encounter histories are then based on subsequent observations of the applied mark (e.g., Pendleton et al. 2006, Baker et al. 2007, Bestgen et al. 2007, Kendall et al. 2009). Marking the animal often requires its capture, or an otherwise invasive procedure, which can be difficult, expensive, and time consuming for many species. Alternatively, obtaining a genetic sample can yield a unique identification via genotype and can be acquired without handling the animal (e.g., Woods et al. 1999, Mowat and Strobeck 2000, Ruell et al. 2009, Williams et al. 2009).

The most challenging C–R model assumption to meet is equal probability of capture, or detection. In wildlife studies, detection is often less than certain (< 1) and variable across the

population (Link 2003). Unmodeled heterogeneity in detection may overstate precision and include bias in parameter estimates (Link 2003). Models can accommodate some variation by grouping attributes with similar values, for example, separating males and females because females are detected at a higher rate than males. Grouping also reduces the number of parameters and thus the sample size needed. Currently, few models can incorporate individual capture heterogeneity (Link 2003). For example, the jackknife estimator (Burnham and Overton 1978) or the maximum likelihood approaches of Pledger (2000) and Huggins (1989) have been shown to be robust where capture heterogeneity is present but small-sample bias in abundance estimates is typical for wildlife studies. Because detection parameters are conditional on the sampling method, sources of capture variation can be eliminated or addressed through study design. Not only should the sampling protocol minimize variation but also achieve the highest detection probability possible and perhaps even ensure that all individuals are detected at least once (Gerber et al. 2014).

C–R models also require that all individuals be observable over multiple occasions, which can be challenging and resource-intensive with rare species. Additionally, sampling methods with multiple occasions may include a trap-response effect. A trap response (a positive or negative behavioral response to the trapping event) is a common source of capture variation that can bias parameter estimates (Pollock et al. 1990). For example, an animal captured on one occasion that is less likely to be recaptured via the same technique presents a ‘trap-shy’ response which results in positive bias in a population estimate. Conversely, a ‘trap-happy’ response, which may ensue when an animal receives a reward, such as food, can negatively bias estimates (see Williams et al. 2002). Capture variation can be reduced by choosing a sampling method that does not include a trap-response effect or by applying multiple sampling methods. For instance,

cage traps may be used to capture and mark animals during the first sampling period and a lure site or a scat-collection design might be used during subsequent sampling occasions.

Currently, cougars, like many other species, are sampled during hunter harvest (Garshelis and Visser 1997, Diefenbach et al. 2004, Nicolai et al. 2005, Dreher et al. 2007, Colorado Parks and Wildlife unpublished data) and via methods that usually involve their pursuit and/or capture. Cage traps, foot-hold snares, and the use of hounds are common sampling techniques that can be termed 'invasive' and may result in a declining recapture probability due to a 'trap-shy' response. Because cougars leave sign and exhibit behaviors like territoriality and curiosity, a noninvasive-sampling method may be an appropriate alternative (Long et al. 2008). Noninvasive methods allow individuals within a population to be sampled without needing to be handled or even observed (Taberlet and Luikart 1999, Garshelis 2006, Beja-Pereira et al. 2009, Pauli et al. 2010), thus reducing the likelihood of encountering a trap effect. When used as a secondary method of detection, noninvasive methods can reduce bias due to the capture variation associated with an individual-survey method (Noyce et al. 2001). In addition, noninvasive sampling has other benefits in that it minimizes stress and disturbance to the study animals; and when successful, it may allow a larger sample size at a lower cost (Pauli et al. 2010).

Many noninvasive techniques have been tried to assess population demographics of large carnivores. Track surveys have been used to verify occupancy or suggest general population trends (Diefenbach et al. 1994, Sargeant et al. 1998, Wilson and Delahay, 2001, Hayward et al. 2002, Choate et al. 2006, Gompper et al. 2006), but track surveys cannot be employed to estimate abundance unless all animals can be individually identified by their tracks (Van Dyke et al. 1986). When track surveys are combined with the collection of genetic material, individual identification is possible (McKelvey et al. 2006, Ulizio et al. 2006). Likewise, camera traps,

when used alone, will not yield an individual identification unless the animal is uniquely marked with an applied tag or displayed a patterned pelage, such as a tiger (Karanth and Nichols 1998). Scat-detection dogs have successfully located genetic material but the use of dogs is generally of greater expense than other noninvasive techniques (Wasser et al. 2004, Smith et al. 2005, Harrison 2006, Long et al. 2007, Wasser et al. 2011).

Snow tracking, finding and following tracks in the snow, has been used to realize various objectives including evaluating movement patterns, confirming species presence, and locating genetic samples (Seidensticker et al. 1973, Hemker et al. 1984, McKelvey et al. 2006, Ulizio et al. 2006). Sawaya et al. (2011) reported that winter tracking cougars under favorable conditions returned hair samples 80% of the time after tracking on average 1.09 km. However because success is largely dependent upon optimal snow conditions and timing after snow fall (Squires et al. 2004), this method is only effective in specific geographic regions.

A scent lure with a scratch-pad-style hair snare is a cost-effective method to survey felids and carnivore communities, in general, but the effectiveness of this technique was low when tested several times (Harrison 1997, Sargeant et al. 1998, McDaniel et al. 2000, Long et al. 2003, Weaver et al. 2005, Choate et al. 2006, Harrison 2006, Schmidt and Kowalczyk 2006, Downey et al. 2007, Long et al. 2007, Ruell and Crooks 2007, Castro-Arellano et al. 2008, Crooks et al. 2008, Sawaya et al. 2011, Lang et al. 2013). These studies used scent lures as the main attractant but felids may exhibit a greater response to auditory cues (Chamberlain et al. 1999) suggesting that a predator call may be a more effective lure. Additionally, most of the hair-snaring devices consisted of a board with a scent-lure-covered carpet pad pierced with nails and secured to a tree (Turbak 1998, McDaniel et al. 2000, Harrison 2006, McKelvey et al. 2006, Schmidt and Kowalczyk 2006, Long et al. 2007, and Sawaya et al. 2011). Scratch pads snagged hair part of

the time, though the quality of the hair and whether the hair was from the target species was inconsistent. Scratch pads tend to snag hair in the telogen phase. Telogen hair is about to be shed and may not have a follicle. Although it is possible to have DNA incorporated into the hair shafts, especially in species that self-groom (Alberts et al. 2010), the quality and quantity of DNA extracted from a hair-shaft source is highly variable (Bengtsson et al. 2012). Obtaining the root tissue, which includes the follicle, is preferred (Bengtsson et al. 2012). Because at least 10 plucked hairs are needed to minimize the chance for genotyping errors, such as allelic dropout (Goossens et al. 1998), improvements in hair-snaring techniques are needed to ensure accurate genotyping.

Barbed wire is a possible alternative hair-snaring mechanism to traditional scratch-pad designs. Barbed wire has been extensively used to collect hair from grizzly and black bears (Woods et al. 1999, Mowat and Strobeck 2000, Poole et al. 2001, Boersen et al. 2003, Belant et al. 2005, Boulanger et al. 2006, Dreher et al. 2007, Kendall et al. 2008, Settlage et al. 2008, Proctor et al. 2010). Barbed wire has also snagged hair from wild boar (Ebert et al. 2009) and white-tailed deer (Belant et al. 2007). I could not find a study that used barbed wire in an attempt to snag hair from a felid species. However, I collected hair suspected to be cougar from a barbed-wire fence during a snow-tracking survey (Yeager unpublished data).

Noninvasive methods are possible due to advancements in genetics. Current laboratory techniques dictate the quality and quantity of genetic material that a NGS method must yield. In the past, evaluating genetic variation often compelled the sacrifice of the study animals in order to obtain the amount or type of tissue needed for the existing techniques, such as protein electrophoresis or restriction fragment length polymorphisms (RFLP) analysis (Avise et al. 1979, Brown and Wright 1979, Lewontin 1991). But with the advent of the polymerase chain reaction

(PCR) (Mullis and Faloona 1987), a minute quantity of DNA extracted from sources such as a single spermatozoid or a hair follicle became sufficient to obtain the genetic information desired (Taberlet et al. 1996). Thus the use of a NGS method, which usually yields small quantities of genetic material, became possible and methods such as snow tracking to find hair samples became viable sampling techniques (Dreher et al. 2007, Sawaya et al. 2011).

DNA can be used to both confirm species and identify individuals (Woods et al. 1999, Kéry et al. 2011). In most instances, motion-sensor cameras or mitochondrial DNA are used to confirm species identification. Individuals are typically identified using nuclear DNA, as it has a high level of variability needed to differentiate individuals (Menotti-Raymond and O'Brien 1995). Menotti-Raymond et al. (1999) developed a genetic linkage map for the domestic cat containing 253 microsatellite loci. These loci can be used to analyze other felids. How many and which microsatellites are used depend upon the degree of genetic diversity between individuals in the population sampled (Woods et al. 1999). Menotti-Raymond et al. (1999), Culver et al. (2000), Ernest et al. (2000), Anderson et al. (2004), and Mondol et al. (2009) reported between seven and 12 loci with a high degree of variability was adequate to express enough heterozygosity to differentiate individuals in their respective studies.

In general, samples collected via NGS methods, such as hair, feathers, and scat, typically have a low quality and quantity of DNA relative to blood and tissue samples (Broquet et al. 2007). The inherently small quantities of DNA are susceptible to sample contamination and degradation in the field and in the laboratory (Taberlet and Luikart 1999). The poor DNA samples may fail to amplify or display genotyping errors by allelic dropout or false alleles exhibiting false homozygotes and heterozygotes respectively (Buchan et al. 2005). Eliminating or accounting for genotyping errors is essential in satisfying the assumption of known identity in

C–R models. Failure to do so can result in an over or under estimation of abundance depending upon the type of error (Lukacs and Burnham 2005). Ernest et al. (2000) reported an 8% allelic dropout rate during fecal amplification compared to a < 1% error rate in blood and muscle assays. Strict data collection and laboratory protocols can minimize genotyping errors (Taberlet et al. 1996). When possible, errors can be observed by comparing NGS results to more reliable profiles generated through blood and tissue analyses of the same individual (Ernest et al. 2000, Mills et al. 2000, Mondol et al. 2009). Running multiple tests on a single sample (multiple tubes approach) will also reveal and greatly reduce genotyping errors as well as provide an estimate of the certainty of the conclusion (Navidi et al. 1992, Taberlet et al. 1996).

Many genotyping techniques currently exist. The exact extraction and amplification protocol needed to maximize the DNA yield will be specific to the samples obtained. For example, a protocol that works well for scat may not work with hair and more precisely, what works with bear hair may not work with cougar hair. How much and what part of the sample is to be used as the DNA source must first be determined. In the case of hair, too few hairs may yield DNA but genotyping errors will likely be high as the probability of allelic dropout increases when DNA concentration falls below 0.05 ng per 10 μL (Gagneux et al. 1997). Too many hairs will contain too much melanin, a PCR inhibiting substance. Fifteen nanograms of melanin, found in a 5 – 10 cm hair shaft, can completely inhibit the PCR process (Uchihi et al. 1992). Although the follicle is the ideal DNA source, DNA is also incorporated in the hair shaft with the highest quantity near the root end, decreasing distally (Heywood et al. 2003). Because cougar hair, in general, is extremely fine with diminutive follicles, using 5 mm of the proximal end of a 10 – 15 hair tuft of fur may yield the maximum amount of DNA. The sample will

include any follicles present and a combined total of 5 – 7.5 cm of hair shaft segments containing the most DNA without inhibiting the PCR process.

The phenol–chloroform method (P–C) and a silica-based extraction via a Qiagen® kit are two commonly used extraction protocols. P–C has been used to extract DNA from telogen hairs (Higuchi et al. 1988, Hellmann et al. 2001) or from hair shafts where follicles were purposefully removed (Alberts et al. 2010). P–C uses extremely toxic chemicals and is laborious but it has been shown to yield relatively high DNA recovery in degraded forensic samples (Carracedo 2005). The P–C protocol usually includes ethylene diamine tetraacetic acid (EDTA) in the buffer system. However, with hair, substituting $Ca^{2+}$ ($CaCl_2$) for EDTA may increase DNA yield (Hellmann et al. 2001). Silica-based methods, such as Qiagen® kits, are simple to use and highly effective in extracting DNA from a variety of tissue types (Janjua et al. 2014). When applied to Asiatic black bear hair, Qiagen® kits (65%) extracted DNA slightly more effectively than P–C (55%: Janjua et al. 2014), though the protocol of Janjua et al. (2014) used EDTA. With regard to cougar hair, a comparison of DNA quantities extracted via Qiagen® kits and P–C using $Ca^{2+}$ in the buffer system is needed to determine which extraction protocol yields the highest quality DNA.

