Colorado Division of Parks and Wildlife
July 2014 - June 2015

WILDLIFE RESEARCH REPORT

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		:	Along the Urban-Exurban Front Range of
		:	Colorado
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ABSTRACT

The use of telomeres as a method to determine the age structure of bear and cougar populations has continued to be examined. The age-to-length relationship for bears is near completion and should be completed in the coming year for cougars. We have completed the fourth year of a Ph.D. project with the University of Wisconsin to examine telomeres in detail for bears. This project will also look at stable isotopes to examine foraging ecology and bear use of human food sources. We have also completed a M.S. project with the University of Wisconsin to examine telomeres and stable isotopes for cougars relative to predation on domestic animals and cougar foraging ecology.

Our principal research objective is to assess cougar population ecology, prey use, movements, and interactions with humans along the urban-exurban Front Range of Colorado. This year capture efforts focused on re-collaring previously collared cougars, and capturing previously unmarked independent-age cougars and cubs. In addition to re-collaring cougars we collared 4 new cougars, primarily younger individuals. Mortality was high over the year with 6 mortalities for independent age cougars (predominantly human related and natural causes) (Table 1). Home-range patterns remained consistent to previous years. Relocation of cougars as a management tool has had limited assessment, but given some success, still warrants further investigation. Mule deer are the predominant prey in cougar

diets, although cougars will also utilize elk regularly. The majority of this project has been completed and the focus of this year's efforts was on noninvasive sampling of cougars and bobcats.

WILDLIFE RESEARCH REPORT

COUGAR AND BEAR DEMOGRAPHICS AND HUMAN INTERACTIONS IN COLORADO

MATHEW W. ALLDREDGE

PROJECT NARRITIVE OBJECTIVE

1. To assess cougar (*Puma concolor*) population demographic rates, movements, habitat use, prey selectivity and human interactions along the urban-exurban Front Range of Colorado.
2. Develop methods for delineating population structure of cougars and black bears (*Ursus americanus*), assessing diet composition and estimating population densities of cougars for the state of Colorado.

SEGMENT OBJECTIVES

Section A: Telomeres and Stable Isotopes

1. Evaluate the potential to develop a model for estimating age of bears and cougars based on telomere length to be applied in non-invasive sampling efforts.
2. Determine diet composition of bears and cougars using stable isotopes.

Section B: Front Range cougars

3. Capture and mark independent age cougars and cubs to collect data to examine demographic rates for the urban cougar population.
4. Continued assessment of aversive conditioning techniques on cougars within urban/exurban areas, including use of hounds and shotgun-fired bean bags or rubber bullets (Completed).
5. Continue to assess relocation of cougars as a practical management tool.
6. Assess cougar predation rates and diet composition based on GPS cluster data (Completed).
7. Model movement data of cougars to understand how cougars are responding to environmental variables.
8. Develop non-invasive mark-recapture techniques to estimate cougar population size.

SECTION A: BEAR AND COUGAR TELOMERES AND STABLE ISOTOPES

BY M. ALLDREDGE

OVERVIEW

Understanding the age structure of a population is very useful to managers, especially for hunted populations. Age structure can provide indications about the appropriateness of current harvest levels, changes that may need to occur in harvest, and the general health of a population. Typical approaches involve estimating age structure based on sampling harvested animals and obtaining ages based on tooth wear and replacement characteristics or from analyzing tooth annuli. Recently, a new approach has been developed for some species that estimates the age of animals based on examining the length of telomeres in relation to the age of the animals.

Telomeres are repetitive DNA sequences that cap the ends of eukaryotic chromosomes, whose nucleotide sequence $(T_2AG_3)_n$ is highly conserved across vertebrate species (Meyne et al. 1989). During each cell

cycle telomeric repeats are lost because DNA polymerase is unable to completely replicate the 3' end of linear DNA (Watson 1972). Thus, telomeres progressively shorten with each cell division; past research has demonstrated age-related telomere attrition in a variety of laboratory and wild species and has correlated telomere length with individual age (e.g. Hausmann et al. 2003, Hemann and Greider 2000). Using real-time quantitative polymerase chain reaction (Q-PCR; Cawthon 2002), we have demonstrated the potential for quantifying telomere length for black bears of known-age in Colorado (Alldredge 2010).

Understanding diet composition and foraging ecology of bears is also useful to managers, especially in urban areas, as bears continually interact with humans and human derived food sources. The dynamics of this interaction and the extent to which bears utilize human food sources is largely unknown. The use of stable isotope analysis is one approach to understanding the amount and timing of utilization of various food sources within a bear's diet. Examining different tissue types from bears can explain patterns of use for various food sources and will provide managers a better understanding of this problem at a population level.

We have continued a graduate study with the University of Wisconsin and Wisconsin Department of Natural Resources to develop methods of identifying population age structure using telomeres and examining diet composition and foraging ecology using stable isotopes for bears. See attached report for a complete project overview and objectives (Appendix I). Currently one manuscript is in review describing spatial patterns of black bear diets across Colorado based on stable isotope analyses (Appendix I).

During 2011, we collected blood, tissue, hair, and bone samples from 400 bears across the state. These bears were either nuisance bears or hunter harvested bears. Samples from these bears are being utilized for both the telomere and stable isotope components of this project. Preliminary assessments indicate high genetic quality from samples for use in the telomere work. Initial data from stable isotope analyses indicate significant variation in $\delta^{13}C$ and $\delta^{15}N$ (Figure 1) among bears which suggests that differentiation in diets based on stable isotope analysis will be possible. Starting in the summer of 2012, bears along the Front-Range of Colorado were also collared and repeatedly sampled to examine a detailed time series for the shortening of telomeres, especially relative to hibernation.

Similarly, stable isotope analyses for cougars is focused on identifying cougar predation on specific species guilds, identifying the use of small prey items, and determining factors associated with differences in prey utilization. This graduate project has been completed and defended at the University of Wisconsin (Appendix II for abstracts of completed isotope work). We have also continued to examine telomere length to age relationships for cougars (Appendix II).

As an initial step to investigate the utility of using stable isotopes to assess cougar diets, we collected hair samples from prey species found at cougar kills. Additionally, hair samples were collected from domestic animals (llamas, goats, cats, dogs, etc.) that could potentially be preyed on by cougars. Stable isotope analysis has been done on these prey items and findings suggest that examining prey by species guilds does result in significant differences ($P < 0.05$) in $\delta^{13}C$ and $\delta^{15}N$ content (Figure 2).

SECTION B: FRONT RANGE COUGARS

BY M. ALLDREDGE

INTRODUCTION

We are completing the cougar/human interaction study on the Front Range of Colorado. Given that cougars currently coexist with humans within urban/exurban areas along Colorado's Front Range, varying levels of cougar-human interaction are inevitable. CPW is charged with the management of

cougars, with management options ranging from minimal cougar population management, to dealing only with direct cougar-human incidents, to attempted extermination of cougars along the human/cougar spatial interface. Neither inaction nor extermination represent practical options, nor would the majority of the human population agree with these strategies. In the 2005 survey of public opinions and perceptions of cougar issues, 96% of the respondents agreed that it was important to know cougars exist in Colorado, and 93% thought it was important that they exist for future generations (CPW, unpublished data).

There is a growing voice from the public that CPW do more to mitigate potential conflicts, and the leadership of CPW has requested that research efforts be conducted to help minimize future human/cougar conflicts. In order to meet these goals, CPW believes it is necessary to directly test management prescriptions in terms of desired cougar population and individual levels of response.

Long-term study objectives for the Front Range Cougar Research project involve directly testing management responses of cougars at various levels of human interaction, as well as collecting basic information about demographics, movement, habitat use, and prey selection. The Cougar Management Guidelines Working Group (CMGWG) (2005) recommended that part of determining the level of interaction or risk between cougars and humans is to evaluate cougar behavior on a spectrum from natural, to habituated, to overly familiar, to nuisance, to dangerous. The CMGWG (2005) clearly stated that there is no scientific evidence to indicate that cougar habituation to humans affects the risk of attack. We also continue to monitor relocated cougars to determine the effectiveness of relocation as a management tool.

The use of GPS collars (obtaining up to 8 locations per day) also allows for a detailed examination of demographic rates. We are monitoring cougars that utilize natural habitats and cougars that use a mixture of natural and urban habitats. This allows for an assessment of demographic rates, movement patterns, and habitat use among cougars utilizing these two habitat configurations. We have also monitored cubs (approximately 6 months of age or older), primarily to determine survival but potentially to understand movement patterns and dispersal.

The use of GPS collars also allowed us to study predator-prey relationships and diet composition. GPS locations are divided into selection sets based on the likelihood of the set of locations (clusters) representing a kill site. A random sample of these clusters was investigated to determine what a cougar was doing at the site, and whether or not it represented a kill site. Kill sites were thoroughly investigated to determine as much information as possible about what was killed at the site.

Currently GPS collars are being used to assess the effectiveness of lures or calls to attract cougars to hair snag locations as part of the development of noninvasive population estimation techniques. Understanding the effectiveness of these attractants is crucial to the development of these techniques and an assessment of potential biases that may exist. We are attempting to maintain 20 collared cougars for this portion of the study.

STUDY AREA

The original pilot study was conducted in Boulder and Jefferson counties, in an area near Interstate 70 north to approximately Lyons, Colorado, which was also a likely area for addressing long-term research objectives and is the current study area for the development of noninvasive techniques (see Figure 3). The study area for portions of the long term study included this original area but was expanded south to highway 285. Research efforts in the additional southern portion were generally limited to capturing cougars that were in the urban setting and/or had interacted directly with humans. The study

area is comprised of many land ownerships, including private, Boulder city, Boulder County, Jefferson County, and state and federally owned lands. Therefore, we have been directly involved with Boulder city and Boulder and Jefferson county governments to obtain agreements from these entities on conduct of research and protocols for dealing with potential human/cougar interactions prior to conducting any research efforts. We have also acquired permission to access numerous private properties to investigate cougar clusters and to trap cougars.

METHODS

Baiting, using deer and elk carcasses, has been conducted throughout the year, with a focus on areas that do not allow the use of hounds. Bait sites are monitored using digital trail cameras to determine bait site activity. Cage traps were generally used for capture when cougars removed the bait and cached it. Hounds have been the primary method of capture lately as cougars are wary of cage traps and cougars are easy to recapture with hounds. Snares were used in situations where hounds could not be used and cougars would not enter cage traps. Captured cougars were anesthetized, monitored for vital signs, aged, measured, and ear-tagged. All independent cougars (> 18 months old) were fitted with GPS collars. All cubs greater than 15 kg (approximately 6 months or older) were ear-tagged with 22 g ear-tag VHF transmitters or 22g ear-tag ptt Argos transmitters.

When cougars interact with humans and elicit a response from CPW District Wildlife Managers (DWMs), they are potential candidates to be collared for the study. Most incidents prompting response from a DWM occur in neighborhoods, where relocating the cougar is necessary prior to release. For these situations, all treatments require the relocation of the offending individual to an adjacent open-space property or similar area.

Cougars are only relocated for management purposes, generally in conjunction with human conflict. Research cougars that have been collared for other purposes of the study may also become part of the relocation group if their levels of human interaction warrant such a management action. Because only a few cougars are relocated each year, we collar and monitor all cougars that are relocated in the northeast region. Cougars are ear-tagged and fitted with a telemetry collar (VHF or GPS collars may be used depending on the situation).

Release area is critical to the success of any relocation; however, suitable relocation areas may be difficult to find. Such an area must be far enough from the problem area, have suitable prey and be remote enough so that the individual will not be presented with problem opportunities at or near the release site. Understanding the minimum release distance that has a reasonable chance for relocation success is useful for both logistical reasons and to increase the number of potential release sites.

We have concluded the evaluation of cougar diet composition by using GPS location data to identify likely kill sites. Characteristics of clusters of GPS locations representing cougar-killed ungulate sites (Anderson and Lindzey 2003, Logan 2006) were used to develop a standard algorithm to group GPS points together, to provide a sound sampling frame from which statistical inference could be made about clusters that were not physically investigated. GPS collars collected locations 7 to 8 times/24 hrs to reflect time periods when cougars were both active and inactive.

The clustering routine was designed to identify clusters in five unique selection sets (S_1, S_2,…, S_5) in order to identify clusters containing two or more points, those that contained missing GPS locations, and those that were represented by single points. S_1 clusters consist of multiple GPS locations with a 4 day window and within 200 m, while other sets are single points close together in time within varying distance bands. The clustering algorithm was written in Visual Basic and was designed to run

within ARCGIS (Alldredge and Schuette, CDOW unpubl. data 2006). The widths of the spatial and temporal sampling windows were user specified, in order to meet multiple applications and research needs. This also enabled adjustment of the sampling frames to improve cluster specifications as needed.

We used the following protocol to investigate cougar GPS clusters in the field. For S_1 clusters, we investigated each cougar GPS location in the cluster by spiraling out a minimum of 20 m from the GPS waypoint, while using the GPS unit as a guide, and visually inspected overlapping view fields in the area for prey remains. Normally, this was sufficient to detect prey remains and other cougar sign (e.g., tracks, beds, toilets) associated with the cougar. If prey remains were not detected within 20 m radius of the cluster waypoints, we then expanded our searches to a minimum of 50 m radius around each waypoint. For S_2 through S_5 clusters, we went to each cougar GPS location and spiraled out 50 m around each waypoint, while using the GPS unit as a guide. Depending on the number of locations, topography, and vegetation type and density, we spent a minimum of 1 hour and up to 3 hours per cluster to judge whether the cluster was a kill site.

Kevin Blecha has finished his M.S. research on predator-prey dynamics related to the sampling described above. He is specifically looking at predator-prey relationships relative to various habitat types and levels of human density across the landscape. An assessment of prey availability or reliability is also being made through the use of camera traps within these habitat types and levels of human density. Finally, an assessment of cougar use on domestic animals (livestock and pets) is being made (see Appendix III for his thesis summary).

Joe Halseth and Matt Strauser have concluded another study to examine prey selection and kill site dynamics with regard to conspecifics and scavenging. Kill sites were investigated within 24 hours of the kill to determine prey species, to place cameras and to sample ungulates for age and to test for CWD. Some work has indicated that cougars may select for CWD positive animals, but sample sizes have been limited. We intended to sample a large number of ungulates and address this topic further. Additionally, we documented significant amounts of prey sharing among cougars and significant amounts of scavenging from cougar kills. Understanding these kill site dynamics will provide information on kill rates, consumption rates and intra/interspecific interactions (see Appendix IV for more details).