Once extracted, DNA is typically amplified via PCR but various protocols exist. A primer, or a short, complimentary base-pair sequence, is a key component in the replication process. After the DNA is denatured, a primer is needed to anneal to the targeted segment of DNA for replication, or amplification, of the specific microsatellite loci to occur. Primers are also labeled with a fluorescent dye to make the PCR product observable. Two common types of primers are individually dye-labeled primers specific to each loci and a universal primer, such as the M13–tailed forward primers (Boutin-Ganache et al. 2001), that can be used to amplify any

loci. Universal primers are cost effective thus commonly used in genetics laboratories, especially with analyses using many, many markers. With regard to noninvasively collected samples, M13–tailed forward primers successfully amplified DNA extracted from felid scat samples but have not yet proven useful with felid hair (Ruell and Crooks 2007, S. Oyler-McCance personal communication). If further testing of universal primers does not yield a measureable, accurate PCR product, individually dye-labeled primers may be needed to effectively amplify DNA obtained from felid telogen hair.

Despite the extensive investment of resources prior to this study into the development of noninvasive methods to sample cougars, a method capable of reliably obtaining an individual identification over multiple occasions has not been realized. My primary objective was to develop and evaluate an NGS method with little variation in detection and capable of producing the detection histories, via genotypes, needed for C–R models. I chose to test attractants, specifically scent lures and auditory predator calls, in conjunction with hair snares in two study areas over two winter field seasons.

# DEDICATION

*For: Tyler, Logan, Owen, Paiton, Colby, Alana, and Kasey*

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# A NONINVASIVE METHOD USING AUDITORY PREDATOR CALLS AND HAIR SNARES TO DETECT AND GENETICALLY SAMPLE COUGARS (*Puma concolor*)

## INTRODUCTION

Effectively managing natural resources to meet the essential and recreational demands of a growing human population is crucial to maintaining ecosystem diversity, the conservation of species sensitive to anthropogenic factors, and to ensuring resource longevity for future generations (McKinney 2002, McKinney 2005). Land use change through the urbanization of agricultural lands, open spaces, and wilderness is the greatest threat to natural ecosystems (Czech et al. 2000). The resulting dynamic nature of the wildlife urban interface (WUI), or where houses and wildlands meet (Stewart et al. 2007), makes species management and conservation challenging. Furthermore, the effects of human population growth extend beyond the WUI into more remote wilderness through recreational and consumptive activities, such as hiking, hunting, logging, and fossil fuel development.

Large carnivores are potentially affected in both the WUI and in the more remote regions of their large home ranges (Radeloff et al. 2005). The WUI may both benefit and impair an animal. For example, an open dumpster may provide a bear (e.g., *Ursus americanus*, *Ursus arctos*) with a high-caloric food source but garbage-conditioned bears tend to incur conflicts with human, such as property damage and threats to human safety (McCarthy and Seavoy 1994, Peirce and Van Daele 2006). Likewise, deer may be plentiful in the WUI but a cougar (*Puma concolor*) hunting for natural prey may encounter hobby livestock or a domestic dog instead (Colorado Parks and Wildlife unpublished data). Both examples are conflicts that prompt a response by a managing authority, which may include relocation and possibly lethal removal, especially for individuals found to habitually forage within the WUI (Denali National Park and

Preserve 2003, Wyoming Game and Fish Department 2006, Biederbeck et al. 2012). In more remote regions, carnivore interaction with humans is less direct shifting the focus from individual animals to the population scale. In general, populations are monitored or managed to address species conservation concerns. For example, rare or threatened species are managed with the goal of recovery (as per the Endangered Species Act of 1972). Carnivores that are subject to harvest, such as cougars, are managed to minimize human conflicts as well as to maintain a stable population and a persistent resource for hunters (Wyoming Game and Fish Department 2006, Shivik and Sweanor 2014). Whether the interest is to address conflicts in the WUI or to monitor populations over vast expanses of wilderness, understanding more about the biology of an animal will likely aid in any decision making process (Bateman and Fleming 2012).

Population demographics, such as abundance and age structure, are common metrics used to inform various conservation and management decisions. However, accurately and precisely estimating population parameters of cougars, and wild animals in general, is often difficult because all individuals in the population are rarely captured, or detected, with certainty ($p < 1.0$) or equally. When capture heterogeneity is not incorporated into commonly used models, such as closed-capture–recapture (C–R) models (Otis et al. 1978, see Williams et al. 2002), the estimator may overstate precision and include bias (Link 2003).

Much is already known about cougar behavior (see Logan and Sweanor 2001) to suggest that capture variation is possible and likely. Resident adult home-range sizes vary between season (Seidensticker et al. 1973), sex (Dickson and Beier 2002), and female reproductive status (Hornocker 1969). Males generally occupy larger areas than females (Logan and Sweanor 2001), but female home-range sizes are more variable (Hornocker 1969). Females with small

kittens will be less mobile (Seidensticker et al. 1973). Activity varies relative to time of day (Sweanor et al. 2008), and scrapes suggest a pattern in how cougars travel (Hornocker 1969). Movements differ among behaviors such as hunting, feeding, mating (Beier et al. 1995), and transient activity (Lindzey et al.1994).

Detection parameters are conditional on the sampling method applied and may vary by factors such as age, sex, or individual. Accommodating multiple detection parameters requires an adequate sample size which may not be achievable with an elusive species, such as cougars (MacKenzie et al. 2005, Gerber et al. 2014). A few C–R models can incorporate capture heterogeneity but often include small-sample bias in abundance estimates (e.g., Burnham and Overton 1978, Huggins 1989, Pledger 2000, Dorazio and Royle 2003, Link 2003). A trap response (a positive or negative behavioral response to the trapping event) is another common source of capture variation that will bias the estimate of abundance (Pollock et al. 1990). Thus, sampling protocols should strive to achieve the highest possible detection probability by ensuring that all individuals are detected at least once (Gerber et al. 2014), but also attempt to eliminate or greatly reduce sources of capture heterogeneity (Link 2003).

Generally, C-R models also require all animals to be individually identifiable. Several species-specific methods, such as pelage pattern (Karanth and Nichols 1998), an applied mark, (e.g., Pendleton et al. 2006, Baker et al. 2007, Bestgen et al. 2007, Kendall et al. 2009), or the use of DNA (e.g., Woods et al. 1999, Mowat and Strobeck 2000, Ruell et al. 2009, Williams et al. 2009) are used to identify individual animals. With regard to cougars, applying marks and acquiring genetic material in the form of tissue and blood have required that animals be handled, at least briefly, making these methods inherently invasive (Pauli et al. 2010) and a negative experience likely to result in variation in recapture due to a trap response (Pollock 1990). Over

time, capture techniques have improved considerably but observing individual cougars over multiple occasions remains difficult, time consuming, and expensive (Anderson et al. 2004).

Because cougars leave sign, and exhibit behaviors like territoriality and curiosity, a noninvasive-sampling method may be an appropriate alternative (Long et al. 2008). Noninvasive methods allow individuals within a population to be sampled without needing to be handled or observed (Taberlet and Luikart 1999, Garshelis 2006, Beja-Pereira et al. 2009, Pauli et al. 2010). Many noninvasive methods to assess cougar demography are already being used and vary depending on the objective of the study. Track surveys and camera traps can be used to verify occupancy or suggest general population trends (Sargeant et al. 1998, Choate et al. 2006, Gompper et al. 2006). However, individually identifying animals by their tracks is extremely difficult (Van Dyke et al. 1986) and cougars have a uniform pelage thus identification via photographs is typically impossible. Obtaining a genetic sample, such as hair, can provide the DNA necessary to yield an individual identification via genotype (Taberlet and Luikart 1999).

A commonly used NGS method to obtain hair from cougars is to apply an attractant to a hair-snaring device (Long et al. 2008). Many novel hair snares have been tested in combination with scent lures, but their effectiveness remains low. For example, an 11-year study used scent lures to obtain hair samples and confirmed 19 samples as cougar (Lang et al. 2013) but other efforts failed to attract a cougar via scent lures (Long et al. 2003, Choate et al. 2006). Although scents are typically used, felids might exhibit a greater response to auditory cues (Chamberlain et al. 1999), suggesting that a predator call may be a more effective attractant.

Currently, challenges exist when using hair as a DNA source, thus the hair-snaring technique must yield an adequate sample. Overall, hair has less extractable DNA than tissue and blood, resulting in potentially high error rates during the genetic analysis (Taberlet and Luikart

1999). Though it is possible to have DNA incorporated into the hair shafts, especially in species that self-groom (Alberts et al. 2010), the quality and quantity of DNA extracted from a hair-shaft source is highly variable (Bengtsson et al. 2012). Obtaining the root tissue is preferred (Bengtsson et al. 2012). At least 10 plucked hairs are needed to minimize the chance for genotyping errors, such as allelic dropout (Goossens et al. 1998). However, the hair snares generally used to sample felids (McDaniel et al. 2000, Weaver et al. 2005, Harrison 2006, McKelvey et al. 2006, Schmidt and Kowalczyk 2006, Long et al. 2007, Sawaya et al. 2011, Lang et al. 2013) tend to snag shed hair which might not have a follicle. Therefore, improvements in hair-snaring techniques are needed to ensure accurate genotyping.

Prior to this study, extensive resources have been dedicated to the development of noninvasive techniques; however, a cost-effective method that can comprehensively sample a cougar population had yet to be realized. My objective was to find or develop a NGS technique using attractants and hair snares with minimal sampling variation. After initial testing, the most effective lure and hair snare would be assessed by determining the demographic of cougars sampled, estimating detection probabilities, and by the ability to establish capture histories using the method. Here, I describe the components of a noninvasive technique as potential sampling tools to obtain various biological data of a cougar population.

## STUDY AREA

I sampled cougars in two study areas in Colorado, USA (Figure 1). The primary study area was on the Front Range (FR) and a smaller one on the Uncompahgre Plateau (UP). All aspects of this study were approved by the Institutional Animal Care and Use Committee at Colorado Parks and Wildlife (CPW) (protocol #02-2012). The 1,270 km² study area on the Front

Range is located in Boulder, Jefferson, and Gilpin counties. This area is bordered to the east by Highways 36 and 93, to the west by the Peak to Peak Hwy, to the north by the Boulder county line, and to the south by Interstate 70. Though interspersed with private parcels, much of this land is public and managed by Boulder City, Boulder County, Jefferson County, CPW, the Bureau of Land Management (BLM), and the US Forest Service (USFS). The study sites on the Front Range spanned in elevation from 1,690 to 2,868 m. The average monthly precipitation during the study (Feb. – Mar., 2012 and Nov., 2012 – Mar., 2013) was 25.4 mm and the average monthly temperature was -0.2 °C, using climate data collected at 29 National Oceanic and Atmospheric Administration (NOAA) weather stations in or near the study area. The dominant canopy species included ponderosa pine (*Pinus ponderosa*), Douglas fir (*Pseudotsuga menziesii*), and Rocky Mountain juniper (*Juniperus scopulorum*). The understory vegetation included mountain mahogany (*Cercocarpus montanus*), three-leaf sumac (*Rhus trilobata*), bitterbrush (*Purshia tridentata*), Gambel's oak (*Quercus gambelii*), and serviceberry (*Amelanchier alnifolia*). Besides cougars, other medium to large-bodied mammals, such as elk (*Cervus elaphus*), mule deer (*Odocoileus hemionus*), black bears (*Ursus americanus*), coyotes (*Canis latrans*), red foxes (*Vulpes vulpes*), bobcats (*Lynx rufus*), ringtails (*Bassariscus astutus*), American martens (*Martes americana*), and on occasion, unleashed domestic dogs (*Canis lupus familiaris*), occurred in the region.

The 540 km² study area on the Uncompahgre Plateau was located west of the city of Montrose in Montrose and Ouray Counties. Exact boundaries were determined by historical deer and elk winter range data (CPW unpublished data) as well as location data for resident cougars (K. Logan unpublished data). The sites ranged in elevation from 1,704 m to 2,479 m. The average monthly precipitation during the study (Dec., 2012 – Mar., 2013) was 22.3 mm and the

average monthly temperature was -1.3 °C, using data collected at six NOAA weather stations in or near the study area. The vegetation was predominantly pinyon-juniper woodlands with Gambel's oak, serviceberry, and mountain mahogany understory, and a mix of ponderosa pine at higher elevations and sagebrush (*Artemisia tridentata*) at lower elevations. The area was comprised largely of public lands managed by the BLM or the USFS. Similar to the Front Range, other medium to large-bodied mammals, such as elk, mule deer, coyotes, red foxes, bobcats, ringtails, and domestic dogs, occurred in the area.

## METHODS

### *YEAR ONE*

#### *Attractant and hair snare evaluation*

From February to April, 2012, I tested attractants and hair snares at lure sites in the Front Range study area. I selected the sites by first choosing four easily-accessible, 100-km² quadrats (Figure 2). Each quadrat was comprised of twenty-five 4-km² parcels. Using a spatially balanced random sampling function in ArcGIS® v.10.0 (Esri, Redlands, California, USA) (Stevens and Olsen 2004, Theobald et al. 2007), I chose 4 – 6 parcels in each quadrat. I selected the exact site location to avoid areas with human activity, to include specific landscape features (ridgelines, saddles, drainages, canopy cover, and tree line edges), and to comply with restrictions imposed by city and county officials.

I assessed scent lures and auditory calls by establishing four types of sites that varied by whether they included a scent lure, a call, neither, or both (Table 1) and tested hair-snaring techniques at each type of site. When possible, the attractants were assigned randomly so that each quadrat had at least one of each site type. Random allocation of attractants was not possible

in certain locations with moderate to high human use due to permit restrictions prohibiting the placement of auditory calls. I tested two scent lures, Pikauba® (Leurres Forget Lures, Mauricie, Quebec, CA), a scent used to attract lynx (G. Merrill personal communication), and Russ Carman's Canine Call® (Sterling Fur Company, Sterling, Ohio, USA), a lure that has proven useful for attracting carnivores (Crooks 2002). I applied the lure to a scratch pad that consisted of a board (14 cm x 14 cm); cotton batting or a carpet swatch; and a piece of metal altered to snag hair (Turbak 1998; Figure 3). Scratch pads were nailed to a tree at an average height of 55 cm. I also placed a scratch pad with catnip (*Nepeta cataria*) and a visual lure (aluminum pie pan or compact disc) at each site.

At selected sites, I used a predator call with a distressed fawn sound set to play a five-second recording at 30-second intervals as the auditory attractant (Wasatch Wildlife Product® FurFindR®, Magna, Utah, USA). The calls transmitted at a frequency of 2.5 – 3.8 kHz, at an average decibel level of 103.5 dB, and were equipped with light sensors which turned them off during daylight hours. Calls were secured to a tree in plain view at a height of ≈ 2.5 m and were initially used as attractants only. During year one, no hair snare was associated with the device.

I tested a hair snare (metal mesh cube with a 25 cm edge and open on one end) with a 0.1 – 0.2 kg piece of meat wired in the back (Figure 4). To snag hair, I attached a 20 cm spring and a 13 cm barrel cleaning brush. The snares were secured to trees and the height was altered relative to cougar response (0 – 95 cm). I deployed this hair-snaring device at all sites, thus all sites included the natural scent of carrion.

I documented cougar activity through photographs obtained via infrared motion-sensor cameras (Reconyx® PC85 Rapidfire® or PC800 Hyperfire®, Holmen, Wisconsin, USA) programmed to take five photos in rapid succession when triggered. All sites were checked

weekly and as necessary, hair samples were collected, baits and scents were replenished, and new batteries were placed in cameras and calls.

I counted detections (site visits) by site per cougar per night and confirmed with photographs. I counted an individually marked cougar that returned multiple times to the same site within a single night (dawn till dusk) as one detection. Similarly, because I could not differentiate unmarked cougars via photograph, I assumed all activity by unmarked individuals in one night was by the same animal and counted its activity once. I counted adults traveling together separately but considered females with dependent kittens as a single detection. To ensure the camera was functioning properly, I programmed the time lapse on each camera to take a nighttime photo at 00:00 and a daytime photo at 12:00 daily and confirmed that my activity triggered the motion-sensor. I centered the camera's field of view on the entry way of the cubby. A cougar approaching the site from the opposite side might not have been photographed, thus undocumented visits were possible.

In addition to confirming visits by cougars, I used photographs to verify the most effective lure and snare combination by quantifying the number of times the cougar responded to the attractant in a manner such that a sample could have been acquired, e.g., a cougar photographed at the site was observed rubbing the scratch pad.

*YEAR TWO*

*Attractant and hair snare evaluation continued*

November, 2012 to April, 2013, I continued testing select lures and hair snares but modified the method based on year one observations. I used the same 100-km² quadrats and site selection protocol to choose 12 new site locations for each of three more sampling periods. The combined sampling effort for years one and two, included 68 sites established over four

sampling periods. The average length of each sampling period was 31.6 days (2,149 survey nights total).

I did not observe a rubbing response to Pikauba® and Canine Call® during year one; therefore, I applied beaver (*Castor canadensis*) castoreum (Minnesota Trapline Products, Inc. Pennock, Minnesota, USA), a scent found to attract cougars and other felids (McDaniel et al 2000, K. Logan personal communication) to the scratch pads described above. In addition, I simplified the sites by discontinuing the use of catnip and visual lures. Initial observations suggested these components were minimally effective, and the visual lures drew people to the sites.

Photographs indicated cougar interest in the calls during year one, so I incorporated a hair-snaring technique with the calls the following year. I constructed stick cubbies to conceal the call hidden in the back (Figure 5). To snare hair, I placed a two or four-pronged strand of barbed wire and a cable with a roller (15 cm long ¾" PVC pipe) coated with a sticky substance (Tree Tanglefoot®, Grand Rapids, Michigan, USA) across the cubby entry and modified the height based on cougar response (Figure 6). At the end of the study, the average height of the wire and roller was ≈ 28 cm. To further entice a cougar to enter the cubby, I added a 0.1 – 0.2 kg piece of meat and suspended a feather inside the cubby.

Year one observations suggested the baited cube snare was ineffective; therefore, I discontinued testing the device during year two. However, I maintained the bait only sites with inaccessible bait and placed a small piece of bait near the scratch pads so that all sites still had a bait component, consistent with year one.

*Predator call and cubby evaluation*

Year one observations suggested auditory calls and cubbies were the most effective hair-snaring combination. Therefore, I applied the technique on a broader scale to include two study areas, the Front Range (FR) and the Uncompahgre Plateau (UP) in addition to the continued testing of the various attractants described above. In total, I established 148 sites. Each site was active an average of 28.5 days (ranging from 20 – 36 days) for a total of 4,214 sampling nights. Specifically, on the FR, I sampled cougars over four sampling periods. The sites were active an average of 28.4, 29.9, 27.7, and 28.1 days respectively for a total of 2,679 sampling nights. On the UP, I sampled cougars over three sampling periods. The sites were active an average of 29.8, 29.0, and 26.4 days respectively for a total of 1,535 sampling nights. Less effort was applied to the UP study area due to resource limitations.

I chose site locations by first overlaying a grid with 4 km² cells (Figure 1). I randomly selected grid cells without replacement using the spatially balanced points function in ArcGIS® 10.0 (Esri, Redlands, California, USA; Stevens and Olsen 2004, Theobald et al. 2007). During concurrent studies conducted by CPW researchers, cougars were previously captured, marked with unique ear tags, and collared with devices containing global positioning system (GPS) technology (M. Alldredge unpublished data, K. Logan unpublished data). On the FR, more collared animals were monitored in the eastern half of the study area. Because I used the location data of previously marked cougars to estimate detection probabilities, I divided the study area north to south and concentrated most of my effort in the eastern region. I established 15 – 19 sites in the eastern sector (763 km²) and six sites in the western sector (507 km²) per sampling period for a total of 94 sites. On the UP, I applied the same spatially balanced points function to select 18 sites per sampling period for a total of 54 sites. As above, I selected the

exact site location to include specific landscape features (canyon rims, canyon bottoms, ridgelines, saddles, drainages, and tree line edges), to comply with research permit restrictions, such as raptor nesting closures, and to avoid areas with high human activity. In general, I avoided residential areas, trails, and highways to evade negative human interactions and traffic related mortality. Access limitations due to winter snow conditions also influenced site placement. Furthermore, to reduce capture variation, keep the attractants novel, and attempt to accommodate the activity patterns of all cougars, I moved the sites between sampling periods (Mowat and Strobeck 2000, Boulanger et al. 2006).

The site components were consistent in both regions and all sites were designed and constructed by me. Auditory predator calls (Wasatch Wildlife Product® FurFindR®, Magna, Utah, USA) were secured to a large object, such as the base of a tree or a log, and concealed within a stick cubby. Hair-snaring devices stretched across the entry way at an average height of ≈ 28 cm included a four-pronged strand of barbed wire and a cable with one or two rollers (15 cm long ¾" PVC pipe) coated with Tree Tanglefoot® (Grand Rapids, Michigan, USA) (Figures 6 & 7). As above, I added a 0.1 – 0.2 kg piece of deer meat and suspended a feather. I documented cougar activity using photographs (see Photograph Supplement). The cameras were programmed and the sites were checked consistent with the methods described above.

To obtain a rough estimate of the effective distance of the calls I considered detections relative to a cougar's proximity to a site. The GPS collars were programmed to record a location every three or four hours. I determined the closest recorded GPS location of the individual to a site during the entire time the site was active and noted whether it was ultimately detected (Figure 8). The distance at which the probability of attracting a cougar to a site dropped to almost zero was considered the maximum effective distance of the calls. I assessed whether the

approximated effective distance was a reasonable estimate by comparing the hearing capability of the domestic cat (*Felis catus*) (Heffner and Heffner 1985) to the predicted decibel level. Sound decay was predicted using the model

$$L_2 = L_1 - 10\ log_{10}\ \left(\frac{{r_2}^2}{{r_1}^2}\right)$$

($L_1$ is a reference decibel level at distance $r_1$ and $L_2$ is the predicted decibel level at distance $r_2$ (Crocker 1998)).

*Genetic analysis*

Hair was collected and analyzed via strict protocols. To minimize sample contamination and degradation. I used sterile tweezers and re-sterilized the barbs by fire (Kendall et al. 2008, Settlage et al. 2008). I considered the hair on a single barb as a discrete sample and placed each in a separate paper envelope (Poole et al. 2001, Dreher et al. 2007). I collected hair from each roller separately and replaced contaminated rollers. I then put the hair samples in paper envelopes in a plastic bag with a desiccant and stored the bags at room temperature (Taberlet and Luikart 1999).

I attempted to genotype each hair sample at least twice using extraction and amplification protocols effective for felid scat analysis. Because 8 U template DNA is needed to achieve a correct genotype at a 99% confidence level (Taberlet et al. 1996) and 1 U is equivalent to the DNA content of one diploid cell, when possible, I extracted DNA from ≈ 10 hairs (Goossens et al. 1998, Boersen et al. 2003) using Qiagen DNeasy® Tissue Kits (Qiagen Inc., Valencia, CA). However, the barbed wire occasionally caught tufts of extremely fine hair that appeared to include skin cells (see Photograph Supplement). Whole tufts with ≥ 20 hairs were used in the extractions for these samples. I genotyped the samples using microsatellite loci from the domestic cat (Menotti-Raymond et al. 1999) at 10 loci (FCA096, FCA035, FCA043, FCA149,

FCA026, FCA057, FCA090, FCA132, FCA254, and B207-2) shown to have high variability in cougars (Menotti-Raymond and O'Brien 1995, Ernest et al. 2000, Sinclair et al. 2001, Anderson et al. 2004). I amplified the DNA by polymerase chain reaction (PCR) using the cost-effective, universal M13–tailed forward primers (Boutin-Ganache et al. 2001) found to be effective at amplifying DNA from cougar scat samples (S. Oyler-McCance personal communication). I analyzed the PCR amplicons via GeneMapper®. In general, the resulting PCR product from the hair samples did not yield clear genotypes. Therefore, I tried individual dye-labeled primers specific to each loci and my results improved but not for the samples where DNA was extracted from tufts containing > 20 hairs, possibly due to an excess of melanin, a PCR inhibiting substance (Uchihi et al. 1992). When possible, I reran these samples with 10 – 15 hairs. To assess error, I compared the genotypes obtained from the hair samples with archived blood and tissue samples previously collected from the GPS-collared cougars. Additionally, I estimated the probability of successfully genotyping the hair samples collected ($\hat{p}_G$). This estimate excludes family groups and considers the outcome for samples that were exhausted during the protocol development.

*Estimation of detection probability*

I used the marked cougars to estimate detection probabilities. Ear tags allowed animals to be visually identifiable, and GPS location data verified a cougar's position in or out of the study area and relative to each site. I used location data to estimate the probability of detecting an independent (adult) marked cougar by camera, given it was available to be detected on three temporal scales.