We have also completed the M.S. project with Bill Kendall at CSU through the Fish, Wildlife, and Conservation Biology Department to examine techniques to develop non-invasive population estimation methodology for cougars (Appendix V). This study has been completed and publications are in review. The current study to develop techniques for noninvasive sampling of cougars and bobcats (Appendix VI) is based on many of these results. We completed the second year of this population sampling and results are still positive.

RESULTS AND DISCUSSION

Collared cougars from the previous year were captured and re-collared to replace exhausted batteries throughout the year. An additional 4 independent-age cougars were also captured and collared during the year (Table 1). Currently there are 11 independent-age cougars in the study with functioning GPS collars.

There were a total of 6 mortalities for adult collared cougars during the 2014-15 year (Table 1). Causes of death included natural mortality (2) and management or hunting related deaths (4).

Relocation of cougars is also a management technique that we have evaluated in the past and has shown mixed results relative to age, sex and relocation distance. The NE region has expressed renewed

interest in this and we will begin pilot work to investigate this in more detail. We will evaluate relocation distance relative to Directive W2 and the distance recommendations made for management as well as some more long-distance relocations. As this proceeds, we will develop a more detailed study to thoroughly investigate cougar relocation parameters.

The prey selection and kill site dynamics study was initiated in January, 2012 (see Appendix IV for study objectives and preliminary results) and all data for this study have been collected. To date, we have collected over 100 individual samples from deer-killed by cougars and tested these for CWD. A proportion of these have been positive for CWD, primarily those collected during the spring. We have investigated numerous potential kill sites and placed cameras on fresh kill sites to document the activity. We have documented multiple occasions when multiple cougars shared a kill and several scavenging events. Many scavenging events occur after the cougar has consumed the prey and has left. Other scavenging events have occurred while the cougar was still consuming the prey item, including cases where bears have usurped the prey item killed by the cougar. These data are currently being prepared for publication.

Starting in November, 2011, we began investigating snow tracking and lures as potential techniques to estimate cougar abundance. Snow tracking proved to be very difficult because there was limited snow throughout the winter and snow conditions were poor. When snow tracking was feasible tracks of collared cougars were followed and samples (primarily hair) were collected. This approach is highly dependent upon environmental conditions and therefore may not be broadly applicable.

Efforts documented in the literature to lure cougars to specific locations and capture an individual with either a photograph or genetic sample have been limited and relatively unsuccessful. We have finished testing various options to lure cougars to specific locations and extract genetic samples. One option that had not been tested in other studies is the use of game calls to attract cougars. We placed 4 different types of attractant sites at random locations to determine which types of lures or combinations of lures (bait, bait and scent, bait and call, bait, scent and call) would be the most reliable method of attracting cougars. We found that calls have been significantly more effective at attracting cougars to a site (see Appendix V for abstracts of prepared manuscripts).

Although we were relatively effective at luring cougars to a specific location with calls, initial efforts were not successful in extracting genetic samples at these locations. Cougars appeared to ignore scratch pads and were hesitant to take any meat reward left at the site. Cougars did seem interested in the calls and on several occasions investigated the call or stole the call from the site. We investigated methods of extracting genetic samples from cougars approaching the call using cubbies and barbed wire hair snags. Study efforts for this approach included both the Front-Range of Colorado and the Uncompahgre Plateau (see Appendix V for abstracts of prepared manuscripts).

Following on the success of the development of noninvasive techniques for sampling cougars we initiated a three-year study to continue to develop noninvasive methods for sampling cougars and bobcats. Sites were built in November and December, 2013, and were monitored for 12 weeks during January – April, 2014 (see study plan for details, Appendix VI). This year sites were built during November and monitored for three months starting the 1st of December and continuing through the first week of March.

Sites were modified this year to use vertical hair snags instead of horizontal snags in an attempt to get more animals to enter the cubbies and to create a snag that could obtain samples from both bobcats and cougars. The number of unique observations of cougars decreased from 86 in 2014 to 42 in 2015, while observations of bobcats increased from 31 to 68 across years (Table 3). Hair samples for cougars decreased accordingly from 55 in 2014 to 32 the following year. Hair samples from bobcats increased

from 5 the first year to 12 the second year. Genotypes from bobcat hair has not been successful but is somewhat successful for cougars.

SUMMARY

The use of telomeres as a method to determine the age structure of bear and cougar populations is promising and will be investigated further in the coming year to develop the relationship in more detail with regard to covariates. Further refinement of the age-to-length relationship for both species is warranted. In addition, length relationships relative to genetic relatedness and individual stressors will give further insight into interpreting results from future data. We will also be investigating the effects of hibernation on telomere length using wild bears.

The use of stable isotopes from bears and cougars is beginning to show some very interesting results. Examining stable isotopes from various bear tissue types will help elucidate temporal patterns in diet composition, including the use of human foods by bears. It has also become clear that stable isotopes will be a useful tool in examining cougar diets, especially in the use of small prey items that are likely overlooked with other traditional techniques.

In addition to re-collaring previously collared cougars, an additional 4 independent age cougars were collared during the year. Mortality remained high over the year, with 6 collared cougar mortalities. Relocation of cougars as a management tool has had limited assessment, but given some success, still warrants further investigation. Mule deer are the predominant prey in cougar diets, although some utilize elk regularly. We will continue to assess population estimation techniques during the coming year and analyze and publish existing data.

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Prepared by _____

Mathew W. Alldredge, Wildlife Researcher

Table 1: Capture history, aversive conditioning treatments and current status of all independent age cougars captured as part of the Front Range cougar study.

Cougar ID	Sex	Age	Date	Location	Occurrence	Capture	Release Loc	Conditioning	Status
AM02	M	1	6/14/07	Lacey Prop.	Baiting	Cage	On-site	NA	Alive
		1.5	1/10/08	White Ranch	Capture effort	Hounds	On-site	NA	Alive
		1.5	2/9/08	Coal Creek	Intraspecific mortality				Dead
AM04	M	7	7/14/07	White Ranch	Baiting	Cage	On-site	NA	Alive
		7	10/17/07	Eldorado Springs	Livestock depredation	Cage	White Ranch	Beanbag	Alive
		8	4/29/08	Magnolia/Flagstaff	Replace Collar	Hounds	On-site	NA	Alive
		8	5/5/08	South Boulder	Seen in town	Free-dart	Lindsey	Beanbag	Alive
		8	8/4/08	North Boulder	Killed deer in town	Cage	Centennial Cone	Beanbag	Alive
		9	2/24/09	Boulder Canyon	Punctured intestine				Dead
AM06	M	5	11/21/07	Heil Valley Ranch	Capture effort	Hounds	On-site	NA	Alive
		6	12/30/08	Heil Valley Ranch	Replace Collar	Hounds	On-site	NA	Alive
		7	2/2/10	Reynolds Ranch	Replace Collar	Hounds	On-site	NA	Alive
		7	2/15/10	White Ranch	Hunter				Dead
AF03	F	4	11/29/07	Flagstaff	Deer kill	Cage	On-site	NA	Alive
AF01	F	2	12/17/07	Table Mesa	Deer kill	Cage	On-site	NA	Alive
		4.5	12/15/10	White Ranch	Replace Collar	Hounds	On-site	NA	Alive
			3/12/12	BCOS Lindsey	Deer kill	Free-dart	On-site	NA	Alive
		7	11/5/14	Boulder	In town (natural mort)				Dead
AM05	M	2	12/19/07	White Ranch	Capture effort	Hounds	On-site	NA	Alive
		4	12/4/09	White Ranch	Replace collar	Hounds	On-site	NA	Alive
		5	4/4/10	Golden	Roadkill				Dead
AM07	M	1.5	12/26/07	Heil Valley Ranch	Capture effort	Hounds	On-site	NA	Alive
			4/19/08	Highway 7	Roadkill				Dead
AF08	F	1.5	12/26/07	Heil Valley Ranch	Capture effort	Hounds	On-site	NA	Alive
		3	6/18/09	West Horsetooth	Deer kill-remove collar	Cage	On-site	NA	Alive
AM09	M	1.5	12/28/07	Heil Valley Ranch	Capture effort	Hounds	On-site	NA	Alive
		2.5	12/27/08	Hwy 34 (mile 70)	Roadkill				Dead
AF10	F	7	1/15/08	Apex Open Space	Deer Kill	Cage	On-site	NA	Alive

			2/13/08	I-70	Roadkill				Dead
AF19	F	8+	3/4/08	Heil Valley Ranch	Capture effort	Hounds	On-site	NA	Alive
		8+	3/18/09	North Boulder	Deer Kill	Cage	Heil Valley Ranch	Beanbag	Alive
			4/13/09	Left Hand Canyon	Deer Kill	Cage	Heil Valley Ranch	NA	Alive
		8+	1/20/09	Dowe Flats	Deer Kill	Cage	On-site	NA	Alive
			11/5/10	Foothills Hwy, N. Boulder	Roadkill				Dead
AF11	F	1.5	3/5/08	South Table Mesa	Deer Kill	Cage	On-site	NA	Alive
			8/20/08	US-40/Empire	Roadkill				Dead
AM20	M	4	3/6/08	White Ranch	Capture effort	Hounds	On-site	NA	Alive
			5/18/08	West of White Ranch	Livestock Depredation	Shot			Dead
AF15	F	6	3/18/08	Coffin Top	Capture effort	Hounds	On-site	NA	Alive
		7	4/2/09	Hall Ranch	Replace Collar	Hounds	On-site	NA	Alive
			3/25/10	Coffin Tip	Replace Collar	Hounds	On-site	NA	Alive
		8-9	2/4/11	Hall Ranch	Deer Kill	Snare	On-site	NA	Alive
		9+	2/2/12	Longmont Dam Rd	Deer Kill	Snare	On-site	NA	Alive
		9+	11/8/12	Button Rock	Natural Mortality				Dead
AF17	F	9+	3/29/08	Sugarloaf	Pet depredation	Cage	Within 1 mile	Beanbag	Alive
			5/20/08	Four-mile Canyon	Unknown mortality				Dead
AF12	F	2	5/8/08	N. Boulder	Deer Kill	Cage	US Forest Boulder Canyon	Beanbag	Alive
			5/29/08	N. Boulder	Livestock depredation	Cage	Near Ward	Beanbag	Alive
			2/13/09	N. Boulder	Deer Kill/Shot	Snare	None		Dead
AM13	M	2	5/8/08	Sugarloaf	Livestock depredation	Cage	On-site	Beanbag	Alive
			12/17/08	Heil Valley Ranch	Replace Collar	Hounds	On-site	NA	Alive
		3	12/17/09	Heil Valley Ranch	Replace Collar	Hounds	On-site	NA	Alive
			3/27/12	Hall Ranch	Detected by camera				Alive
			5/30/13	Apple Valley Rd.	Shot/depredation				Dead
AM14	M	2	5/15/08	South Boulder	Seen under deck	Free-dart	Lindsey	None	Alive
			5/20/08	South Boulder	Deer kill	Free-dart	West of Rollinsville	Beanbag	Alive
			4/14/09	Rollins Pass	Replace Collar	Hounds	On-site	NA	Alive
		3	2/16/10	Left Hand Canyon	Replace Collar	Hounds	On-site	NA	Alive

		4.5	6/22/11	Allenspark	Elk Kill	Cage	On-site	NA	Alive
		4-5	11/9/11	Hwy 72	Raccoon Kill	Free-dart	On-site	NA	Alive
		4-5	12/4/11	Allenspark	Shot/depredation				Dead
AF34	F	1.5	12/5/08	Heil Valley Ranch	Capture effort	Hounds	On-site	NA	Alive
			3/18/09	N. Boulder	Deer kill	Cage	Heil Valley Ranch	Beanbag	Alive
		2.5	1/4/10	Heil Valley Ranch	Replace Collar	Hounds	On-site	NA	Alive
		3.5	12/31/10	Hall Ranch	Replace Collar	Hounds	On-site	NA	Alive
		4.5	12/28/11	Hall Ranch	Replace Collar	Hounds	On-site	NA	Alive
		5.5	2/13/12	W of Hall Ranch	Unknown mortality				Dead
AM18	M	1.5	12/24/08	Evergreen	Deer kill	Cage	Mt. Evans SWA	None	Alive
			3/14/09	Evergreen	Livestock depredation	Cage	None		Dead
AF16	F	3	12/29/08	Evergreen	Deer Kill	Snare	Flying J Open Space	None	Alive
			3/20/09	Evergreen	Livestock depredation	Cage	Mt. Evans SWA	Beanbag	Alive
AF45	F	5	1/2/09	Gold Hill	Deer kill	Cage	On-site	NA	Alive
			11/24/10	N.Boulder	Euthanized/Lisa Wolfe			NA	Dead
AF40	F	1.5	1/27/09	White Ranch	Capture effort	Hounds	On-site	NA	Alive
		1.5	1/28/09	White Ranch	Replace Collar	Hounds	On-site	NA	Alive
		2.5	2/22/10	White Ranch	Replace Collar	Snare	On-site	NA	Alive
		4-5	3/4/12	Idaho Springs	Fawn Kill	Snare	On-site	NA	Alive
		5	10/13/12	Idaho Springs	Shot by hunter				Dead
AF24	F	10+	2/12/09	North Boulder	Deer Kill	Cage	Hall Ranch	None	Alive
			2/25/09	Hwy 7	Replace Collar	Hounds	On-site	NA	Alive
			4/4/09	North Boulder	Raccoon Kill	Free-dart	Heil Valley Ranch	None	Alive
			5/31/09	North Boulder	Encounter	Shot			Dead
AM31	M	1.5	12/31/08	Evergreen	Chicken coop	Hounds	On-site	None	Alive
			3/29-09	Conifer	Livestock depredation	Cage	Mt. Evans SWA	None	Alive
		2.5	2/16/10	Douglas, WY	Hunter				Dead
AF37	F	1.5	12/31/08	Evergreen	Chicken coop	Free-dart	On-site	None	Alive
			8/11/09	I-70	Roadkill				Dead
AM21*	M	1.5	8/29/09	N. Boulder	Encounter	Free-dart	Ward	None	Alive
		2	3/01/10	Loveland	Livestock depredation				Dead
AF32	F	1.5	9/28/09	Indian Hills	Livestock depredation	Cage	Within 1 mile	None	Alive