Foremost, I estimated a study-wide detection probability for a superpopulation of animals moving freely on and off of the grid for each study area, with no assumption of closure. I used

the total number of marked animals (*N*) confirmed to be in the study area at any time during the study and the number detected (*n*), via photograph, at least once to estimate the detection probability (*p*) via the simple binomial model,

$$\hat{p} = \frac{n}{N}$$

GPS location data revealed that many cougars were not available, or within the boundaries of the study area, 100 % of time. Because the density of animals in a defined area is often of interest (Ivan et al. 2013), I estimated the probability of detection adjusted for partial availability using the model,

$$\hat{p}_{adj} = \frac{n}{\sum_{i=1}^{N} a_i}$$

where $a_i$ is the proportion of time cougar *i* was in the study area.

Because the sites were moved between sampling periods, I also estimated the probability of detecting a cougar at least once per sampling period (≈ 28.5 days) with no assumption of closure. I used the total number of marked animals confirmed to be in the study area at any time during the sampling period (superpopulation) and the number detected, by photograph, at least once.

In addition to the study-wide and sampling-period estimates, I considered detections relative to individual sites. On the FR, I estimated the probability of photographing a cougar at least once at a site given it recorded at least one GPS data point within a 2-km buffer region around the site. Two kilometers was chosen based on the distance between sites and due to cougar movement relative to the fix rates of the collars. I estimated the average detection probability among sites ($\hat{p}$), using only sites *i* with available cougars (*x* = 65).

$$\hat{p} = \frac{\sum_{i=1}^{x} \hat{p}_i}{x}$$

I estimated the variance across all sites using a variance components approach where process variation [$var\ (p_i) = \sigma^2$] was estimated by subtracting the sampling variance [$var\ (\hat{p}_i|p_i)$] from the total variance [$\hat{var}\ (\hat{p})$].

$$\sigma^2 = \hat{var}\ (\hat{p}) - E[var\ (\hat{p}_i|p_i)]$$

where

$$\hat{var}\ (\hat{p}) = \frac{\sum_{i=1}^{X}(\hat{p}_i - \hat{\bar{p}})^2}{(x-1)}$$

and

$$E[var\ (\hat{p}_i \mid p_i)] = \frac{\sum_{i=1}^{x}\left(\frac{\hat{p}_i(1-\hat{p}_i)}{N_i}\right)}{x}$$

(Gould and Nichols 1998).

This estimator did not consider the degree of availability. For example, a cougar that recorded one data point within the buffer zone was tallied once and a cougar that recorded 10 data points was also considered available once. Likewise, a cougar that visited a site multiple times was only counted as detected once. Because this estimate was site-specific, a cougar visiting two different sites was counted once per site. Due to a lower fix rate of the GPS collars in the UP study area, fine scale cougar movements relative to the sites could not be determined. As a result, I was unable to conduct the site-specific analysis with the UP data.

The GPS collars were programmed to record location data at the optimal rate to meet the primary research objectives of the concurrent study. On the FR, the GPS collars were programmed to record seven data points per day (1:00, 4:00, 7:00, 11:00, 15:00, 19:00, and 22:00). Occasionally, data points were not recorded, introducing a potential source of error in the detection probability estimates. For the duration of this study, the success rate for the 16

deployed GPS collars ranged from 82% to 99%. On the UP, the collars were programmed to record a location twice daily, 07:00 and 19:00 but the incidence of missed fixes was high for some collars making fine scale availability difficult to determine. Here, the success rate for the 10 deployed GPS collars ranged from 38% to 89%. Consequently, collars failed to record both daily GPS data points, on average, 13% ± 12% (1 SE) of the time. To address the gaps in the location data, I assumed availability based on the location of the individual prior to and after the missing data. For example, if the cougar was clearly in the study area the day before and the day after the void, it was considered available all days. Detections were counted as described above.

Although some of the components of this technique were fluid throughout the study due to methods development, using the Front Range data set, I estimated the overall conditional probability of identifying, via genotype, any cougar in the study area ($\hat{p}_{\mathrm{I}}$).

$$\hat{p}_{\mathrm{I}} = \hat{p} * \hat{p}_E * \hat{p}_H * \hat{p}_G$$

$\hat{p}_I$ is the product of: the probability that a cougar in the superpopulation was observed via photograph at least once for the duration of the study with no assumption of closure ($\hat{p}$); the probability that the cougar entered the cubby given that it was photographed ($\hat{p}_E$); the probability of obtaining a hair sample given that the animal entered the cubby ($\hat{p}_H$; using only sampling periods 2 – 4); and the probability of accurately genotyping the hair sample ($\hat{p}_G$). The variance ($\hat{var}(\hat{p}_I)$) was estimated using an unbiased estimator for the variance of a product (see Goodman 1960). Additionally, because the assumption of closure was not met during the study due to deaths, periodic marking events, and movement across study area boundaries, I assessed the number of detections relative to availability, or the proportion of time in the study area.

Various goals for operational monitoring programs exist. Such objectives include observing unique cougars only once or re-sighting individuals multiple times. Therefore, I

assessed whether a temporal trend existed for: the probability of first observing an individual marked cougar; the overall occurrence of site visits by all independent adult cougars; and the incidence of initial site visits relative to re-sights.

## RESULTS

### *ATTRACTANT AND HAIR SNARE EVALUATION*

Photographs recorded 57 detections by 14 uniquely marked adults, an unknown number of unmarked cougars, and one known sibling group. Marked adults accounted for 34 of the 57 detections (2.43 detections per adult), unmarked cougars comprised 21 detections, and the known sibling group visited twice. Photos documented five detections at sites with just bait, 12 at sites with bait and a scent, 15 at sites with bait and a call, and 25 at sites with all three components (Table 1). Seven of the 12 detections at sites with bait and a scent occurred at a single site over a short time period. Three of these seven visits were by the same marked subadult male and the other four were suspected to be by the same unmarked subadult. I detected cougars at 24% of sites with bait only, 28% of sites with bait and scent, 50% of sites with bait and calls, and 59% of sites with all three. Of the four types of sites, I compared the probability of detecting $\geq 1$ cougar at a site where a call was present ($0.55 \pm 0.09$) to the same probability of detection at a site without a call ($0.26 \pm 0.08$). The 95% confidence interval for the difference (0.07, 0.51) suggested that adding a call significantly increased the probability of detecting a cougar in this study. I also compared the probability of detecting $\geq 1$ cougar at a site where a scent was present ($0.43 \pm 0.08$) to the same probability of detection at a site without a scent ($0.36 \pm 0.08$). Although this suggests a slight benefit to using a scent lure, the 95% confidence interval for this difference (-0.16, 0.30) overlapped zero and thus indicated adding a

scent was not statistically significant. Of the 14 known adults detected, I observed two at sites with bait only, three at sites with bait and scent, seven at sites with bait and calls, and nine at sites with all three attractants (bait, scent, and call). Some cougars visited multiple sites, and 13 of the 14 individuals observed during the study approached sites where a call was present.

I used photographs to observe a cougar's response to the various lure-snare combinations (see Photograph Supplement). Consistent with visit patterns, behavior recorded in photos also indicated a greater interest in calls than scents. During the first sampling period, I secured calls high in a tree to broadcast the sound farther. Photos documented cougars looking up towards the calls and/or attempting to climb the tree. On several occasions, photos indicated cougars playing with or carrying the calls away. In contrast, photos showed little visible interest in scents. Many scratch pads seemed to be ignored during a cougar visit. Of the 37 detections at sites with the scent, I only observed the cheek-rubbing response characteristic of felids (Reiger 1979) on two occasions; I obtained hair both times. Of the detections at sites with just bait, a cougar attempted to get the bait during three of the five site visits. However, the cube-hair-snaring device was ineffective (see Photograph Supplement).

The cubby hair snare-design was more comprehensively evaluated during year two. The results are described below. A subset of the overall call and cubby dataset obtained via 24 selected sites was used for the attractant and hair-snare analysis described here. The sites were chosen as they were located within the four 100-km$^2$ quadrats established for the purpose of testing the attractants and hair snares. Additionally, a hair snare was not associated with the call during the first year of the study, so cougar response to the call-snare combination could only be assessed in the second year, once the sampling method had evolved to hide the call in a cubby. Thus, at the 24 selected sites established during year two, photos recorded 23 visits. Of the 23

site visits, a cougar responded to the call by entering the cubby 16 times; 14 out of 16 yielded hair samples.

*PREDATOR CALL AND CUBBY EVALUATION*

*Front Range*

Cameras detected cougars at 49 out of the 94 sites. Photos documented cougars during all four sampling periods noting on average, 4.64 detections per week. Photos confirmed 98 detections by 13 independent marked cougars (11 females and two males), two sibling groups, and an unknown number of unmarked animals. Independent marked adults accounted for 37 of the 98 detections, the sibling groups were detected four times, and the unmarked cougars comprised the remaining 57 detections (Table 2). Many of the independent, marked adults were photographed multiple times. Cameras documented two cougars five times, two animals four times, five individuals three times, and four were observed once. Also, four of the independent adults observed were females with kittens.

I determined the approximate effective distance of the calls, given the fix rate of the collars (every three or four hours), to be ≈ 550 m radius of the site (Figure 8). A cougar with the closest recorded GPS location to a site ≥ 550 m, in general, was not detected. The probability of detecting a marked cougar at least once at a site, given that it was known to be < 550 m of that site was 0.38 ± 0.07. The domestic cat can hear a frequency of 2 – 4 kHz at ≈ 2 dB (Heffner and Heffner 1985), which is considerably less than the predicted decibel level of ≈ 14 dB at 600 m. The predicted level was optimal as the estimate does not include environmental variables such as vegetation, humidity, and wind. However, given the predicted sound decay level along with the lack of detections of cougars passing beyond ≈ 550 m, the estimated effective distance of 550 m was probably reasonable.

I estimated the probability of detecting an independent marked cougar (n = 20, superpopulation) given that it was in the study area at any time during the study (year two) to be 0.65 ± 0.11 (1 SE). Not all cougars were in the study area the entire time (Table 3). Three of the seven cougars not detected (AM49, AM124, and AM98) were marked mid-study making these individuals available for a limited period (9, 11, and 31 days out of a possible 148 days), but it is possible that these cougars visited a site prior to being captured and marked. Likewise, two of the cougars not detected (AM74 & AF62) died during the study (available 46 and 94 days) and one cougar (AM13) had a nonfunctional collar so the actual time in the study area could not be confirmed. Only one cougar not detected (AF73) was available 100% of the time. The probability of detection adjusted for partial availability (used in density estimation) was 0.83 ± 0.10.

I detected six or seven unique adult marked cougars per sampling period. The total number of detections was similar for the first, second, and third sampling periods (27, 30, and 25) but declined during the fourth sampling period (16) (Table 4). The probability of detecting an independent marked cougar for each sampling period (28 – 30 days) with no assumption of closure (n = 4; ± 1 SE) was 0.38 ± 0.15, 0.39 ± 0.13, 0.35 ± 0.13, and 0.35 ± 0.13.

The probability of detecting a cougar at a site at least once during the time the site was active given that it recorded at least one GPS data point within 2 km of the site was $\hat{p}$ = 0.14. I estimated the variance components as: $\hat{\text{var}}(\hat{p}) = 0.077$, $\text{E}[\text{var}(\hat{p}_i|p_i)] = 0.018$, and $\sigma^2 = 0.059$, indicating that most of the variation observed was due to process variance ($\sigma^2$) and not sampling variance ($\text{E}[\text{var}(\hat{p}_i|p_i)]$). Only sites where at least one cougar was available to be detected were used in the analysis (65 out of 94 sites). I observed four instances where a cougar was present in the study area but did not record a data point within the 2 km buffered zone of any

site during the sampling period. As a result, given the density of the sites, moving the sites between sampling periods was necessary to ensure all marked cougars were available to be detected.

Of the 98 detections, I collected 52 hair samples. Ten samples may have included hair from multiple cougars, as more than one cougar visited and entered the site over the seven-day period between site checks. Photographs indicated that not all cougars entered or exited the cubby through the entry way. The probability of a cougar passing through the entry way given that it was detected ($\hat{p}_E$) was 0.77 ± 0.04 (1 SE). Unmarked cougars (0.90 ± 0.04) had a higher probability of entering the cubby than marked animals (0.65 ± 0.08) (Table 2). To assess temporal variation, I estimated the probability of a cougar entering the site per sampling period (Table 4). Because I modified the hair-snaring technique after period one by changing the configuration of the wire, switching from two-pronged to four-pronged barbed wire, and lowering the height of the wire and cable, the probability of obtaining a sample more than doubled for the remainder of the study (Table 4). Using only observations from sampling periods 2 – 4, the probability of obtaining a hair sample given that the animal entered the site ($\hat{p}_H$) was 0.93 ± 0.03. The approximate probability of genotyping a hair sample ($\hat{p}_G$) was 0.76 ± 0.06. The overall conditional probability of identifying ($\hat{p}_I$), via genotype, any cougar in the study area at least once for the duration of the study, was 0.35 ± 0.07. The probability of identifying a cougar per sampling period was 0.20 ± 0.07. Additionally, the plot of detections relative to availability illustrates that in general, cougars were more likely to be detected as availability on the sampling grid increased (Figure 9).

The probability of first observing a unique marked cougar declined considerably after the second sampling period (Figure 10). Almost all of the marked cougar observations during the

third and fourth sampling periods were of previously sighted cougars (Figure 11: B). Cougars continued to visit the sites throughout the study, with the incidence of site visits declining slightly over time (Figure 11: A).

*Uncompahgre Plateau*

Cougars were photographed during all three sampling periods and were observed at 12 out of the 54 sites. On average, I observed 1.24 detections per week. Cameras documented 18 detections (Table 5) by seven uniquely marked cougars (six females and one male) and no unmarked cougars (Table 6). Five cougars were photographed multiple times. Eleven marked cougars used the study area but no cougar was in it the entire time. The time spent ranged from 2 – 98 days (out of a possible 102 days). The four cougars not detected were available from 2 – 18 days. Those detected were available ≥ 31 days (range 31 – 98) or in the study area ≥ 30% of the time. The probability of detecting a marked cougar in the superpopulation during the study given that it used the study area at least part of the time (n = 11) was 0.64 ± 0.15. The detection probability estimates included the two cougars harvested within the first few weeks of the study (F152 & M179) and the five cougars fitted with GPS collars mid-study. Consequently, due to the low availability of many cougars, the probability of detection adjusted for partial availability was estimated to be 1.0. The probability of detecting an independent marked cougar for each sampling period with no assumption of closure (n = 3; ± 1 SE) was 0.57 ± 0.22, 0.71 ± 0.20, and 0.33 ± 0.18. Unlike the FR analysis, availability was not considered relative to each site due to too few GPS location data points recorded per day. The probability of a cougar entering or exiting the cubby through the entry way given that it was detected via photograph was 0.56 ± 0.04 and the probability of obtaining a hair sample given that the cougar passed by the hair

snaring devices was 0.80 ± 0.15. I collected seven hair samples. One potentially contained hair from multiple cougars.

*Genetic analysis*

In total, I collected 59 hair samples. I discarded eleven as they potentially contained the DNA of multiple cougars. One sample was lost. I processed 47 single-cougar samples. I genotyped 32 samples at ≥ 8 loci. The remaining 15 samples either produced partial genotypes or were exhausted during the first attempt when the M13–tailed forward primers and tufts > 20 hairs were used. I identified 26 unique cougars. Sixteen were unmarked animals and 10 were marked. I identified five cougars multiple times. I observed one unmarked cougar at three different sites. I compared the genotypes of 13 hair samples of marked cougars to genotypes derived from blood samples. Using all 10 loci, I found five single-locus errors, or mismatches, resulting in an error rate of 0.06 ± 0.03. After censoring one loci (B207-2), where three of the five mismatches were observed, the error rate was 0.03 ± 0.02.

## DISCUSSION

This study was the first to consistently detect and obtain hair samples from cougars at lure sites using attractants and hair snares. The optimal sampling technique to both attract a cougar to a site and obtain a genetic sample (hair) in this study was an auditory call concealed within a stick cubby. Past studies using scent lures have yielded few if any cougar visits, and as a result, precise population questions were unable to be addressed (Long et al. 2003, Choate et al. 2006, Sawaya et al. 2011, Lang et al. 2013). I found that randomization in site placement was possible using an auditory call with a cubby, thus eliminating the bias associated with using prior knowledge and convenience sampling approaches (Anderson 2001). The closed C–R models

commonly used to estimate abundance require encounter histories for each individual over two or more occasions (see Williams et al. 2002). Calls with cubbies sampled many different individuals throughout the population, even given the elusive nature and large home-range sizes typical of cougars. Many individuals were detected on multiple occasions. Although I do not attempt to estimate population parameters here, I found that, given the successful genotyping of hair samples, it may be possible to build the encounter histories needed for population assessments using calls and hair snares. Furthermore, certain components of this noninvasive method may be useful in addressing other research objectives. For example, hair can also be used to assess the dietary components of an individual via stable isotopes (Hopkins et al. 2012, Moss et al. 2015) and a telomere analysis of the DNA may indicate age (Pauli et al. 2011).

I photographed cougars and obtained hair samples in both the FR and the UP study areas. Consistent in both regions, almost every marked cougar confirmed to be in the study area > 30 % of the time was detected via cameras at least once. Many were detected multiple times and at various sites, increasing the potential for estimating *p* with minimal trap response. Only one cougar with 100 % availability was not photographed. However, GPS location information suggested that the cougar may have approached several sites but did not come within the camera's field of view, so the individual was never photographed. Detections were confirmed via photographs alone, and the camera was positioned on only part of the cubby. As a result, undocumented cougar visits were possible and the frequency of this was unknown.

The number of detections observed on the UP was not proportional to that observed on the FR given the size difference of the two study areas. In addition, once attracted to the site, cougars on the UP were less likely to enter the cubbies. Cougars may have been detected and sampled at different rates for various reasons. Based on observations during intensive trapping

efforts in both study areas, fewer cougars were observed on the UP (K. Logan unpublished data) than on the FR (M. Alldredge unpublished data). As a result, the sample size of available marked cougars was considerably smaller on the UP; and as expected, the UP results were more variable. Cougar populations may vary, in part, due to differences in the type of human interaction cougars encounter in each region. For example, harvest pressure, in general, is greater on the UP than on the FR. The UP consists of great expanses of accessible public lands necessary for running hounds. Currently, hunting via hounds is the primary method of pursuit to capture and take cougars. The FR consists of a higher proportion of private property, creating barriers to hunting efforts. However, cougar mortality due to interactions, such as livestock depredation, exists in both study areas (CPW unpublished data).

I detected both marked and unmarked cougars but detection probabilities were estimated using only the availability of marked animals. Marked and unmarked cougars were probably equally likely to investigate the auditory attractant, but once at the site, photographs suggested that marked cougars, generally, were more cautious than unmarked animals. For example, a marked cougar might vacillate by circling the site and may or may not enter; whereas an unmarked cougar would enter the cubby without hesitation. The behavioral variation may be due to a trap response from a previous handling event. In order to mark a cougar, the individual must first be captured via cage trap, foot snare, or pursued by hounds, resulting in a significantly negative interaction with humans. Because I did not attempt to minimize human scent when constructing and visiting the sites, it is possible that bias was introduced in the detection probability estimates through a negative trap response, and that the detection probability of an unmarked cougar may be slightly higher. Furthermore, I observed a higher probability of an unmarked cougar entering the cubby than a marked cougar. Therefore, I suspect that, in general,

previously captured cougars were more leery of the lure sites. Although both marked and unmarked cougars were detected, this technique may be slightly more effective when sampling cougars that were not previously captured, especially those captured via cage traps.

My results suggest caution when interpreting the effect of scent on attracting cougars to sites. Consistent with the limited success of past studies using scent lures (Long et al. 2003, Choate 2006, Lang et al. 2013), I collected only two hair samples, and photographs did not suggest cougar interest in scents. Although I documented more site visits at sites with both scents and calls (25) than at sites with only a scent (12) or only a call (15), I found that adding a scent lure did not significantly increase the probability of detecting a cougar. Additionally, I did not observe a rubbing response to Pikauba® in this study despite the positive responses by lynx to scent lures (G. Merrill personal communication). I recorded the same lack of response to Canine Call®, another lure that has proven useful for attracting carnivores (Crooks 2002). However, beaver castoreum (McDaniel et al. 2000) elicited two rubbing responses. Therefore, I suggest that including a scratch pad with beaver castoreum at each site could potentially yield more site visits and possibly a few more hair samples, while adding little expense.

Besides scents and calls, I included bait at each site, possibly confounding the effect of scents and calls. Variability in detection can occur due to the trap response (trap happy) that can arise when a food item is used as bait, potentially influencing the probability of detecting the individual (Pollock et al. 1990). In general with a trap happy response, the probability of detecting an individual is higher after its initial detection. Failure to consider a trap happy response can lead to a negatively biased abundance estimate (see Williams et al. 2002). Because the cube hair-snaring device required bait, I limited the size of the bait to 0.1 – 0.2 kg to minimize the potential for a trap response. To reduce bias between site types, a small piece of

meat was regularly added to all sites. Consistent with other studies, such as those using bait to capture animals (M. Alldredge unpublished data, Schemnitz 1996), I observed cougars at sites with only bait. However, adding a scent and/or a call not only yielded more site visits but also hair samples. No hair samples were obtained from the bait-only sites.

Uniquely identifying an individual via lure sites was a multi-step process which included attracting a cougar to a site, obtaining a genetic sample, and genetically analyzing the sample without error. With almost all marked individuals being detected by camera and an average of 4.64 site visits per week (on the FR), I concluded early in the study that an auditory call was an effective attractant. However, I did not obtain a hair sample with every site visit. Cougars did not always enter the cubby and if they did enter, the hair snares were not always effective, indicating that improvements in the hair-snaring component were needed. Based on cougar activity observed via photographs, I modified the sites after the first sampling period, and the probability of obtaining hair more than doubled for the remainder of the study. Nevertheless, increasing the probability of obtaining a hair sample or improving the quality of the sample acquired may be achieved by using alternative hair-snaring mechanisms in the entry way of the cubby, or by using auditory calls with a hair-snaring design other than a cubby.

The assumption of closure was not met due to various reasons such as death, animals being marked mid-study, and normal movements across large home ranges that spanned the study area boundaries. As a result, many of the marked animals used to estimate the various detection probabilities were not in the study area the entire time and represented a superpopulation of animals that could be detected by the sites. The estimate for $\hat{p}$ of 0.65 ± 0.11 (FR) is the same for all individuals in the superpopulation. For example, in this analysis, an animal on the sampling grid 10% of the time had the same probability of being detected as an

individual that was on the grid 100% of the time. In reality, a cougar that was in the study area less than 30 % of the time was generally not detected. Thus the probability of detection for each animal was absolutely conditional on the time spent on the sampling grid (Figure 9). As a result, when considering only cougars with a high proportion of time on the grid, calls could be deemed more effective than this analysis suggests. In addition, because the density of animals within a defined area is often of interest (Ivan et al. 2013), I estimated a detection probability adjusted for partial time on the grid.

Not all hair samples collected yielded a unique identification when genetically analyzed. Nineteen percent of the samples obtained potentially contained hair from multiple cougars, as photographs indicated that more than one individual entered the cubby between site checks. The DNA extraction and amplification protocols I employed cannot distinguish multiple individuals within a single sample. As a result, many samples were discarded prior to the genetic analysis. I checked the sites approximately once per week. A more frequent check schedule would have reduced the probability of obtaining a sample with DNA from multiple cougars, but only in some instances. Mixed samples due to cougars traveling in groups would be inevitable using this technique. Identifying females with dependent kittens was especially challenging, because family groups almost always visited the sites together. I regularly observed the kittens entering and exiting the sites multiple times. Hence, as expected, the samples contained hair from multiple individuals. It may be possible to obtain a genotype for all individuals by analyzing single hairs from a mixed sample. However, hair inherently has little extractable DNA, and the potential for genotyping errors is high (Taberlet and Luikart 1999). To identify all individuals and minimize genotyping error, many single-hair subsamples would need to be analyzed. Due to

resource limitations, I was unable to process the number of subsamples needed to genotype each unique cougar in a mixed sample without error.

Because hair has a lower quality and quantity of DNA than other sources, such as blood and tissue, the lab protocol attempted to maximize DNA yield and minimize genotyping error. Extraction and amplification protocols were not tried concurrently but rather as trial and error. The original samples varied from a few hairs to several large tufts (see Photograph Supplement). Because cougar hair is extremely fine with tiny follicles, for some samples I attempted to extract DNA from small tufts with > 20 hairs. In general, the tuft samples did not amplify, possibly due to an excess of melanin, a PCR inhibitor. Though DNA can be incorporated into the hair shaft with the greatest amount in the proximal end (Heywood et al. 2003), as little as 15 ng of melanin, found in a 5 – 10 cm hair shaft, can completely inhibit the PCR process (Uchihi et al. 1992). In future efforts, I suggest extracting DNA from 5 mm of the proximal end of a 10 – 15 hair tuft of fur. The sample will include any follicles present and a combined total of 5 – 7.5 cm of hair shaft segments containing the most DNA without inhibiting the PCR process. Of the two amplification protocols that I tried, the individual dye-labeled primers produced the best results. Despite the success of M13–tailed forward primers to amplify DNA extracted from scat samples (Ruell and Crooks, 2007, S. Oyler-McCance personal communication), the protocol using this universal primer was not effective with felid hair, which supports the results of Ruell and Crooks (2007). In the development of the genotyping protocol, I exhausted several samples that may have otherwise yielded complete genotypes.