		3.5	11/28/10	Golden	In neighborhood	Free-dart	White Ranch	None	Alive
		3.5	12/1/10	Golden	In neighborhood	Cage	Radium	None	Alive
			9/23/11	Green Mtn. Res.	Found dead				Dead
AM46	M	2	11/13/09	Evergreen	Elk kill	Cage	On-site	None	Alive
			3/5/10	Genesee	Livestock depredation	Shot			Dead
AF50	F	3	11/24/09	West of Boulder	Deer kill	Cage	On-site	NA	Alive
AM44	M	6	12/15/09	White Ranch	Capture effort	Hounds	On-site	NA	Alive
			3/18/10	White Ranch	Replace collar	Hounds	On-site	NA	Alive
		7-8	3/20/11	White Ranch	Elk kill	Snare	On-site	NA	Alive
		9	5/30/12	SW of White Ranch	Shot/depredation				Dead
AM606	M	2	1/6/10	Boulder	Seen in town	Free-dart	MacGregor Ranch	None	Alive
			9/23/11	Laporte	Shot killing goat				Dead
AF54	F	4	1/14/10	White Ranch	Capture effort	Hounds	On-site	NA	Alive
			5/16/11	White Ranch	Deer Kill/Replace Collar	Cage	On-site	NA	Alive
		7	3/14/13	White Ranch	Replace Collar	Hounds	On-site	NA	Alive
			4/18/14	White Ranch	Livestock depredation	Shot			Dead
AF52	F	4	1/28/10	Hall Ranch	Capture effort	Hounds	On-site	NA	Alive
		5-6	3/24/11	Hall Ranch	Deer Kill	Cage	On-site	NA	Alive
AM51	M	1.5	1/28/10	Hall Ranch	Capture effort	Hounds	On-site	NA	Alive
			1/6/2014	Larimer Cty	Hunter Harvest	Hounds			Dead
AF56	F	1.5	2/22/10	Conifer	Livestock depredation	Cage	Mt. Evans SWA	Beanbag	Alive
			5/24/12	Conifer	Shot				Dead
AF55	F	4	2/23/10	Conifer	Livestock depredation	Cage	Mt. Evans SWA	Beanbag	Alive
			3/13/10	Conifer	Pet Depredation	Cage		Euthanized	Dead
AM53	M	4	3/13/10	Genesee	Elk Kill	Cage	On-site	NA	Alive
			3/3/11	Medved property	Shot/hunter				Dead
AM60	M	2	3/29/10	Walker Ranch	Baiting	Cage	On-site	NA	Alive
AF58	F	1.5	4/4/10	Table Mesa	Baiting	Cage	On-site	NA	Alive
			6/3/10		Roadkill				Dead
AF62	F	5	4/13/10	Walker Ranch	Elk Kill	Cage	On-site	NA	Alive
		6	4/13/11	Walker Ranch	Baiting	Cage	On-site	NA	Alive
			12/10/11	Gross Dam	Non-target/released	Cage	On-site	NA	Alive

		6	11/14/12	Walker Ranch	Recollar	Cage	On-site	NA	Alive
			2/16/12	Walker Ranch	Natural Mortality				Dead
AF59	F	5	4/22/10	Blue Jay/Jamestown	Deer Kill	Cage	On-site	NA	Alive
		5	1/6/11	N. Boulder	Deer Kill	Cage	On-site	NA	Alive
		5-6	12/29/11	Sunshine Canyon	Deer Kill	Free-dart	On-site	NA	Alive
		6	3/6/12	NW of Boulder	Unknown mortality				Dead
AM63	M	1	9/22/10	Paradise Park	Deer Kill	Cage	White Ranch	None	Alive
			9/30/10		Road Kill				Dead
AF57	F	3	11/3/10	Lacy Property	Baiting	Snare	On-site	NA	Alive
		4-5	2/4/12	JCOS Ralston Buttes	Replace Collar	Hounds	On-site	NA	Alive
		5-6	3/5/13	Boulder/OSMP	Recollar	Cage	On-site	NA	Alive
			1/15/14	Lacy property	Recollar	Cage	On-site	NA	Alive
AF61	F	4-5	11/18/10	Flagstaff	Deer Kill	Free-dart	On-site	NA	Alive
		4-5	3/2/11	Coal Creek Canyon	Raccoon Kill	Cage	Walker Ranch	None	Alive
		5	12/10/11	Gross Dam Rd	Baiting	Snare	On-site	NA	Alive
			1/27/14	Magnolia	Recollar	Hounds	On-site	NA	Alive
AF64	F	1.5	1/20/11	Heil Valley Ranch	Baiting	Cage	On-site	NA	Alive
		3-4	7/19/12	N of Nugget Hill	Kill	Snare	On-site	NA	Alive
AM67	M	1.2	12/16/10	White Ranch	Baiting	Cage	On-site	NA	Alive
		5							
			3/4/12	Big Thompson	Shot/Depredation	Snare			Dead
AF69	F	1.5	12/1/10	N. Boulder	Deer Kill	Free-dart	On-site	NA	Alive
		2	4/6/11	N.Boulder/Town	Deer Kill	Free-dart	Reynolds Ranch	None	Alive
		4	3/31/12	Wonderland	Deer Kill	Cage	On-site	NA	Alive
AM70	M	2	1/23/11	Gold Hill	Deer Kill	Cage	On-site	NA	Alive
			3/2/11	Boulder Heights	Dog Kill	Cage	Reynolds Ranch	None	Alive
		3	2/26/12	Buckhorn Rd	Unknown mortality				Dead
AM71	M	2	1/27/11	Heil Valley Ranch	Baiting	Cage	On-site	NA	Alive
		3	12/23/11	Casper, WY	Shot/hunter	Hounds			Dead
AM72	M	4	2/6/11	Heil Valley Ranch	Baiting	Snare	On-site	NA	Alive

		5	5/2/12	Heil Valley Ranch	Unknown mortality				Dead
AF73	F	4	3/6/11	Sunshine Canyon	Baiting	Cage	On-site	NA	Alive
		3-4	10/28/11	Four Mile Canyon	Deer Kill	Cage	On-site	NA	Alive
		4-5	3/27/13	Magnolia	Recollar	Hounds	On-site	NA	Alive
			1/25/14	Magnolia	Unknown Mort.				Dead
AM74	M	4	2/23/11	White Ranch	Baiting	Cage	On-site	NA	Alive
		5	3/7/12	Golden Gate Canyon	Deer Kill	Snare	On-site	NA	Alive
			12/31/12	Crawford Gultch	Shot				Dead
AM76	M	2-3	3/6/11	Heil Valley Ranch	Baiting	Cage	On-site	NA	Alive
		3	12/27/11	Heil Ranch	Replace collar	Hounds	On-site	NA	Alive
		4	2/13/13	Heil Ranch	Recollar	Snare	On-site	NA	Alive
			12/12/13	Heil Ranch	Unknown Mort.				Dead
AF77	F	5	3/9/11	Morrison Mountain	Baiting	Cage	On-site	NA	Alive
		5	11/15/12	Indian Hills	Recollar	Snare	On-site	NA	Alive
AM78	M	2	3/18/11	W. Evergreen	Deer Kill	Cage	On-site	NA	Alive
			5/12/11	Soda Creel/I-70	Road Kill				Dead
AF23	F	2.5	12/8/12	Lacy Property	Initial Collar	Cage	On-site	NA	Alive
			3/27/14	Booth Property	Recollar	Hounds	On-site	NA	Alive
AF79	F	4	3/18/11	Mt. Evans	Dumpsite	Cage	On-site	NA	Alive
		4-5	2/17/12	Mt. Evans	Replace Collar	Hounds	On-site	NA	Alive
AM80	M	1.7 5	3/18/11	Mt. Evans	Dumpsite	Cage	On-site	NA	Alive
AM84	M	2	4/9/11	Shield Park HOA	Sheep depredation	Cage	Deer Creek Canyon	None	Alive
		3	5/4/12	S. Deer Creek	Shot/depredation				Dead
AF86	F	1.5	3/13/12	Gross Dam Road	Recollar	Snare	On-site	NA	Alive
		2	1/31/13	Flagstaff	Recollar	Cage	On-site	NA	Alive
			3/5/14	Walker Ranch	Recollar	Hounds	On-site	NA	Alive
			5/22/15	Walker Ranch	Recollar	Cage	On-site	NA	Alive
AF91	F	1.5	2/4/12	Cotter Mine	Capture effort	Hounds	On-site	NA	Alive
		2	7/20/12	I-70	Road Kill				Dead
AF22	F	1.5	2/29/12	Golden	Baiting	Cage	On-site	NA	Alive
		2	10/5/12	Idaho Springs	Road Kill				Dead

			4/3/15	Golden	Hunting				Dead
AF87	F	4-5	11/18/11	Heil Ranch	Baiting	Snare	On-site	NA	Alive
		4	12/7/11	Hall Ranch	Deer Kill	Cage	On-site	NA	Alive
		5	3/11/13	Hall Ranch	Recollar	Hounds	On-site	NA	Alive
AF88	F	1.5	10/14/11	N. Boulder	Deer Kill	Cage	On-site	NA	Alive
		2	1/11/12	White Ranch	Possible Intraspecific				Dead
AF26	F	1.5	2/27/13	White Ranch	Initial Collar	Hounds	On-site	NA	Alive
AF27	F	1.5	10/31/12	White Ranch	Initial Collar	Cage	On-site	NA	Alive
			1/26/13	White Ranch	Non-target	Snare	On-site	NA	Alive
			2/14/13	Ralston Creek	Non-target	Cage	On-site	NA	Alive
AM49	M	3	4/1/13	Ralston	Initial Collar	Hounds	On-site	NA	Alive
			1/4/14	Lacy Property	Recollar	Hounds	On-site	NA	Alive
			2/17/15	Gultra Property	Shot/Hunter				Dead
AM98	M	1.5	1/4/13	Eldorado Springs	Deer Kill	Cage	On-site	NA	Alive
			5/31/13	Big Thompson	Unknown Mortality				Dead
AM99	M	1.5	12/2/12	Lyons	Human conflict	Free dart	New Hall	None	Alive
			1/6/13	Lyons	Human conflict	Free dart	HWY 72	None	Alive
			1/16/13	Boulder	Human Conflict	Free dart	Buckhorn Rd.	None	Alive
			1/31/13	Livermore	Depredation/Shot				Dead
AM100	M	2	12/23/12	Boulder	Initial Collar	Cage	On-site	None	Alive
			5/27/12	Boulder	DWM Capture Mort	Dart			Dead
AM109	M	1.5	7/23/13	Sugarloaf	Initial Collar	Cage	On-site	None	Alive
			12/16/13	Coal Creek	Recollar	Cage	On-site	NA	Alive
			1/18/15	Idaho Springs	Human Conflict	Cage		Euthanized	Dead
AF122	F	1.5	3/19/13	Hall Ranch	Initial Collar	Cage	On-site	NA	Alive
			1/8/14	Hall Ranch	Recollar	Hounds	On-site	NA	Alive
			3/5/15	Hall Ranch	Recollar	Hounds	On-site	NA	Alive
AM123	M	1.5	3/19/13	Hall Ranch	Initial Collar	Cage	On-site	NA	Alive
			1/13/14	W. Loveland	Human Conflict	Shot			Dead
AM124	M	2	3/30/13	Hall Ranch	Initial Collar	Cage	On-site	NA	Alive
			3/25/14	Heil Ranch	Unknown Mort.				Dead
AF126	F	1	5/16/13	W. Boulder	Human Conflict	Cage	Sugarloaf	None	Alive

SW023	F	1	4/9/09		Rehab	Release	Pike forest	None	Alive
			11/14/09	Lost Valley Ranch	Found dead				Dead
SW026	M	1	10/20/09		Rehab	Release	Hermit Park	NA	Alive
		3	8/19/11	New Mexico	Shot/hunter				Dead
SW107	M	1	5/7/10		Rehab	Release	Radium	NA	Unkn
			3/22/11		Shot/hunter				Dead
AF995	F	1	8/25/11		Rehab	Release	Reynolds Ranch	NA	Alive
		2	6/23/12	Sunshine Canyon	Road Kill				Dead
AF110	F	1	4/25/14	Flagstaff	Initial Collar	Cage	On-site	NA	Alive
			1/4/15	Hwy 7	Shot/Hunter				Dead
AM110	M	1.5	12/19/13	Marietta	Initial Collar	Cage	On-site	NA	Alive
AM111	M	2	1/9/14	Hall Ranch	Initial Collar	Cage	On-site	NA	Alive
AM117	M	4	1/17/15	Heil Valley	Initial Collar	Cage	On-site	NA	Alive
AF43	F	4	1/23/15	Heil Valley	Initial Collar	Cage	On-site	NA	Alive
359	F	2	8/16/14	Boulder	Management	Cage	Boulder Creek	NA	Alive
			4/7/15	Heil Valley	Initial Collar	Cage	On-site	NA	Alive
AM108	M	3	4/10/15	Heil Valley	Initial Collar	Cage	On-site	NA	Alive
AM92	M	1	5/1/15	Walker	Not collared	Cage	On-site	NA	Alive

Table 2: Capture history, aversive conditioning treatments and current status of all cougar cubs captured as part of the Front Range cougar study.

Cougar ID	Sex	Age	Mother	Date	Location	Occurrence	Capture	Release Loc	Conditioning	Status
AF35	F	3	AF16	12/29/08	Evergreen	Deer Kill	Cage	Flying J Open Space		Alive
				12/31/08	Evergreen	Roadkill				Dead
AM36	M	3	AF16	12/29/08	Evergreen	Deer Kill	Cage	Flying J Open Space		Alive
				1/8/09	Evergreen	Starvation				Dead
AM30	M	8	AF01	1/30/09	S. Boulder	Deer Kill	Cage	On-site		Alive
										Dead
AM38	M	8	AF01	1/30/09	S. Boulder	Deer Kill	Cage	On-site		Alive

				3/27/09	S. Boulder	Encounter	Free-dart	Lindsey	Beanbag	Alive
				3/30/09	S. Boulder	Pet Depredation	Free-dart	Centennial Cone	None	Alive
				4/9/09	Morrison	Encounter	Free-dart	None	Euthanized	Dead
AM29	M	6	Euth.	2/11/09	N. Boulder	Deer Kill	Free-dart	Hall Ranch	None	Alive
		12		6/15/09	N. Boulder	Encounter	Free-dart	Masonville	Beanbag	Alive
				10/23/09	Big Thompson	Goat Depredation	Shot			Dead
AM21* collared	M	12	Unkn	3/25/09	Table Mesa	Baiting	Cage	On-site	NA	Alive Dead
AM25	M	12	Unkn	5/22/09	Indian Hills	Deer Kill	Cage	On-site	None	Alive
				9/13/09		Raccoon	Free-dart	Perforated intestine		Dead
AM41	M	12	Unkn	5/22/09	Indian Hills	Deer Kill	Free-dart	On-site	None	Alive
					Indian Hills	Encounter	Shot			Dead
AM65	M	4-5	AF32	11/28/10	Golden	In Neighborhood	Free-dart	White Ranch	None	Alive
AM66	M	4-5	AF32	11/28/10	Golden	In Neighborhood	Free-dart	White Ranch	None	Alive
				12/1/10	White Ranch	Recapture	Hounds	Radium	None	Alive
AF68	F	10	AF50	2/9/11	Flagstaff	Deer Kill	Cage	On-site	NA	Alive
AM83	M	9	AF52	3/24/11	Hall Ranch	Deer Kill	Cage	On-site	NA	Alive
AM85	M	9	AF62	4/13/11	Walker Ranch	Baiting	Cage	On-site	NA	Alive
AF86* collared	F	9	AF62	4/13/11	Walker Ranch	Baiting	Snare	On-site	NA	Alive Alive

Table 3: Noninvasive hair snag capture results for bobcats and cougars. Number of animals seen, number of hair samples collected and number of successful genotypes.