The frequency at which I checked the sites was dictated by the battery life of the cameras and calls and by the necessity of collecting the hair sample before it was contaminated with hair from another cougar. I monitored the sites once per week, which was adequate to maintain

functional electronics. However, a more frequent schedule might have yielded fewer multiple-cougar samples. This study was conducted in the winter to avoid the complication of attracting bears but winter conditions made access to many sites time-consuming and difficult. Also, heavy snow events buried the cubbies filling in the entry way and muffling the calls. Because the sites needed to be checked regularly, they generally were not placed in extremely remote locations but in more accessible regions within the randomly selected quadrats. Additionally, I avoided residential areas and roads in order to minimize the potential for human conflicts. Because cougars occupy such large home ranges, the fine-scale choice in site placement should not have biased the detection probability estimates. The main factors contributing to the overall expense of the project were technician's salaries, sample processing, cameras, and vehicle maintenance and gasoline.

## MANAGEMENT IMPLICATIONS

Auditory predator calls, cameras, and hair snares are tools that can be used to noninvasively obtain various biological data from previously marked and unstudied cougar populations needed to inform various management decisions. Lure sites can be adapted to a variety of habitat types, and site density as well as temporal scale can be tailored to meet specific objectives. For example, it is possible that the quality and quantity of hair samples obtained via the cubby hair-snare design are adequate enough to suggest dietary trends, genetic diversity, or the age of an individual. Additionally, in a population where cougars are captured and marked for other purposes, calls and cameras can be used as a secondary method of detection over subsequent sampling occasions providing an estimate of abundance.

With a few modifications to the technique, this method, when used alone, may have great utility in estimating population parameters. Because adult females, adult males, females with kittens, juveniles, and possibly dispersers were observed over multiple occasions, the results suggest that almost all members of the population with high availability will be repeatedly sampled by this technique in an operational monitoring program. However, the overall probability of identifying a cougar via genotype using calls and hair snares alone is still relatively low, thus certain components of the method would need to be modified for precise population estimates to be achievable. For example, the density of sites or the duration they are active can be adjusted to improve the probability of detecting all individuals in the population. Calls were effective attractants but not all site visits yielded a hair sample. Only a few hair snaring devices were tried in the entry ways of the cubbies. Countless more possibilities exist within the imagination of the researcher wanting to implement this technique. In addition, exploring alternative DNA extraction and amplification techniques may improve the overall probability of identifying an individual via genotype in the lab.

Table 1. Summary of effort and results for preliminary attractant evaluation on the Front Range, Colorado (2012). Scents and auditory calls were tested either alone or in combination. Bait was included because a hair snare that utilized bait was tested at all sites. Site visits were confirmed using motion-sensor cameras. Marked cougars were identifiable via unique ear tags and GPS location data. Given the proportions of sites with detections, more cougars were observed at sites where calls were present.

| Attractant(s) | No. of sites | Site active (avg. days) | Camera detections | Unique marked cougars observed ‡ | Proportion of sites w/detections | ± 1 SE |
|---|---|---|---|---|---|---|
| Bait only | 17 | 31.6 | 5 | 2 | 0.24 | ± 0.11 |
| Bait & scent | 18 | 33.3 | 12† | 3 | 0.28 | ± 0.11 |
| Bait & call | 16 | 29.0 | 15 | 7 | 0.50 | ± 0.13 |
| Bait, scent, & call | 17 | 32.2 | 25 | 9 | 0.59 | ± 0.12 |

† Seven detections were at the same site and probably by 2 individuals.
‡ Several individual cougars were detected at multiple site types.

Table 2. Summary of cougars detected at sites with auditory calls via motion-sensor cameras and a cubby hair-snare design on the Front Range, Colorado (2012 – 2013). Marked cougars were identified using ear tags and GPS location data. Unmarked cougars were the most likely to enter the cubbies (0.90 ± 0.04). †Fifty-two hair samples were collected but some samples potentially included hair from more than one cougar. If the cougar entered the cubby, the probability of obtaining hair was high (0.86 ± 0.06 – 1.00).

| | No. of camera detections | Entered/ detected | ± 1 SE | †No. of samples | Sample/ entered | ± 1 SE |
|---|---|---|---|---|---|---|
| Marked | 37 | 0.65 | ± 0.08 | 21 | 0.86 | ± 0.06 |
| Sibling group | 4 | 0.50 | ± 0.29 | 2 | 1.00 | |
| Unmarked | 57 | 0.90 | ± 0.04 | 40 | 0.91 | ± 0.06 |

Table 3. Summary of detections and availability (time in the study area) per sampling period (*n*=4) for all independent adult marked cougars on the Front Range, Colorado (2012 – 2013). The cougar ID indicates sex (AF52/Female, AM76/Male). Some GPS data points were not recorded resulting in a < 1.0 collar success rate suggesting that some error was possible in the availability estimates. Italicized, bold values indicate the cougar was detected. For example, AF01 was available 100 % of the time for all sampling periods but her three detections occurred during period three only. Seven cougars were not detected (in red). † Some cougars were ear-tagged but had non-functional GPS collars. AF50 and AF52 were photographed at least once but availability could not be determined.

| Cougar ID | Days available | No. of detections | GPS collar success rate | Availability (Period 1) | Availability (Period 2) | Availability (Period 3) | Availability (Period 4) | Total Availability |
|---|---|---|---|---|---|---|---|---|
| AF01 | 148 | 3 | 0.86 | 1.00 | 1.00 | ***1.00*** | 1.00 | ***1.00*** |
| AF23 | 123 | 1 | 0.92 | 0.26 | ***1.00*** | 1.00 | 1.00 | ***0.83*** |
| AF54 | 148 | 3 | 0.87 | 1.00 | ***1.00*** | 1.00 | 1.00 | ***1.00*** |
| AF57 | 148 | 5 | 0.89 | 1.00 | ***1.00*** | ***1.00*** | 1.00 | ***1.00*** |
| AF61 | 148 | 1 | 0.95 | ***1.00*** | 1.00 | 1.00 | 1.00 | ***1.00*** |
| AF64 | 148 | 3 | 0.94 | ***1.00*** | 1.00 | 1.00 | ***1.00*** | ***1.00*** |
| AF69 | 115 | 1 | 0.90 | ***0.94*** | 0.76 | 0.55 | 0.83 | ***0.78*** |
| AF86 | 148 | 4 | 0.90 | ***1.00*** | ***1.00*** | ***1.00*** | 1.00 | ***1.00*** |
| AF87 | 148 | 3 | 0.85 | 1.00 | ***1.00*** | ***1.00*** | ***1.00*** | ***1.00*** |
| AM76 | 148 | 3 | 0.92 | ***1.00*** | 1.00 | ***1.00*** | ***1.00*** | ***1.00*** |
| AM100 | 108 | 1 | 0.87 | 0.00 | 0.87 | 1.00 | ***1.00*** | ***0.73*** |
| † AF50 | Unk | 5 | N/A | ***Unk*** | ***Unk*** | Unk | ***Unk*** | ***Unk*** |
| † AF52 | Unk | 4 | N/A | Unk | ***Unk*** | ***Unk*** | ***Unk*** | ***Unk*** |
| AF73 | 148 | 0 | 0.87 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| AF62 | 94 | 0 | 0.96 | 1.00 | 1.00 | 0.47 | 0.00 | 0.64 |
| † AM74 | ≤ 46 | 0 | N/A | Unk | ≤ 0.27 | 0.00 | 0.00 | ≤ 0.31 |
| AM98 | 31 | 0 | 0.99 | 0.00 | 0.60 | 0.18 | 0.00 | 0.21 |
| AM124 | 11 | 0 | 0.82 | 0.00 | 0.00 | 0.00 | 0.31 | 0.07 |
| AM49 | 9 | 0 | 0.98 | 0.00 | 0.00 | 0.00 | 0.26 | 0.06 |
| † AM13 | Unk | 0 | N/A | Unk | Unk | Unk | Unk | Unk |

Table 4. Summary of the number of detections and the effectiveness of the cubby hair-snare design per sampling period ($n$=4) on the Front Range, Colorado (2012 – 2013). The probability of detecting a marked cougar (via motion-sensor cameras) did not decline over time. Not all cougars that visited the sites entered the cubbies. The hair snares were modified after period one. As a result, the probability of obtaining hair approximately doubled.

| | No. of detections | Detected/ available | ± 1 SE | Entered/ detected | ± 1 SE | No. of samples | Samples/ entered | ± 1 SE |
|---|---|---|---|---|---|---|---|---|
| Period 1 | 27 | 0.38 | ± 0.15 | 0.74 | ± 0.09 | 8 | 0.45 | ± 0.10 |
| Period 2 | 30 | 0.39 | ± 0.13 | 0.77 | ± 0.08 | 19 | 0.96 | ± 0.04 |
| Period 3 | 25 | 0.35 | ± 0.13 | 0.84 | ± 0.08 | 16 | 0.86 | ± 0.07 |
| Period 4 | 16 | 0.35 | ± 0.13 | 0.69 | ± 0.12 | 9 | 1.00 | |

Table 5. Summary of the number of detections and the effectiveness of the cubby hair-snare design per sampling period (*n*=3) on the Uncompahgre Plateau, Colorado (2012 – 2013). Cougars were observed (via motion-sensor cameras) during all sampling periods but the activity at the sites was highly variable over time possibly due to the small sample size.

| | No. of detections | Detected/ available | ± 1 SE | Entered/ detected | ± 1 SE | No. of samples | Samples/ entered | ± 1 SE |
|---|---|---|---|---|---|---|---|---|
| Period 1 | 6 | 0.57 | ± 0.22 | 0.50 | ± 0.22 | 2 | 0.67 | ± 0.21 |
| Period 2 | 9 | 0.71 | ± 0.20 | 0.78 | ± 0.15 | 5 | 0.86 | ± 0.12 |
| Period 3 | 3 | 0.33 | ± 0.18 | 0 | | 0 | 0 | |

Table 6. Summary of detections and availability (time in the study area) per sampling period (*n*=3) for all independent adult marked cougars on the Uncompahgre Plateau, Colorado (2012 – 2013). No cougar was in the study area the entire time (102 days). The cougar ID indicates sex (F96/Female, M180/Male). Some GPS data points were not recorded resulting in a < 1.0 collar success rate suggesting that some error was possible in the availability estimates. Italicized, bold values indicate the cougar was detected. For example, F96 was detected during period one even though she was only available 66% of the time. Four cougars were not detected (in red). Those not detected were available ≤ 18% of the time. † One cougar (M180) was uniquely marked with ear tags but did not have a GPS collar. This cougar was photographed at the sites but availability could not be determined.

| Cougar ID | Days available | No. of detections | GPS collar success rate | Availability (Period 1) | Availability (Period 2) | Availability (Period 3) | Total Availability |
|---|---|---|---|---|---|---|---|
| F96 | 60 | 1 | 0.47 | ***0.66*** | 0.14 | 1.00 | ***0.59*** |
| F111 | 98 | 2 | 0.48 | ***0.89*** | 1.00 | ***1.00*** | ***0.96*** |
| F129 | 31 | 2 | 0.81 | 0.00 | ***0.31*** | ***0.63*** | ***0.30*** |
| F137 | 92 | 4 | 0.85 | ***0.89*** | ***1.00*** | ***0.81*** | ***0.90*** |
| F171 | 69 | 1 | 0.38 | 0.83 | ***0.83*** | 0.34 | ***0.68*** |
| F181 | 58 | 5 | 0.71 | 0.00 | ***1.00*** | 0.72 | ***0.57*** |
| †M180 | Unk | 3 | N/A | ***Unk*** | ***Unk*** | Unk | ***Unk*** |
| M179 | 18 | 0 | 0.78 | 0.51 | 0.00 | 0.00 | 0.18 |
| F152 | 10 | 0 | 0.67 | 0.29 | 0.00 | 0.00 | 0.10 |
| F95 | 8 | 0 | 0.89 | 0.00 | 0.00 | 0.25 | 0.08 |
| M183 | 2 | 0 | 0.86 | 0.00 | 0.00 | 0.06 | 0.02 |