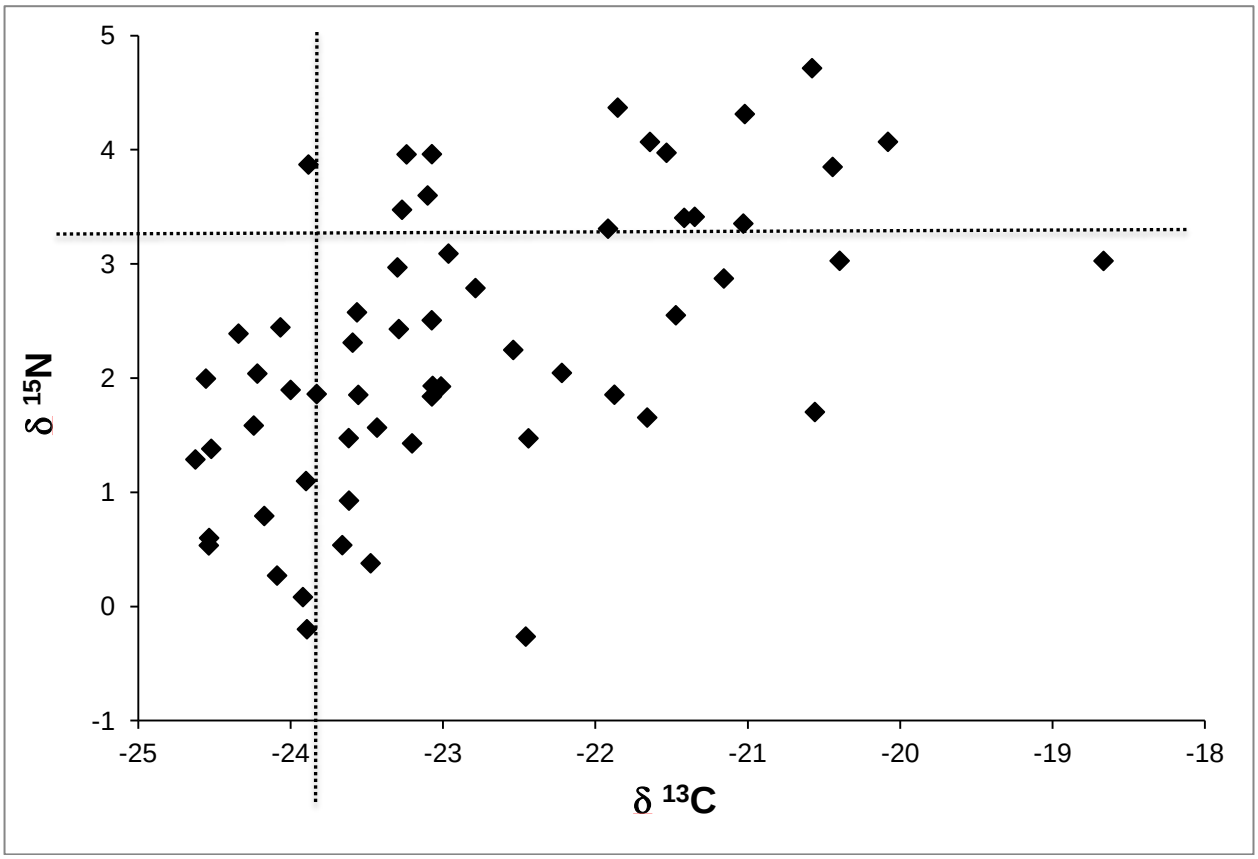
Species	Year	Pictures	Hair Samples	Genotypes
Bobcat	2014	31	5	0
Bobcat	2015	68	12	1
Cougar	2014	86	55	20
Cougar	2015	42	32	11

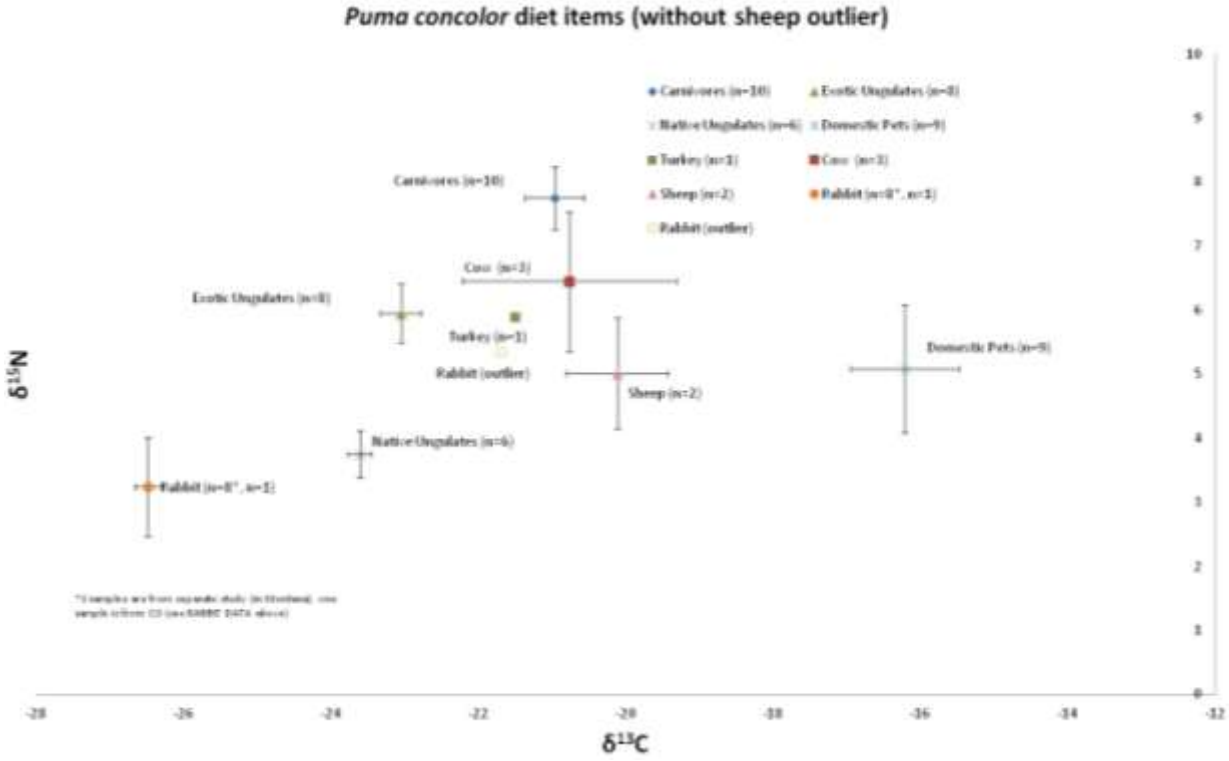
List of figures:

Figure 1: Carbon and nitrogen content in hair from 60 bears harvested in Colorado during the 2011 hunting season showing the variability in concentrations reflecting dietary differences.

Figure 2: Carbon and nitrogen content in hair samples from cougar prey items found in the Front Range of Colorado. Prey items grouped into guilds demonstrates differences in carbon and nitrogen content based on similarities in prey species diet.

Figure 3: Study area for the main Front Range cougar study where most capture effort and field work is conducted.


5
4
3
2
1
0
-1
δ 15N
-25
-24
-23
-22
-21
-20
-19
-18
δ 13C


Puma concolor diet items (without sheep outlier)
Carnivores (n=10)
Exotic Ungulates (n=8)
Native Ungulates (n=6)
Domestic Pets (n=9)
Turkey (n=1)
Cow (n=1)
Sheep (n=2)
Rabbit (n=0*, n=1)
Rabbit (outlier)
δ15N
δ13C
-28
-26
-24
-22
-20
-18
-16
-14
-12
0
1
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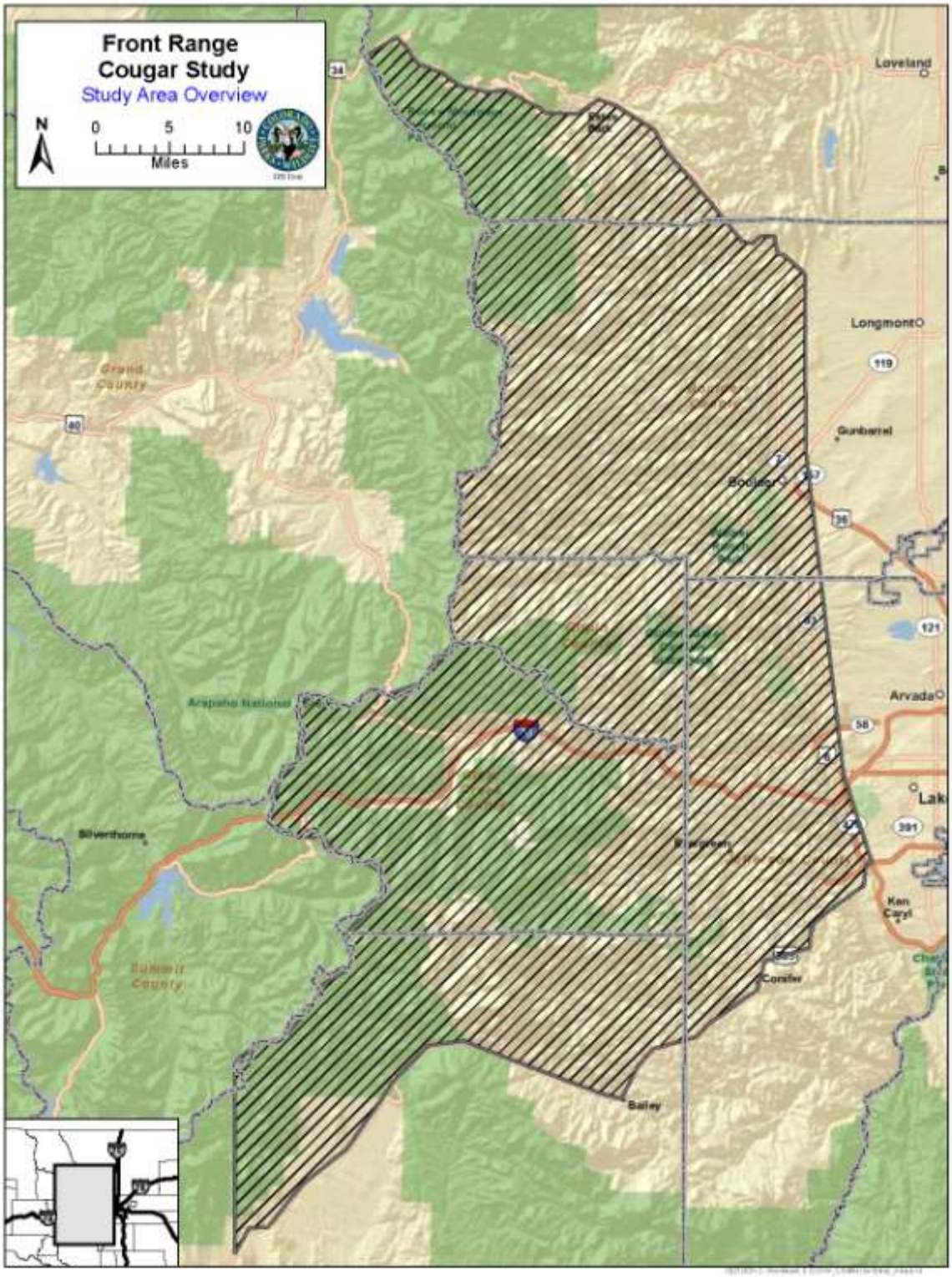
Front Range
Cougar Study
Study Area Overview
N
0
5
10
Miles
Loveland
Longmont
Boulder
Gunbarrel
Arvada
Lak
Silverthorne
Ken Caryl
Conifer
Bailey
Grand County
Arapaho National
34
119
40
121
58
391

Appendix I

Front Range Cougar Research
2014-2015

SPATIO-TEMPORAL PATTERNS OF DIET AND TELOMERE LENGTH IN COLORADO BLACK BEARS

University of Wisconsin - Madison & Colorado Parks and Wildlife

Becky Kirby

Jonathan Pauli

Mat Alldredge

Research Report

SPATIO-TEMPORAL PATTERNS OF DIET AND TELOMERE LENGTH IN COLORADO BLACK BEARS

Becky Kirby, Ph.D. student, UW-Madison

Introduction

The effect of human-derived food on free-ranging wildlife populations is recognized as a growing problem across North America. This has been particularly evident among carnivore populations and especially related to human-wildlife conflict. In the past twenty years, American black bear (*Ursus americanus*) conflicts have expanded along the wildland-urban interface, and are generally attributed to access to human foods (Beckmann et al. 2008; Greenleaf et al. 2009), but still exhibit high geographical and temporal variation (Baruch-Mordo et al. 2008; Beston 2011). Whether increased conflicts are due to growing populations, or alternatively environmental-mediated behavioral changes, remains unknown; and without a thorough understanding of individual, environmental, and population characteristics that contribute to nuisance bears, effective management has proven difficult. As conflicts are predicted to continue to rise, multi-pronged approaches that quantify the influence of anthropogenic foods are needed, as well as those that can assess regional population trends.