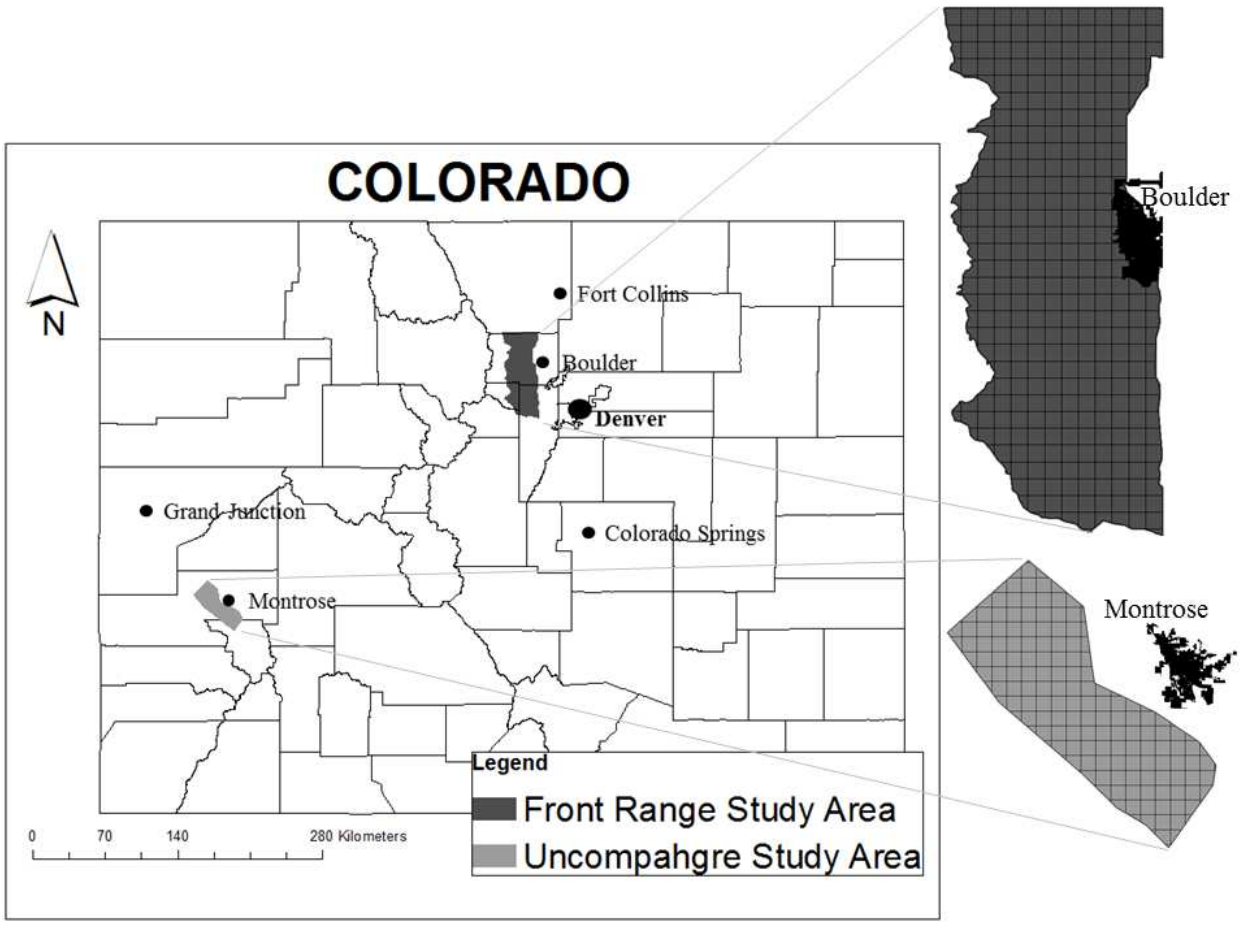


Figure 1. A map of the two study areas in Colorado where lure sites were established to sample cougars (2012 – 2013). A grid with 4 km ² cells was established in both study areas. Cells were randomly selected. Exact site placement was chosen to include specific landscape features, to comply with research permit restrictions, and to evade areas with high human activity.

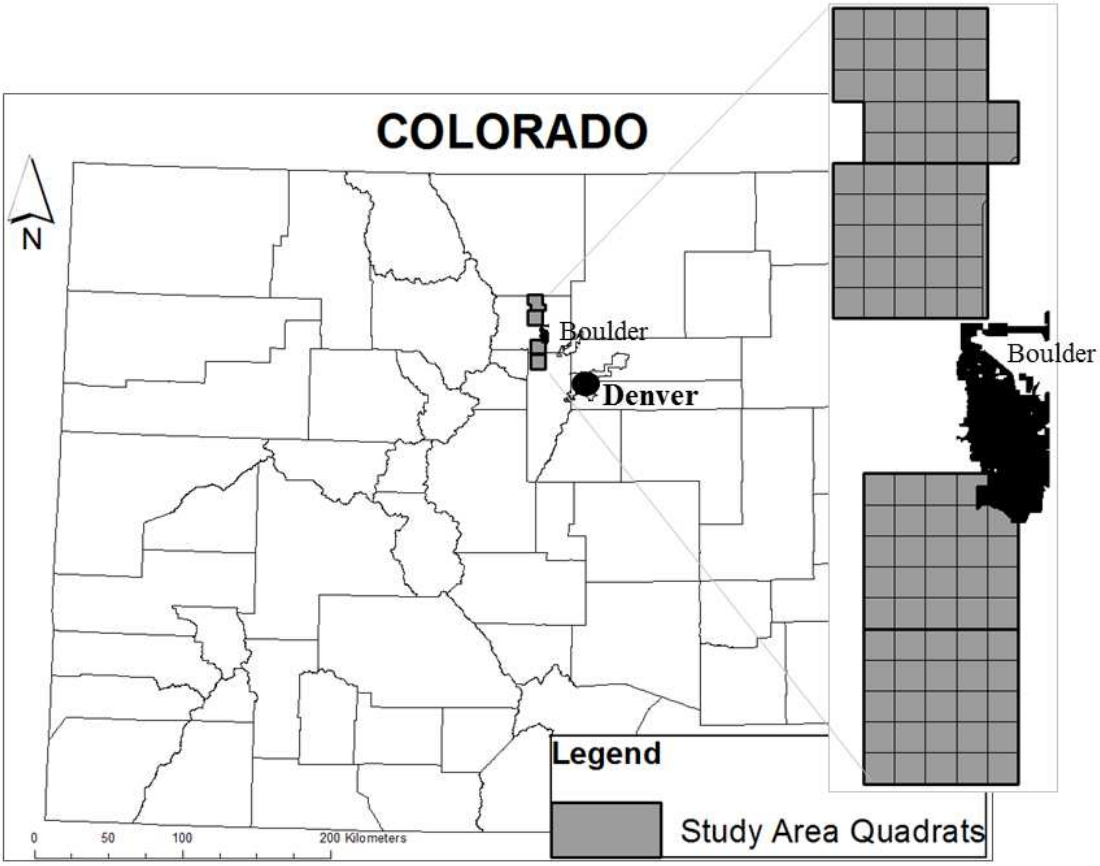


Figure 2. Map of the Front Range study area where various attractants and hair snares were tested. Four 100 km² quadrats were divided into 4 km ² grid cells. Grid cells were randomly selected and attractants were randomly allocated. The exact site locations within each cell were chosen to avoid areas with high human activity, to include specific landscape features, and to comply with permit restrictions.

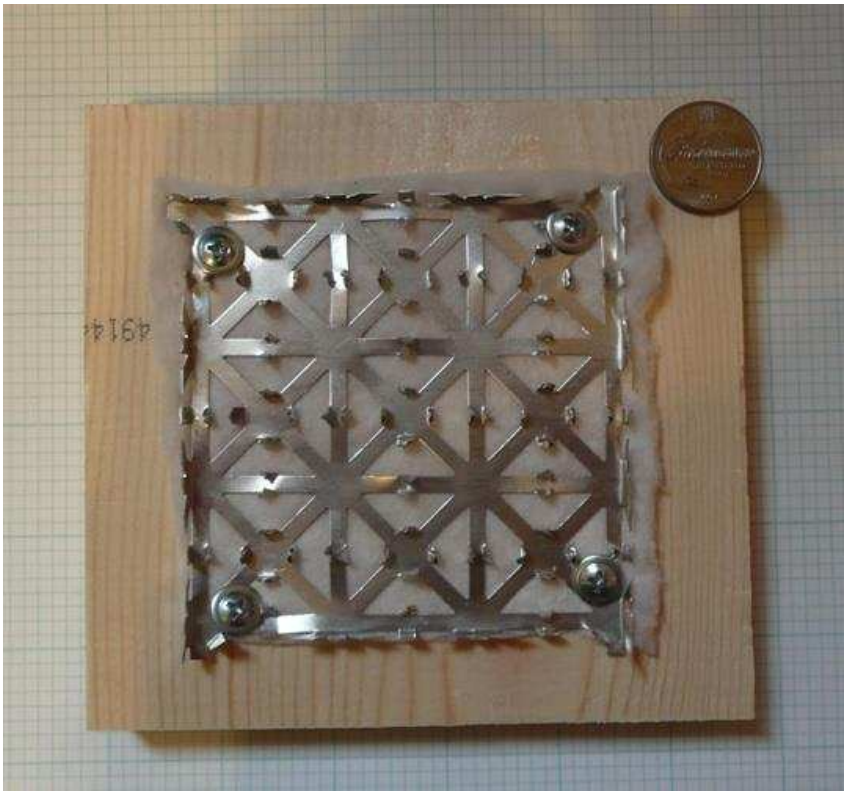

Figure 3. The scratch pad shown was used with various scent lures on the Front Range, Colorado (2012). A single lure was applied on each snare and hair samples were collected approximately once per week. Two hair samples were obtained via scratch pads with the lure, beaver castoreum. The coin was placed to indicate scale.

Figure 4. One of the metal-mesh-cube hair-snaring devices tested on the Front Range, Colorado (2012). A spring and a barrel cleaning brush were attached to snag hair. Bait was wired in the back and replenished approximately once per week. No hair samples were obtained via this device.

Figure 5. One of the stick cubbies constructed on the Uncompahgre Plateau, Colorado (2012 – 2013) with hair-snaring devices in the entry way and an auditory call concealed in the back. A feather was suspended in the cubby and a small piece of bait was added to provide natural deer scent. Bait was replenished, hair samples were collected, and the batteries in the calls were replaced approximately once per week. Many hair samples were obtained via this method.

Figure 6. Pictured are the entries of two cubbies on the Front Range, Colorado (2012 – 2013). Barbed wire and a cable with one or more sticky-coated rollers most effectively snagged hair from cougars. The wires were set at a height of approximately 28 cm.

Figure 7. These photographs obtained via a motion-sensor camera depict a cougar entering and exiting a cubby on the Front Range, Colorado (2012- 2013) in the manner intended. An auditory predator call was placed inside the cubby. A strand of barbed wire and two sticky-coated rollers in the entryway can be seen stretching across the dorsal side of the animal.