To help monitor population trends, knowledge of aging and associated changes in fitness is critical. The age of bears, as well as other mammals, is typically determined by pulling a vestigial premolar and counting cementum annuli (Schroeder and Robb 2005). The estimated age from counts of cementum annuli is highly accurate, but requires the animal to be captured or harvested. With rising numbers of studies using noninvasive sampling for DNA analyses of hair, feather, and scat samples, an aging technique that could be applied to these samples would be desirable. Previous research has demonstrated age-related telomere attrition in a variety of species and has correlated telomere length with individual age (e.g. Hemann and Greider 2000, Haussmann et al. 2003, Pauli et al. 2011). Telomeres are repetitive DNA sequences that cap the ends of eukaryotic chromosomes, whose nucleotide sequence $(T_2AG_3)_n$ is highly conserved across vertebrate species (Meyne et al. 1989). During each cell cycle telomeric repeats are lost because DNA polymerase is unable to completely replicate the 3' end of linear DNA (Watson 1972); thus, telomeres progressively shorten with each cell division. Though the relationship between chronological age and telomere length is highly variable among species, Pauli et al. (2011) successfully demonstrated that after accounting for covariates thought to influence telomere length (sex of the animal, size of the population, and geographic location), they could obtain accurate estimates of age class in martens (*Martes* spp.), and that age estimation via their model in fact exceeded those typically obtained from counts of cementum annuli. Thus, they concluded that quantification of telomere length could be a promising tool to age carnivores and estimate demographic structure for noninvasively collected hair samples (Pauli et al. 2011). Further, even if telomere length is not strongly predictive of chronological age, telomere dynamics can be a valuable indicator of fitness and senescence (Bize et al. 2009).

This project aims to assess broad-scale patterns of diet and telomere length in black bears across Colorado in hunter-harvested and nuisance bears.

Objectives

1. Quantify diet via stable isotopes in hunter-harvested and nuisance bears
2. Quantify telomere length in hunter-harvested bears
3. Investigate individual telomere attrition rate longitudinally in wild bears

Methods

Objective 1: Quantify diet via stable isotopes in hunter-harvested and nuisance bears

In 2011, Colorado Parks and Wildlife opportunistically collected samples from hunter-harvested and nuisance bears. When possible, managers collected 5-10 mls of whole blood and >50 guard hairs (with follicles intact). Hair and blood samples have been analyzed with stable isotopes for diet reconstruction, and hair is being used for telomere length analyses. Other measures collected included sex and head width, as well as age and GPS coordinates of harvest location (Figure 1). Data from 296 hair samples and 113 blood samples are being included in analyses.

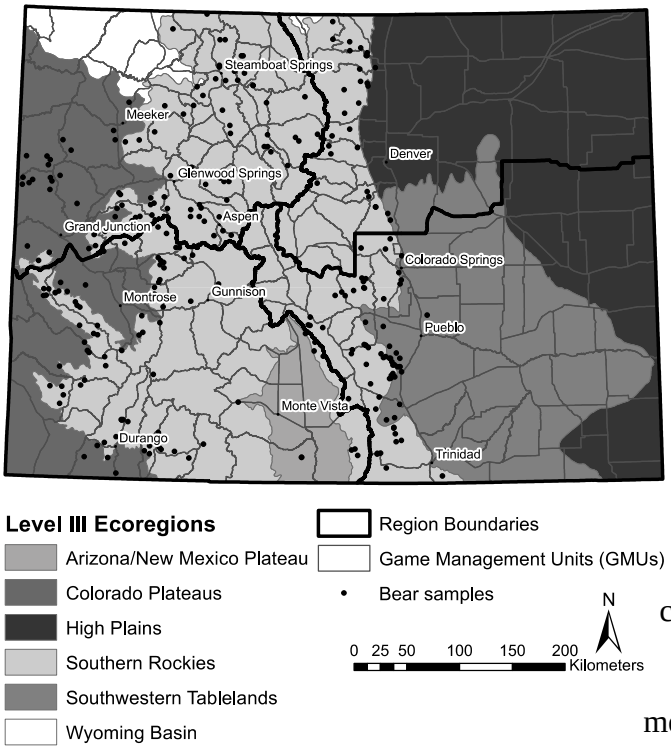

Figure 1. Locations of bear samples collected in fall 2011 by Game Management Unit (GMU), ecoregion, and geographic region.

Stable isotope analysis has yielded significant contributions to wildlife ecology in the last several decades (Kelly 1999, Crawford et al. 2008); of particular interest to managers has been quantifying diet components of free-ranging vertebrates using carbon and nitrogen isotopes. Because corn and sugar utilize a distinct photosynthetic pathway from native plants in temperate North America, corn-dominated human food (waste and agriculture) exhibit distinct carbon ($\delta^{13}C$) values, which can be measured in consumer tissues (Jahren et al. 2006). In addition, measuring nitrogen ($\delta^{15}N$) values can indicate trophic position and animal content in the diet; higher nitrogen values reflect higher trophic positions (Hobson and Welch 1992). Traditional diet reconstruction methods (such as scat or stomach content analyses) tend to underestimate highly digestible resources. Because diet analysis with stable isotopes uses the abundance of two elements (^{13}C and ^{15}N), it avoids this bias. Further, sampling tissues with different metabolic rates allows for higher resolution of temporal patterns of resource use (Hilderbrand et al. 1996). Using isotopic mixing models, we can calculate the percent of diet obtained from native plants, heterotrophs and human-derived food items (Phillips et al. 2005).

Follicles are first clipped off hair samples and placed aside for DNA extraction. The remaining hair shaft is rinsed three times with 2:1 chloroform:methanol solution to remove surface oils (Cryan et al. 2004), dried for 72 hours at 60°C, and homogenized with surgical scissors. Whole blood samples are dried for 72 hours at 60°C, and homogenized with a spatula. Diet samples will also be dried for 72 hours at 60°C and homogenized in a ball mill. For ^{13}C and ^{15}N analysis, samples are weighed, placed in tin capsules and submitted to the Stable Isotope Facility at the University of Wyoming to be analyzed with a Costech 4010 elemental analyzer attached to a Thermo Finnigan DeltaPLUS XP Continuous Flow Isotope Ratio Mass Spectrometer. Results are provided as per mil (parts per thousand [‰]) ratios relative to the international standards of Peedee Belemnite (PDB; $\delta^{13}C$) and atmospheric nitrogen (AIR; $\delta^{15}N$) with calibrated internal laboratory standards.

By quantifying the isotopic signature in tissues of bear and that of diet sources, we can quantify the contribution of isotopically distinct items to the diet of the bear. During 2013, CPW collected potential bear diet samples from 6 different areas: Northern Front Range, Southern Front Range, Uncompahgre Plateau, Piceance Basin, Steamboat Springs, and San Juan Mountains. Samples were collected in early summer and late summer/early fall to obtain herbaceous plants as well as soft/hard mast. Diet samples included items such as cow parsnip, dandelions, chockecherries, raspberries, ants, and acorns (Irwin and Hammond 1985, Raine and Kansa 1990, Baldwin and Bender 2009). Additionally, roadkill deer/elk were opportunistically sampled. A total of 288 vegetation samples and 116 animal matter samples were included in analyses.

To reconstruct diet, we first examined prey samples within geographical regions, and between types. To define a human-derived foods signature, we used human hair samples from across the U.S. (Bowen et al. 2009). Using K nearest-neighbors randomization tests (Rosing et al. 1998) we found that diet items, as expected, collapsed into 3 broad biologically relevant and isotopically distinct classes: native vegetation, animal matter, and human-derived foods, with only minor regional differences (Figure 2).

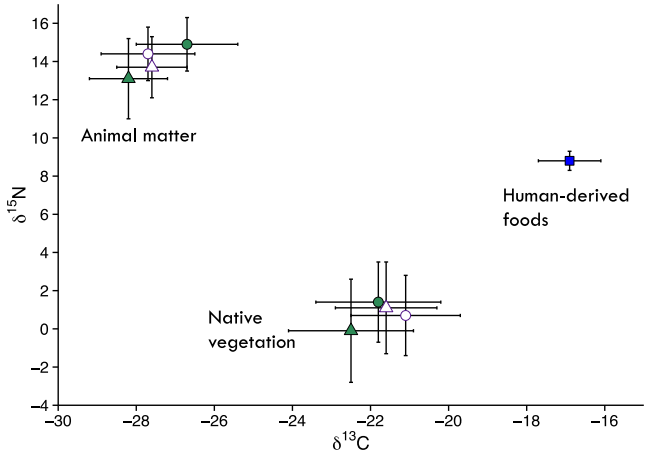

Figure 2. Mean and standard deviation of diet groups used in analyses, corrected for trophic discrimination. Green symbols represent eastern CO, purple symbols represent western CO; triangles represent northern CO, circles represent southern CO.

We estimated proportional importance of each forage group to regional bear populations with Bayesian-based mixing models in the package Stable Isotope Analysis in R (SIAR; Parnell et al. 2010). These models incorporate prior information on variability in isotopic signatures and proportional contributions of sources, resulting in more precise estimates of consumption. We also compared raw isotope values between age, sex, and mortality type.

We then examined how potential variables, specifically demographic class, habitat productivity, and human activity, were correlated with isotopic signature, and therefore diet. Bear harvest location within a Game Management Unit (GMU) was the smallest geographic level to analyze variables representing both habitat productivity and human activity. We compared linear models using $\delta^{13}C$ and $\delta^{15}N$ as response variables, and used Akaike's Information Criterion to select the best models to predict $\delta^{13}C$ and $\delta^{15}N$ separately. Covariates examined include age-sex class (adult female, adult male, subadult female, subadult male), elevation, road density (natural-log transformed), and growing season NDVI.

We then considered conflict bear diet by analyzing samples from bears killed by vehicle collision (n = 14) and lethal nuisance removal by CPW (n = 14), which represent ~16% and 11% of each mortality type in 2011, respectively. We used logistic regression to estimate the odds ratios for mortality types based on isotopic signature, comparing conflict bears to a subset of harvested bears only in the same GMUs (n = 62), to remove geographical bias.

Objective 2: Quantify telomere length in hunter-harvested bears

We are using the same hair samples collected in Objective 1 for telomere length analysis. As telomeres shorten with cellular replication, they are potentially a useful marker for chronological age and a proxy for fitness and senescence (Aydos et al. 2005). Telomerase, a reverse transcriptase, counteracts this loss in the germline, but tends to be far less active in somatic cells; this activity seems to vary with body mass, with larger animals having less telomerase activity (Seluanov et al. 2007). Additionally, as lifestyle-related activities, in particular oxidative stress, can affect telomere length negatively (von Zglinicki 2002, Monaghan and Haussmann 2006), so we aimed to examine what might be the individual and ecological drivers of telomere attrition.

Hair samples were extracted with standard procedures using Qiagen Dneasy tissue extraction kit. We quantified the relative length of telomeres using real-time quantitative polymerase chain reaction (Q-PCR) (Cawthon 2002). This approach has been found to be highly accurate, in particular for within species comparison (Cawthon 2002, Nakagawa et al. 2004). The method determines relative telomere length by comparing the ratio of telomere repeat copy number (T) to single copy gene number (S) in a particular DNA sample to that of an arbitrary reference DNA. Relative differences in telomere length between individuals then, is exhibited by contrasting the T/S ratio of one individual to that of another. Any single copy gene sequence can be employed for standardization, and after exploring several possibilities we found HNRPF specific to bears (Fedorov et al. 2009) to be the most readily amplified. Telomere primers developed by Cawthon (2009) generate a short, fixed length product, reducing variability within sample replicates.

Telomere and single-copy gene PCR are conducted on separate 96-well plates, but preparation is identical except for the primers. Each reaction contains 8 µl sample DNA (diluted to 3 ng/µl), 10 µl SYBR Select Master Mix (Life Technologies - Applied Biosystems), telomere primers (250 nM each final concentration) or single copy gene primers (500 nM each final concentration), and distilled water to total 20 µl reaction volume. Real-time PCR is conducted with an Eppendorf Mastercycler, with the following thermocycling conditions: telomere: 50°C for 2 min, 95°C for 5 min, followed by 2 cycles of 94°C for 15 sec and 49°C for 15 sec, and then 35 cycles of 95°C 15

sec, 62°C 10 sec, 74°C 15 sec (telomere) or 95°C 15 sec, 62°C 15 sec, 72°C 45 sec (HNRPF). Baseline correction is performed on raw fluorescence data in the program LinRegPCR (Ruijter et al. 2009) using its automatic strict baseline correction. Comparative Cq (within LinRegPCR) uses the starting concentration (N0) for each sample calculated within LinRegPCR based on threshold, efficiency, and Cq (N0=Nt/(E^Cq) where Nt is the threshold, E is the mean amplification efficiency, and Cq is the quantification cycle (Ruijter et al. 2009). Ratio of T/S is presented as the relative telomere length (in this study with a C.V. of 13%).

We explored relationships to age and other covariates beginning with simple correlations and t-tests, and linear regression. Potential covariates were extracted at the level of Game Management Unit (GMU) for each harvested bear. We examined how potential variables could influence telomere length in black bears. Specifically we considered three groups of variables measuring individual characteristics, geographic characteristics, and habitat characteristics. Individual variables included age, sex, zygomatic width, and $\delta^{15}N$. Geographic/environmental variables included location of harvest (latitude and longitude in UTMs) and elevation. Habitat characteristics included vegetative productivity, bear population density, and forage quality. Bear harvest location within a Game Management Unit (GMU) was the smallest geographic level to analyze variables representing environmental and habitat quality, all of which we calculated in ArcGIS (ESRI, v.10). Average elevation for each GMU was calculated from the National Elevation Dataset (USGS 2009). Net primary productivity (NPP, g C/km^2 $year^{-1}$) at 1-km spatial resolution was downloaded from the MOD17 data set (Numerical Terradynamic Simulation Group, University of Montana, Missoula, Montana). We calculated average NPP from 2000-2010 at the level of GMU. To create an index of relative bear population density, we calculated proportion of bears harvested within a GMU to the harvest total. We also examined a bear forage quality index used by Colorado Parks and Wildlife. This index is calculated by multiplying a mast production rating (1-5) by a mast potential scale (the number of primary masting species present) (Apker, unpublished data). This estimates availability and potential of common bear mast foods at the level of Bear Data Analysis Unit (a coarser scale than GMU).

We first explored effects of continuous variables on telomere length with simple linear regression analyses. We tested from differences in telomere length between age and sex classes using Welch's 2-sample t-test. We explored relationships among covariates with Pearson correlations. We excluded head size from further analyses as it was highly correlated with age ($r = 0.42$, $n = 202$, $P < 0.0001$). We created a suite of models then from the exploratory analyses to include covariates that appeared to be biologically meaningful, and represented intrinsic, geographic, and habitat characteristics. We included only bears with data for all covariates ($n = 191$). We compared linear models using T/S as the response variable and used Akaike's Information Criterion to select the best models. Covariates examined included age, sex, elevation, latitude, and bear population density (log-transformed).

Objective 3: Investigate individual telomere attrition rate longitudinally in wild bears

Dunshea et al. (2011) recently called for more longitudinal studies to elucidate factors affecting telomere dynamics. Further, recent studies of hibernating rodents have effectively demonstrated that spending more time in torpor retards the rate of telomere attrition (Turbill et al. 2012, 2013). As initial results indicate little relationship with chronological age, understanding what

ecological factors affect telomere attrition is particularly relevant. Thus, we will longitudinally examine telomere length in bears, as well as the relationship between telomere attrition and bear hibernation. To do so, we sampled free-ranging black bears. CPW collared and sampled 6 bears on the Front Range in Colorado during 2012; 4 of which were resampled in the den in winter 2012-13. An additional 2 bears were collared in summer 2013, and 5 total were resampled in winter 2013-14. Telomere length will be measure in these bears (with at least 1 summer and 1 winter sample). Bears were captured and anesthetized using CPW standard protocols in summer and relocated in winter dens.

Hibernation length will be estimated from GPS data. We will use d-ROMs and OXY-Adsorbent tests (Diacron, International, Italy) to measure the oxidative status of each individual in summer and winter (Beaulieu et al. 2011, Stier et al. 2012). Bears that hibernate less, or are using poorer quality habitat/diet during the summer would be expected to be under increased oxidative stress. We will explore relationships between calculated rates of telomere change and oxidative status, and hibernation length using mixed linear models (Beaulieu et al. 2011, Turbill et al. 2013). These results will inform our understanding of environmental factors influencing aging and telomere attrition in black bears.

Preliminary Results and Discussion

Objective 1: Quantify diet via stable isotopes in hunter-harvested and nuisance bears

We have analyzed stable isotope data from 296 hair samples and 113 blood samples, and present preliminary results. Hair samples indicate diet composition during the period of growth (mid-summer through fall), whereas blood samples represent more recent diet (last month). Enriched (higher) $\delta^{13}C$ and $\delta^{15}N$ likely indicate greater consumption of human-derived foods and animal matter, respectively. Preliminary analyses show wide variation among individual bears and a general linear relationship between $\delta^{13}C$ and $\delta^{15}N$

As expected, native vegetation made up the primary summer diet group for Colorado bears in all regions, ranging from a low of 69% in the northeast to a high of 85% in the southwest (Table 1). However, there were strong longitudinal differences in estimates of human food contributions, with eastern bears consuming over 20% human-derived foods, while western bears consumed less than 10%. Because we used region-specific diet samples to parameterize the model, these differences are not based on an isotopic difference in prey base.

Table 1. Assimilated dietary estimates for black bears in the summer and fall seasons, obtained from the isotopic signatures of hair and blood, respectively. Eastern bears consumed more human-derived foods than western bears, regardless of season, but bears consumed less human-derived foods during the fall than the summer. Estimates provided by region of Colorado.

	Mean Proportion (95% CI)			
Diet Groups	NE CO	SE CO	NW CO	SW CO
Hair (n)	29	71	104	92
Vegetation	0.69 (0.65-0.74)	0.72 (0.69-0.76)	0.83 (0.80-0.85)	0.85 (0.82-0.88)
Animal matter	0.04 (0.02-0.07)	0.06 (0.05-0.08)	0.11 (0.09-0.12)	0.11 (0.10-0.13)
Human-derived foods	0.26 (0.21-0.32)	0.22 (0.18-0.26)	0.07 (0.03-0.10)	0.04 (0.00-0.07)
Blood (n)	9	29	37	38
Vegetation	0.64 (0.53-0.76)	0.77 (0.70-0.84)	0.83 (0.80-0.86)	0.86 (0.83-0.88)
Animal matter	0.07 (0.00-0.13)	0.07 (0.05-0.11)	0.15 (0.12-0.17)	0.13 (0.11-0.15)
Human-derived foods	0.29 (0.14-0.45)	0.15 (0.06-0.24)	0.02 (0.00-0.05)	0.01 (0.00-0.04)

For summer diet, age-sex class was an influential predictor of the hair isotopic signature of bears, with all four age-sex groups exhibiting significant differences (Table 2, MANOVA, Wilk's λ = 0.84, $P < 0.001$). Adults were enriched in both $\delta^{13}C$ and $\delta^{15}N$ over subadults, though adult females were the most enriched in $\delta^{13}C$, while adult males were the most enriched in $\delta^{15}N$.

Table 2. Isotopic signatures of hunter-harvested black bear hair by age-sex class. Adults (> 4 years of age) and females are enriched over subadults (1-4 years), with females enriched in carbon and males enriched in nitrogen, suggesting females consumed more human-derived foods while males consumed more animal matter. Different superscripts indicate significantly different groups at $P < 0.05$.

		$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
	n	Mean	SD	Mean	SD
Adult female	71	-21.30^{a}	0.99	5.20^{ab}	1.18
Adult male	93	-21.70^{b}	1.24	5.65^{a}	1.36
Subadult female	39	-21.96^{bc}	1.07	4.64^{b}	1.11
Subadult male	93	-22.16^{c}	0.91	4.90^{b}	1.12

Both top models also identified NDVI as an important covariate, with a slight negative relationship with $\delta^{13}C$ (β = -0.007, P = 0.047) and $\delta^{15}N$ (β = -0.018, $P < 0.001$) (Table 3), suggesting that bears in GMUs with higher productivity consumed more native vegetation. Road density was positively related to $\delta^{13}C$ enrichment (β = 0.533, $P < 0.001$), and thus, to bear reliance on human-derived foods, regardless of age-sex class (Figure 3).

Table 3. Top models to predict carbon and nitrogen isotope hair and blood signatures, representing summer diet and fall diet, respectively. Covariates tested were age-sex class, mean road density (natural-log transformed), growing season productivity (NDVI), and mean elevation (all calculated at the level of Game Management Unit). Models were ranked using AIC (only < 2 ΔAIC are shown).

	AIC	Δ AIC	weight
Hair			
$\delta^{13}C$			
Road density + Age-Sex Class + NDVI	9.75	0.00	0.49
Road density + Age-Sex Class + NDVI + Elevation	10.98	1.23	0.27
$\delta^{15}N$			
NDVI + Age-Sex Class	102.38	0.00	0.44
NDVI + Age-Sex Class + Elevation	103.53	1.15	0.25
NDVI + Age-Sex Class + Road density	103.91	1.53	0.20
Blood			
$\delta^{13}C$			
Road density + NDVI	60.53	0.00	0.62
Road density + NDVI + Elevation	62.34	1.81	0.25
$\delta^{15}N$			
NDVI	42.94	0.00	0.34
NDVI + Road	44.22	1.28	0.18
NDVI + Elevation	44.73	1.79	0.14

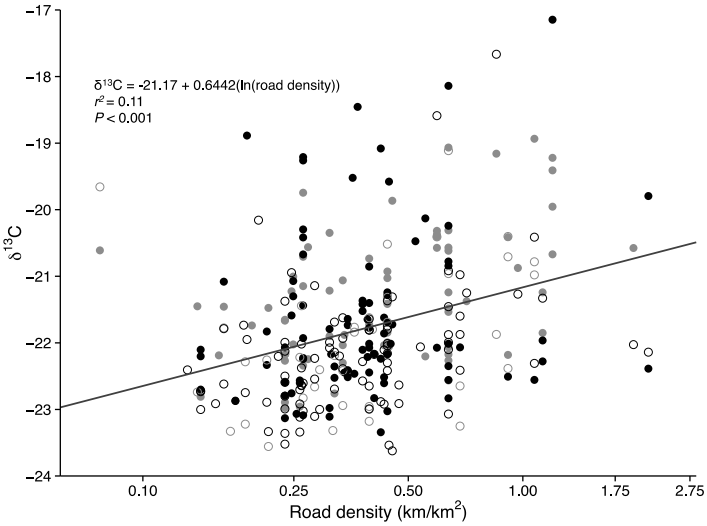

Figure 3. Linear regression of $\delta^{13}C$ on road density (natural-log transformed), showing a positive relationship between increased road density and $\delta^{13}C$ enrichment, with points representing age-sex classes: adult male (*filled black circles*), adult female (*filled gray circles*), subadult male (*open black circles*), subadult female (*open gray circles*).

Hair samples from "conflict" bears (nuisance removals or vehicle collisions) were typically enriched in isotopic signature compared to hunter-harvested bears (Table 4, MANOVA, Wilk's $\lambda = 0.93$, $P = 0.04$), with nuisance bears being the most enriched. Enrichment in $\delta^{13}C$ is related to an increased probability of being a nuisance bear, as opposed to a hunter-harvested bear. The odds of being a nuisance bear increased by 60% for each per mil increase in $\delta^{13}C$ (odds-ratio: 1.6, 95% CI: 1.1-2.51, $P = 0.02$).

Table 4. Isotopic signatures of bear hair by mortality type. Conflict bears (including both vehicle collisions and nuisance removals) were significantly enriched in carbon and nitrogen compared to hunter-harvested bears, suggesting greater human-derived food consumption. Of the conflict bears, nuisance bears were the most enriched group in carbon and nitrogen.

		δ^{13}C (‰)		δ^{15}N (‰)	
	n	Mean	SD	Mean	SD
Hunter-harvested	62	-21.17	1.32	5.39	1.26
Vehicle collisions	14	-20.87	1.27	5.81	1.41
Nuisance removal	14	-20.12	1.61	6.25	1.10

At the landscape scale, then, human activity (as indexed by road density) appeared to be the strongest predictor of human-derived food consumption. This relationship held regardless of age-sex class, tissue type, or native vegetative productivity. Further, use of food subsidies was predictive of conflict, confirming that lethally removed nuisance bears, as well as roadkill bears, consumed more human-derived foods than hunter-harvested bears.

This work has been prepared as a manuscript and is in the review process with *Oecologia*.

Objective 2: Quantify telomere length in hunter-harvested bears

Quantifying telomere length accurately is based on careful optimization of the qPCR reactions and choice of a reliably amplifiable single copy gene. For reactions to be considered adequate, efficiencies of both the telomere and the single copy gene reactions need to consistent. Reactions are run in triplicate and we use the mean T/S as relative telomere length in subsequent analyses.

We quantified relative telomere lengths (T/S) of hunter-harvested bears across Colorado (n= 245). Relative telomere lengths averaged 3.43 (range: 12.8 – 6.99), across bears aged 1 – 21 (152 males and 93 females). We did not detect any relationship between telomere length and individual covariates including age ($F_{1,217} = 0.39$, $P = 0.54$), age class ($t_{219} = 0.57$, $P = 0.57$), zygomatic width ($F_{1,223} = 0.59$, $P = 0.44$), or sex ($t_{186} = 0.49$, $P = 0.63$), suggesting intrinsic covariates have little influence on bear telomere length (Figure 4).

Telomere length declined with increasing latitudes ($F_{1,219} = 20.98$, $P < 0.0001$) and increasing elevation ($F_{1,243} = 7.71$, $P = 0.0059$), suggesting a geographical relationship with telomere length. We did not detect a relationship with NPP and telomere length ($F_{1,243} = 0.14$, $P = 0.71$) or δ^{15}N and telomere length ($F_{1,240} = 0.70$, $P = 0.40$). However, telomere length increased with bear population density ($F_{1,241} = 4.63$, $P = 0.03$), as well as bear forage quality ($F_{1,239} = 5.21$, $P = 0.023$). Both these covariates are measures of habitat quality, suggesting a positive effect of habitat quality on telomere length.

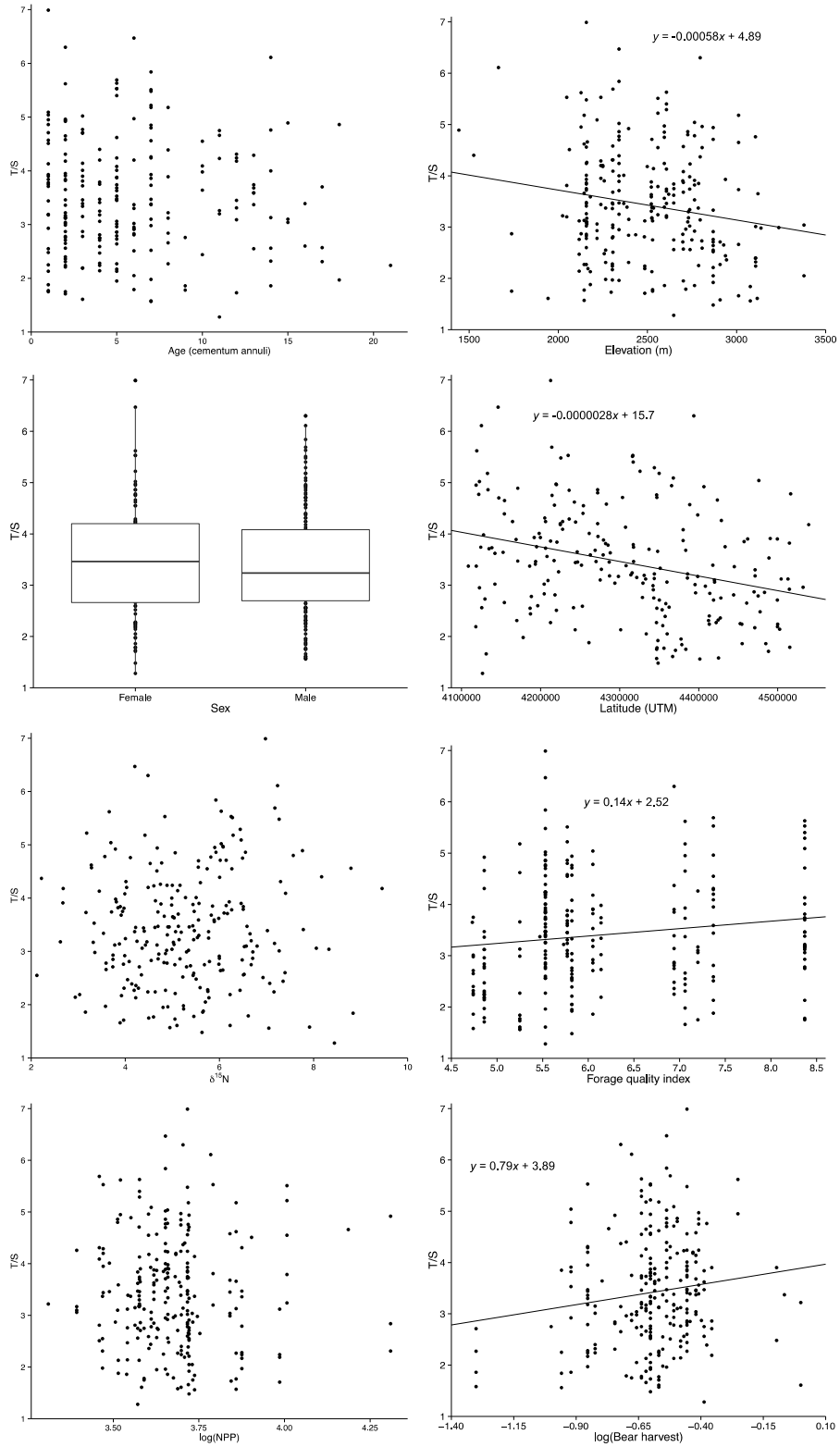

Figure 4. Relationship of relative telomere length (T/S) with individual covariates that we predicted could influence telomere lengths in Colorado black bears. Regressions shown for significant relationships.