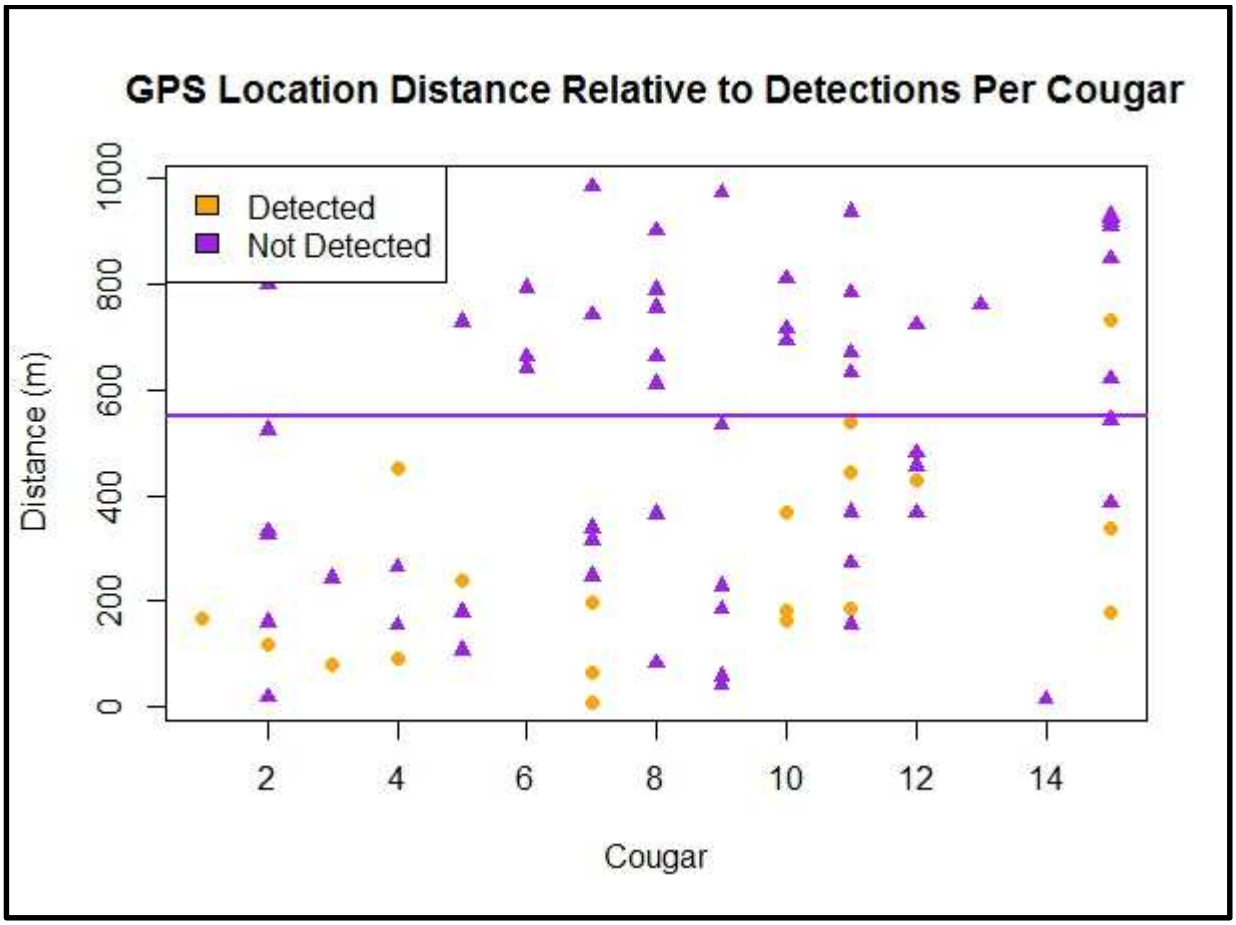


Figure 8. Using the GPS location data for 15 independent marked cougars, the closest recorded location to each site relative to whether the individual was photographed at the site (yellow circle) was used to determine the effective distance of the calls. Beyond approximately 550 m, the probability of observing a cougar at a site was almost zero. For example, cougar '10' recorded a GPS data point within 1000 m of six different sites. When cougar '10' was known to be within 550 m of a site, it was photographed on all three occasions (yellow circles). However, when the closest location for cougar '10' exceeded 550 m, it was not observed (purple triangles).

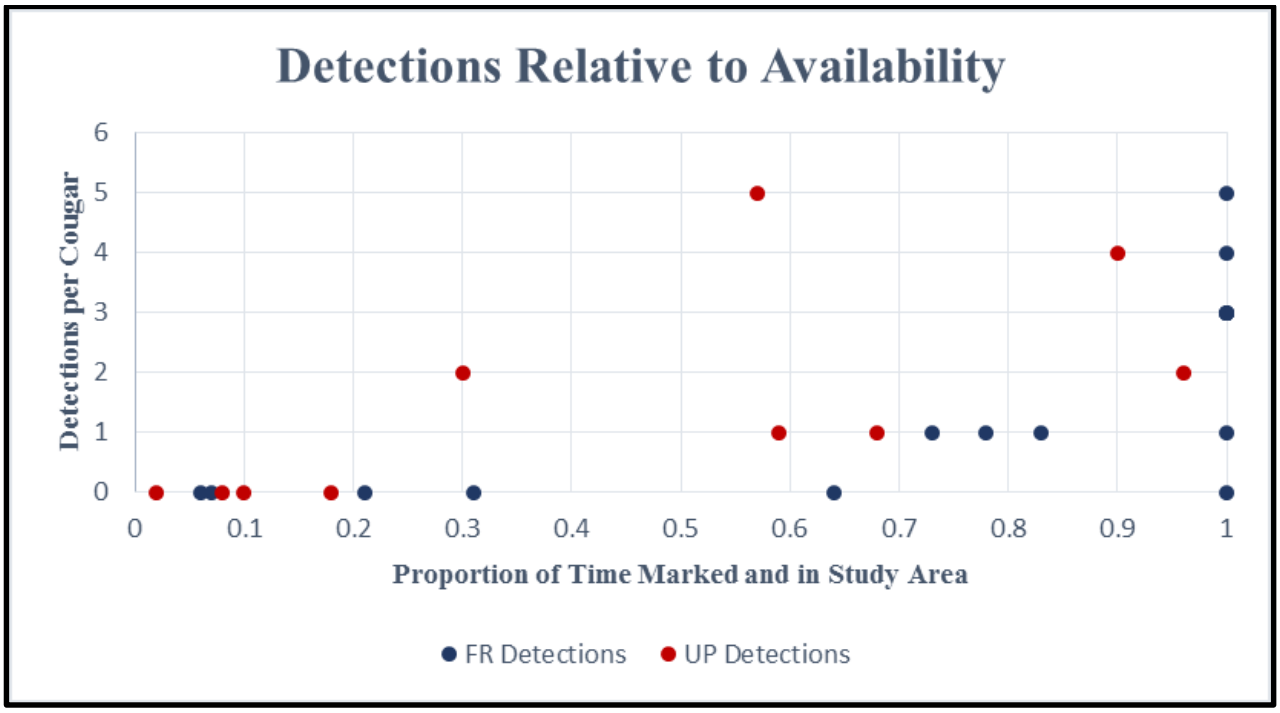


Figure 9. Cougar detections relative to the time spent on the sampling grid in both study areas, the Front Range and Uncompahgre Plateau, Colorado (2012 – 2013). In general, cougars that were available > 30 % of the time, were detected at least once.

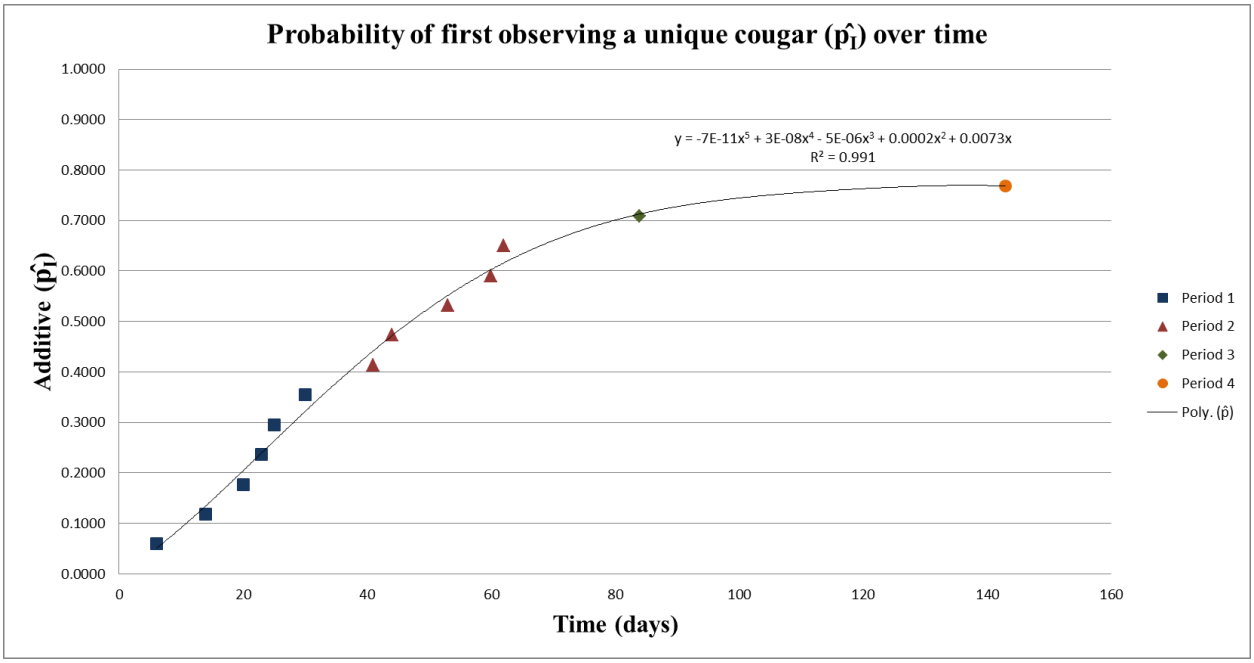


Figure 10. The probability of first observing an independent marked cougars in the Front Range study area over time. Cougars were sampled during four discrete sampling periods (2012 – 2013). Most of the marked animals were first observed during the first half of the study, or within 75 days. By day 62, the probability of first observing an animal was 0.65.

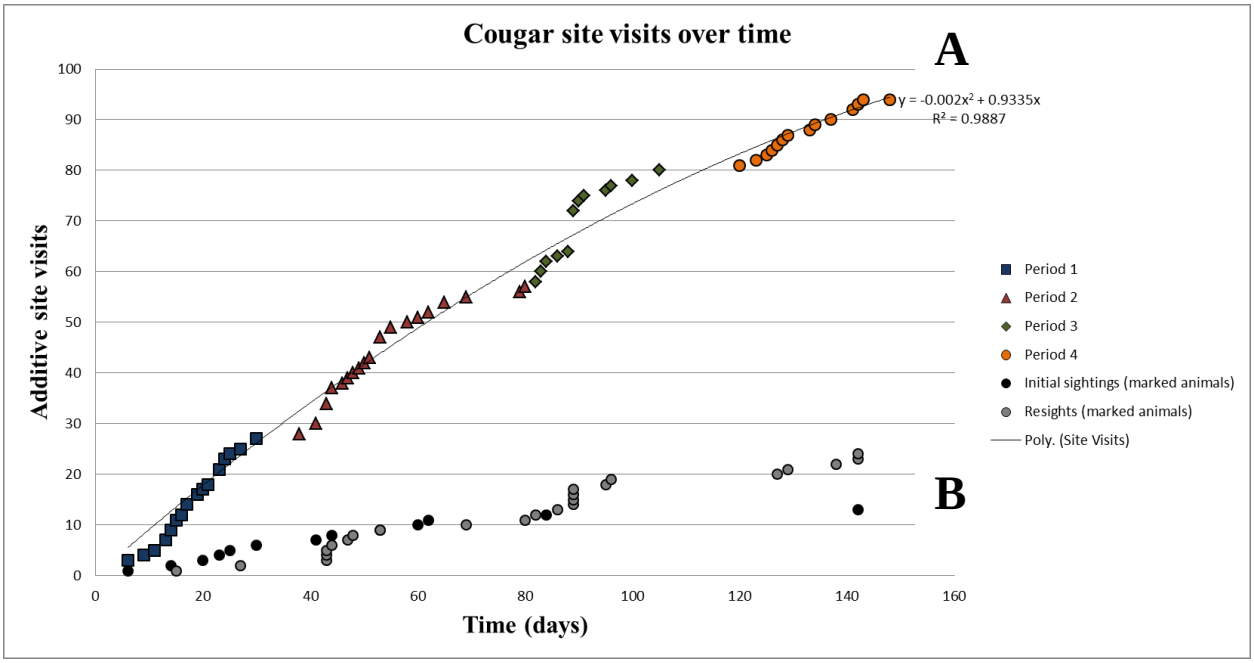


Figure 11. Site visits over time using the data obtained in the Front Range study area. Auditory predator calls were used as the attractant. Cougars were sampled via motion-sensor cameras over four sampling periods and many animals were observed multiple times. Each data point in dataset "A" represents a single cougar visit by both marked and unmarked animals. The incidence of site visits declined only slightly over time. Each data point within dataset "B" represents a single marked cougar visit differentiated by whether the observation was the initial sighting (black) or a re-sighting (grey). Most marked cougars were initially observed within the first half of the study (within 75 days). Consequently, although re-sights were observed throughout the study, most of the marked animal observations during the second half of the study were re-sights.

PHOTOGRAPH SUPPLEMENT

Photo I. A cougar appears to incidentally walk past the site while not approaching any of the attractants (scratchpad with catnip, scratchpad with Pikauba®, or the bait in the cube snare).

Photo II. A cougar is displaying the cheek rubbing response characteristic to felids.

Photo III. A cougar approaches the baited cube snare from the wrong direction.

Photo IV. A cougar approaches the hair snare from the appropriate angle but does not place its head far enough in the snare necessary to obtain a sample.

Photo V. The call is secured to the tree above the baited cube snare and out of the camera's field of view.

Photo VI. The call is secured in the tree above the cougar and out of view.

Photo VII.  A cougar looks up towards the call secured to the tree branch above

Photo VIII.  A juvenile cougar with the call in its mouth will eventually carry it off.


Photo IX. Examples of hair samples obtained via the barbed wire and sticky rollers. The sample in the bottom right photo was not typical.

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