The top linear model included age and latitude as influential covariates. Latitude was a significant covariate in all of the top 6 models (Table 5). Though the betas were not significant, age was present in the top three models (Table 6), with a negative relationship with telomere length. Bears harvested in northern Colorado had shorter relative telomere lengths than those harvested in southern Colorado.

Table 5. Top models to predict relative telomere length (T/S). Covariates included age, latitude, mean elevation and bear population density. Elevation and population density were estimated at the level of Game Management Unit. Models were ranked using AIC (only < 2 ΔAIC are shown).

	AIC	Δ AIC	weight
Age + Latitude	5.94	0.00	0.23
Age + Latitude + Bear population density	6.53	0.59	0.17
Age + Latitude + Elevation	6.77	0.83	0.15
Latitude	7.31	1.38	0.12
Latitude + Elevation	7.66	1.72	0.10
Latitude + Bear population density	7.86	1.92	0.09
Age + Latitude + Elevation + Bear population density	7.91	1.96	0.09

Table 6. Covariate estimates for top 3 models predicting relative telomere length.

	Estimate (β)	*P*
Model 1		
Age	-0.03	0.07
Latitude	-0.0000028	<0.0001
Model 2		
Age	-0.003	0.07
Latitude	-0.0000025	0.0004
Bear population density	0.49	0.24
Model 3		
Age	-0.03	0.09
Latitude	-0.0000027	<0.0001
Elevation	-0.00027	0.29

We then wanted to test if there was underlying genetic structure that could be driving the latitudinal pattern in telomere lengths. CPW analyzed the CO bear population in 2008 and found that it was all one panmictic population. We confirmed this with a subset of 100 individuals from our dataset. We genotyped the bears at 4 microsatellite loci and used the program STRUCTURE for analysis. As expected, they did not exhibit any geographic structure, and the latitudinal patterns are unlikely to be solely a result of an underlying genetic structure. Thus, we posit that these patterns reflect differences in important environmental conditions, particularly those driving physiological stress and characteristics of hibernation, that are overwhelming potential relationships to typical predictors of telomere lengths.

This work is currently in preparation as a manuscript.

Objective 3: Investigate individual telomere attrition rate longitudinally in wild bears

Samples have been collected, with a total of 6 bears with samples from at least 2 time periods. Having optimized the telomere length reaction and have been examining potential influences on telomere length, we are proceeding with laboratory analyses of these bear samples. We are quantifying quantify telomere length in each blood sample from these bears, as well as oxidative stress, to examine differences between hibernation characteristics, stress, and aging.

Continuing Plans

Objective 1 has been prepared as a manuscript, and is in the review process with *Oecologia.* Objective 2 is currently in preparation as a manuscript, with the intention to submit within a few months. Objective 3 is in the process of laboratory analyses, intended to be completed this year. Ultimately this study intends to enhance our understanding of patterns of bear diet and telomeres across Colorado in order to inform future management.

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The diet of black bears tracks the human footprint across a heterogeneous landscape

Our large-scale analysis of >240 black bears sampled across the Colorado landscape reveals how large carnivores can specialize on resource subsidies regionally. This work is the first to examine bear use of subsidies at this scale, and highlights the fidelity of stable isotopes ($\delta^{13}C$) as a tracer of human foods. Our findings provide insights and methodological approaches for the growing guild of synanthropic species, reveal a large-scale subsidization of ecological communities, and emphasize the need for understanding how behaviorally flexible species respond to available food subsidies.

Abstract Food subsidies have become a widely available and predictable resource in human-modified landscapes for many vertebrate species. Such resources can alter individual foraging behavior of animals, and induce population-wide changes. Yet little consensus exists about the relative influence of the availabilities of native and human food subsidies to wildlife foraging throughout altered landscapes. We explored this unresolved question by analyzing the effects of landscape factors on American black bear (*Ursus americanus*) diet across Colorado. We estimated assimilated diet using stable isotope analysis of harvested black bear tissues to determine the contribution of human-derived foods to bear diets throughout Colorado, as well as how increasing reliance on human-derived food subsidies increases the risk of conflict. We found that bears (n = 296) showed strong regional diet variability, but substantial use of human-derived food subsidies in eastern Colorado (>30% assimilated diet). The age-sex class of the bear, and road density and crop cover of its harvest location were the most influential predictors of ^{13}C enrichment (a tracer of human food subsidies). Furthermore, foraging on subsidies increased risk of conflict; the odds of being a nuisance bear increased by 60% for each ~ 1 ‰ increase in $\delta^{13}C$. Our study confirms the efficacy of $\delta^{13}C$ as a proxy for human activity, and indicates that while demographic differences play a clear role in the foraging ecology of bears, availability of subsidies coincident with varying levels of human activity appears to be a principal driver in predicting diet of Colorado bears.

Appendix III

Front-range Cougar Research
2014

Effect of human activity on cougar diet and age structure: non-invasive approaches

UW-Wisconsin & Colorado Parks and Wildlife

Wynne Moss

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Research Report

Novel habitats present novel challenges for an apex carnivore (*Puma concolor*)

Abstract: Human-modified landscapes are now the most common global covertype. Species persistence in these landscapes hinges upon adaptability, including the capacity to exploit novel food resources and habitats. Yet, for large carnivores, there may be significant costs of such a strategy. Cougars (*Puma concolor*), though generally considered specialists of large ungulates, are capable of preying upon a variety species, which could be advantageous in novel ecosystems like developed landscapes. However, these areas also represent a landscape of heightened risk of conflict with humans. We examined the tradeoff between dietary flexibility and survival in a population of cougars inhabiting Colorado's urban-wildland interface. We monitored space use of GPS-collared cougars and related this to estimates of diet from stable isotope analysis. Our population-wide estimate of diet revealed that native herbivores constituted the bulk of assimilated biomass (64-79%), though there was considerable variation among individuals. Cougars using the most highly developed areas obtained 20% more of their diet from alternative prey (synanthropic wildlife and domestic animals) than those in the least developed areas. Adult males and subadults consumed more alternative prey compared to adult female cougars. Use of developed areas significantly increased risk of mortality for both males and females. Thus, though cougars displayed a highly plastic foraging strategy in developed areas, they were less likely to survive. Our findings reveal that, despite their dietary flexibility, the heightened risk from human conflict is likely to inhibit cougar population recoveries in densely populated areas.

Niche sprawl in an opportunistic apex predator (*Puma concolor*)

Abstract

Urban areas are dramatic examples of landscape change and increasingly identified as systems in which to promote ecological complexity and conservation. Yet, little is known about the processes that regulate highly developed ecosystems, or the behaviours employed by species adapting to them. We evaluated the isotopic niche of an ecologically important apex carnivore, the cougar (*Puma concolor*), over broad spatiotemporal scales and in a region characterized by rapid human growth. We detected a niche expansion, from specialization on native herbivores in wildlands to enhanced reliance on exotic and invasive species by cougars in contemporary urban interfaces. We show that 25 years ago, cougars inhabiting these same urban interfaces possessed diets that more closely resembled their wildland counterparts, suggesting foraging adaptations are recent. Thus, urban sprawl has been accompanied by a niche sprawl over both time and space, indicating that an important top predator is interacting in novel ways. Thus, adaptations to urbanization could alter the ecological role of apex carnivores, and while human-dominated landscapes may maintain these species, their functional relationships are unlikely to remain the same.

INTRODUCTION

The conservation and management of large carnivores depends upon a solid understanding of their foraging ecology and diet composition. Large carnivore foraging behavior is inherently related to their risk of conflict (Mattson et al. 1992, Mishra 1997), and is, therefore, of interest to agencies concerned with human-carnivore interactions. In addition, quantifying the diets of large carnivores is essential to predicting their impact on prey populations, and, indeed on entire ecosystem processes. This information is particularly lacking in urban ecosystems, where large carnivores are at high risk of conflict (Beckmann and Lackey 2008, Kertson, Spencer, Marzluff, et al. 2011), and where their interactions with prey species may shift dramatically (Lowry et al. 2012).

Cougars (*Puma concolor*) are an extremely plastic predator, capable of preying upon a variety of species, from small mammals to large-bodied ungulates (Murphy and Ruth 2010). This, coupled with their cryptic nature, makes it difficult to predict their diet, and differences in methodologies between studies further complicate our ability to understand cougar diet across a wide geographic scale. All recent studies of North American cougar populations, however, indicate that native ungulates are the primary prey (Anderson and Lindzey 2003, Knopff et al. 2010, Kertson, Spencer, and Grue 2011, White et al. 2011). This degree of reliance is variable, both across and within populations. Factors that influence cougar diet composition include prey availability (Knopff 2010), demographic class (Anderson and Lindzey 2003), and habitat type (Magioli et al. 2014). All of these factors strongly differ in urban relative to wildland habitats, indicating that cougar diet may be altered in urban ecosystems. In fact, cougars in urban areas have been shown to rely more heavily upon alternative, smaller-bodied prey items (Kertson, Spencer, and Grue 2011). Yet, the importance of these small-bodied prey has likely been underestimated by use of kill site investigations, which are biased towards larger-bodied prey. Further, there is significant within-population variability in diet (Knopff and Boyce 2007); the factors generating such variability are not well understood but would aid in predicting the extent of dietary shifts and identifying the individuals who are likely to depredate domestic species.

Monitoring the age-sex structure of cougar populations is important for understanding their relationship to prey species, as well as to forecasting demographic trends and setting sustainable harvest goals. Demographic classes tend to prey upon different species (Anderson and Lindzey 2003, White et al. 2011). However, it is not known whether or not demographic classes differ in diet in urban areas (Kertson, Spencer, and Grue 2011), nor how the age-sex structure of cougar populations might be altered by urbanization and high rates of human conflict. For instance, young male cougars are more likely to utilize urban and exurban habitat, and therefore might be expected to rely more heavily upon domestic species (Kertson et al. 2013); therefore younger cougar populations could be expected to undergo higher rates of conflict and more depredation events. As such, understanding the link between age-sex structure and cougar diet in urban areas is an essential aspect of reducing cougar-human conflict.

Current methods of estimating cougar age (using gum recession and tooth wear, cementum annuli counts, or individual history) can only be carried out on captured or necropsied individuals; as a result, the structure of cougar populations is generally extrapolated from harvest

composition or from research studies conducted in limited geographic areas. Therefore, current methods provide an incomplete picture of cougar population ecology. Alternatively, the quantification of telomere length could provide a non-invasive method to identify cougar age class, since telomere DNA can be extracted from non-invasively collected samples like hair snags. Telomeres are repetitive DNA sequences that cap the ends of eukaryotic chromosomes and shorten with each round of cellular replication and animal age (Watson 1972). A predictable relationship between telomere length and age has been found for several species (Haussmann et al. 2003, Pauli et al. 2011), indicating the potential for using telomere length as an indicator of age. The relationship between cougar age and telomere length has not been explored. If cougar telomere length can be modeled as a function of age and other available covariates, this could represent a valuable tool for aging this species.

To understand how urbanization alters cougar foraging ecology and diet composition, we will compare the diets of two populations in Colorado, one in an urban-exurban population (the Front Range), and one in a wildland area (the Uncompahgre Plateau). We will also investigate variation in cougar diet within the Front Range population to determine what factors predispose individuals to use non-ungulate prey. In doing so, we will develop and evaluate non-invasive techniques to quantify cougar diet and demographic class.

Our objectives are:

(1) Compare population-wide differences in diet between urban-exurban (Front Range) and wildland (Uncompahgre Plateau) cougar populations using a non-invasive approach, stable isotope analysis.
(2) Investigate the factors that impact cougar reliance on primary prey in the Front Range population, including habitat use, demographic class, and body condition.
(3) Develop a noninvasive method to identify cougar demographic class.

METHODS

All cougar captures and sampling have been done as part of ongoing CPW projects: *Cougar Demographics and Human Interactions Along the Urban-Exurban Front Range of Colorado* and *Puma Population Structure and Vital Rates on the Uncompahgre Plateau.*

Objective 1: Compare population-wide differences in diet between two cougar populations

We sampled hair from GPS-collared individuals who are captured as part of the Front Range and Uncompahgre Plateau cougar projects. These samples were the basis for stable isotope analysis to quantify diet composition. We used hair samples because this type of tissue was easily obtained at captures and would easily apply to future non-invasive work. Analyzing the diet of individuals from both of these study areas provided us with a unique opportunity to compare resource use over wide geographic areas with differing levels of human density. The Front Range study area has a higher proportion of urban-exurban habitat and greater human density than the Uncompahgre Plateau; these differences in habitat and human density may drive shifts in diet.

Prey hair was collected from roadkills and cougar kill sites, as well as from shed hair. We sampled prey species separately for each study site, because isotopic signature can vary with geographic area. If we did not find differences in isotope signature between study site for a given prey species, we pooled samples. We collected species that were commonly found at kill sites or were identified as important components of cougar diet by other studies.

All hair samples were washed three times with 2:1 chloroform: methanol to remove surface oils and debris (Cryan et al. 2004), homogenized, and dried for 72 hours at 55°C. Samples were weighed into tin combustion capsules and analyzed with a Thermo Finnigan Delta Plus XP Elemental Analyzer. Results are provided as per mil (parts per thousand [‰]) ratios relative to the international standards of Peedee Belemnite (PDB; $\delta^{13}C$) and atmospheric nitrogen (AIR; $\delta^{15}N$) with calibrated internal laboratory standards.

Diet reconstruction with stable isotopes relies upon comparing the isotopic signature of the consumer to the signatures of potential diet items, which are classified into biologically relevant and isotopically distinct groups. To group prey samples, we applied a K nearest-neighbor randomization test (Rosing et al. 1998). Diet composition was estimated as proportional use of each of these prey groups using Bayesian mixing models in the software package SIAR (Parnell et al. 2008). This analysis estimates the distribution in possible diet compositions for each individual or for entire populations (Parnell et al. 2010). We estimated a population-wide estimate of diet composition for both populations, as well as estimates at the individual level and between demographic classes for the Front Range population.

Objective 2: Investigate the factors influencing diet in the Front Range population

For GPS-collared cougars on the Front Range, we investigated the effects of numerous covariates (age-sex class, body condition, habitat use, and interactions between covariates) on diet. Using isotope mixing models (see above), we demonstrated that increasing values of $\delta^{13}C$ and $\delta^{15}N$ were associated with greater use of alternative, urban-associated prey items; therefore linear models to predict isotope signature also predict diet composition.

To measure habitat use during foraging behavior, we used housing density at nighttime GPS locations. Housing density was log-transformed for linear models to meet assumptions of normality, and we took the median value of each individual's nighttime locations as a measure of that individual's exposure to human influence. We assigned an index of body condition to each cougar using the residual from a population-wide body length-to-mass regression (Jakob et al. 1996). Measures of demographic class included age in months, age-sex class (adult male, adult female, subadult male, or subadult female), and sex. We used linear mixing models with $\delta^{13}C$ and $\delta^{15}N$ as response variables, and selected the best model to predict carbon and nitrogen separately, using Akaike's Information Criterion, corrected for small sample sizes (AIC_c).

Objective 3: Develop methods for and evaluate the accuracy of using telomere length to non-invasively age cougars.

Because tissue types differ in their rate of cellular replication, we will examine telomere length in two commonly sampled tissue types, blood and hair. Blood and hair have collected from

cougars captured on the Front Range research study and from necropsied individuals at the Wildlife Health Laboratory. We derived a "known" age via gum-line recession, tooth wear, capture history, and cementum annuli counts.

DNA was extracted from blood and hair follicles using Qiagen DNeasy kit and Qiagen DNA micro kits (Qiagen, Valencia, CA). Telomere length will be quantified with real-time quantitative polymerase chain reaction (Q-PCR) (Cawthon 2002). This method determines relative telomere length by calculating the ratio of telomere repeat copy number (T) to single copy gene number (S), standardized by an arbitrary reference DNA. We will compare standardized T/S ratios among individuals. For a single copy reference gene, we will use the nuclear gene 36B4, which is highly conserved across vertebrates and was used to quantify telomere length in humans (Cawthon 2002).

We will run telomere and single copy gene q-PCRs using similar PCR protocols, with the only difference being the primer set. To generate a standard curve, we will dilute DNA from an arbitrarily chosen individual to 1 ng/µl, 2.5 ng/µl, 4 ng/µl, and 6 ng/µl and amplify these concentrations in adjacent wells. Each reaction will contain 8 µl sample DNA (diluted to 3 ng/µl), 10 µl SYBR Select Master Mix (Life Technologies), telomere primers (250 nM each final concentration) or single copy gene primers (500 nM each final concentration), and distilled water to total 20 µl reaction volume. Real-time PCR will be conducted with an Eppendorf Mastercycler, with the following thermocycling conditions: 50°C for 2 min, 95°C for 5 min, followed by 2 cycles of 94°C for 15 sec and 49°C for 15 sec, and then 35 cycles of 95°C 15 sec, 62°C 10 sec, 74°C 15 sec (telomere) or 95°C 15 sec, 62°C 15 sec, 72°C 45 sec (36B4). Based on the fluorescent signal of SYBR Green and the standard curve, the telomere-to-single copy gene will be calculated, and presented as relative telomere length (T/S).

We will explore the relationship between T/S and age using linear regression, for blood and hair separately. To improve the model, we will explore the use of other covariates, like sex or isotopic signature. If a predictable relationship can be found, we will develop a model that can assign age to non-invasively sampled cougars using telomere length and other available covariates.

PRELIMINARY RESULTS AND DISCUSSION

Objective 1: Compare population-wide differences in diet between two cougar populations

Prey items were grouped into four distinct classes, which differed in isotopic signature as well as ecological role. Native herbivores (elk, mule deer, and rabbits) were the most depleted in isotope signature (Table 1). The other three groups (large domestics, synanthropic wildlife, and small domestics) consist of prey items associated with human development; these groups are enriched in isotopic signature, suggesting an input from corn-derived nutrients and a slightly higher trophic level than native herbivores. Therefore, our analysis of prey isotopic signatures demonstrates that $\delta^{13}C$ or $\delta^{15}N$ can be used as indicators of wildland vs. urban food webs.

We also analyzed the isotopic signature of 38 GPS-collared individuals from the Front Range and 63 individuals from the Uncompahgre Plateau. Cougars in the Front Range had a wider variation in isotopic signature than those in the Uncompahgre Plateau (Table 1). This translated to a much higher variation in diet. Front Range cougars obtained 67-76% (95% CI) of assimilated diet from native herbivores, mostly elk and deer, and the other third from alternative prey (Figure 2A). In the Uncompahgre Plateau population, nearly all of assimilated diet (98-100%) was obtained from native herbivores, with no other diet sources providing significant input (Figure 2B). Future work will use mixing models with Bayesian prior probabilities to reduce the amount of error in these estimates. We will also describe cougar isotope signature in terms of niche space to calculate measures of niche overlap between the two populations.

The Front Range population was much more diverse in diet, utilizing alternative prey items like domestic species and urban-associated wildlife. These items are likely to be more abundant in urban areas (Prange and Gehrt 2004), and therefore cougars may simply be responding to higher availability. However, these alternative prey are also smaller-bodied, and therefore require less handling time. Thus, use of these prey could minimize time spent at kill sites, an important strategy to mitigate risk of foraging in urban areas.

Objective 2:
Because cougars in the Front Range had a more heterogeneous diet than in the Uncompahgre Plateau, we investigated some of the drivers that influence diet within this population. The top model for both $\delta^{13}C$ and $\delta^{15}N$ included housing density and sex; all of the top models (< 2 ΔAIC_C) included housing density and some combination of age and/or sex (Table 2). Therefore habitat use and demographic class were the most important indicators of cougar diet.

Parameters estimated from the top linear model show that for every unit increase in log-housing density, $\delta^{13}C$ increases by 0.20 ‰ (P =0.01) and $\delta^{15}N$ increases by 0.35 ‰ ($P < 0.001$). These differences in isotopic signature translate to non-negligible differences in diet. For every unit increase in log-housing density, proportional contribution of native herbivores decreases by 5.0% (Figure 2; $P < 0.001$; $R^2 = 0.38$).

Demographic class was also an important predictor of diet; this result is primarily driven by the differences between adult females and other demographic classes. In the top linear models, the estimated effect of sex on $\delta^{13}C$ was 0.30 ‰ ($P = 0.17$), meaning at the same level of housing density, males were enriched relative to females by 0.30 ‰, though not significantly so. For $\delta^{15}N$, the effect of sex was 0.62 ‰ ($P = 0.01$), with males similarly enriched relative to females. Using mixing models, adult females were estimated to consume the highest proportions of native herbivore prey (Figure 3A), whereas adult males and subadults showed a lower reliance on native herbivores and were not significantly different in diet (Figure 3B, 3C). There were no significant differences in habitat use by demographic classes (ANOVA; $P = 0.37$) therefore, differences in diet between age-sex classes were independent from habitat use.

Our investigation into covariates of isotope signature demonstrates that habitat use drives variation in diet, with a smaller effect of demographic class. Cougars who forage in urban areas rely much less heavily upon primary prey, which follows, on a smaller scale, the pattern between the Uncompahgre Plateau and Front Range. Adult females in our population showed the highest

reliance on ungulates across all levels of housing density, which differs from earlier studies which indicated that adult males most heavily use ungulate prey (Knopff 2010). Notably, body condition did not appear as an important covariate in any model; therefore smaller-bodied prey are not simply utilized by individuals who are nutritionally stressed, but may be an important nutritional input to cougars in this population.

Objective 3: Develop methods for and evaluate the accuracy of using telomere length to non-invasively age cougars

We have gathered hair and blood samples from live-captured and necropsied individuals to evaluate telomere analysis. In addition to the 38 hair samples from live-captured, Front Range cougars (described above), we obtained 29 hair samples from uncollared, necropsied cougars in the Front Range area. We also sampled blood from 104 cougar captures, which represent 73 unique individuals. For live cougars, we estimated age from tooth wear and gum recession, reproductive status, and known capture history. For necropsied individuals, ages were estimated via tooth wear and gum recession; additionally, for a subset of these individuals, we are obtained age estimates with cementum annuli counts.

DNA extraction from hair follicles and blood is ongoing. We have successfully extracted sufficient quantities of DNA (5-30 ng/ul) from all blood samples. To date, DNA from 15 hair samples has been extracted, though yields of DNA have been much lower (<5 ng/ul) and therefore may be difficult to amplify using Q-PCR. In the upcoming year, we will modify the extraction process to attempt to boost yields of usable DNA from hair samples.

We have successfully amplified telomeric DNA from blood samples though have not yet begun amplifying these samples with Q-PCR. Using previously extracted DNA from cougar blood sampled from Wyoming, however, we have had success in using Q-PCR to amplify telomeres. Amplification efficiencies were consistent for both single copy and telomere genes, and we were able to obtain robust estimates of relative telomere length. For a given age, there was considerable variation in telomere length between individuals; however, preliminary regression analysis indicates a relationship between age and relative telomere length, with telomere length declining with age.

SUMMARY AND FUTURE PLANS

During FY2013-2014 we completed the isotopic analysis of cougar and prey samples, and translated isotopic signature into estimates of diet using uninformed Bayesian mixing models. We began preliminary analyses to describe differences in diet between urban-exurban and wildland cougar populations; thus far, our data indicate that urban-exurban cougars have a much more diverse diet. We also explored numerous covariates to predict isotopic signature and found that housing density and demographic class are important influences of cougar diet in the Front Range population. Future analyses in the upcoming year will compare estimates of diet from stable isotopes to those from kill-site compositions to corroborate the accuracy of this non-invasive technique. We will also attempt to reduce the error in our estimates by using Bayesian prior probabilities derived from kill site compositions.

We also successfully extracted DNA from cougar blood samples for telomere analysis, but have not yet obtained sufficient quantities of DNA from hair samples to quantify telomere length. Future work will utilize Q-PCR to measure relative telomere length in blood and hair to determine the relationship between telomere length and age in different tissue types.

This study will yield novel insights into cougar foraging ecology, primarily how diet is affected by human density and demographic class. Such information is vital to understanding cougar predator-prey relationships and to reducing livestock and pet depredation. Further, this study uses stable isotopes to assess cougar diet; this technique will be useful in non-invasive studies of diet and is an alternative technique to expensive, time-consuming kill site analysis. Finally, we will assess a genetic technique for aging cougars, which, if effective, would enable non-invasive monitoring of cougar population structure.

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Table 1. Stable isotope values for cougars and their potential prey in the Front Range (FR) and Uncompahgre Plateau (UP) study areas, 2007-2013. Isotope values are given in ‰, relative to international standards and are not corrected for trophic discrimination. When prey signatures were not different between study sites, they were grouped. The Front Range population has higher variability in isotopic signature, and therefore diet.

		$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Sample	*n*	Mean ± SD	Mean ± SD
Cougar			
FR	41	-21.3±0.7	8.1±0.8
UP	63	-21.6±0.5	8.5±0.5
Prey			
Small domestics	29	-16.7±2.4	6.2±1.3
Synanthropic wildlife	38	-20.6±1.3	7.4±1.4
Large domestics	26	-22.5±1.4	6.9±1.6
Native herbivores (FR)	48	-24.4±1.0	3.8±1.5
Native herbivores (UP)	15	-24.1±0.4	5.0±1.1

[1]Small domestics: cat (*Felis catus*), dog (*Canis familiaris*), chicken (*Gallus domesticus*)
[2]Synanthropic wildlife: raccoon (*Procyon lotor*), skunk *(Mephitis mephitis*), fox (*Vulpes vulpes*), coyote (*Canis latrans*), squirrel (*Sciurus spp.*)
[3]Large domestics: llama (*Llama glama*), sheep (*Ovis aries*), goat (*Capra aegagrus*), alpaca (*Vicugna pacos*)
[4]Native herbivores: mule deer (*Odocoileus hemionus*), elk (*Cervus elaphus*), rabbit (*Sylvilagus nuttallii*)

Table 2. Results of stepwise model selection to predict isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of mountain lions (*Puma concolor*) in the Front Range study area, 2007-2013. Potential covariates were selected *a priori* and include housing density at foraging sites (HD), sex, age-sex class, age in months, body condition (BCI), and an interaction between age-sex class and housing density. Due to low sample size (n = 36), the corrected AIC_c was used to select the top-ranking models. Housing density and demographic class (either age, sex, or a combination) are the most important covariates.

$\delta^{13}C$ model	*k*	AIC	ΔAIC	L	w
HD + Sex	-39.9	-38.6	-	4	1.000	0.258
HD	-38.8	-38.1	0.51	3	0.775	0.200
HD + Age (months)	-39.1	-37.8	0.73	4	0.694	0.179
HD + Sex + Age (months)	-38.5	-36.5	2.04	5	0.360	0.093
HD + BCI	-37.8	-36.5	2.04	4	0.360	0.093
HD + Sex + BCI	-37.9	-35.9	2.65	5	0.265	0.068
Age (months)	-34.9	-34.2	4.40	3	0.111	0.029
HD + Age-sex class	-36.8	-33.9	4.66	6	0.098	0.025
Sex	-34.6	-33.9	4.67	3	0.097	0.025
HD + Sex + Age-sex class	-36.8	-32.8	5.76	7	0.056	0.014
BCI	-33.3	-32.5	6.03	3	0.049	0.013
Age-sex class	-31.8	-29.8	8.74	5	0.013	0.003
$\delta^{15}N$ model						
HD + Sex	-35.9	-34.6	-	4	1.000	0.454
HD + Age-sex class	-36.7	-33.8	0.79	6	0.673	0.305
HD + Age-sex class + BCI	-34.8	-30.8	3.79	7	0.151	0.068
HD + Age-sex class + Age (months)	-34.7	-30.7	3.90	7	0.143	0.065
HD + Age (months)	-31.3	-30.0	4.54	4	0.103	0.047
HD	-30.5	-29.7	4.84	3	0.089	0.040
HD + BCI	-28.6	-27.3	7.31	4	0.026	0.012
HD + Age-sex class + HD:Age-sex class	-33.4	-26.4	8.12	9	0.017	0.008
Sex	-21.7	-20.9	13.66	3	0.001	0.000
Age (months)	-20.5	-19.71	14.85	3	0.001	0.000
Null model	-19.5	-19.1	15.43	2	0.000	0.000
Age-sex class	-20.1	-18.1	16.50	5	0.000	0.000
BCI	-17.5	-16.8	17.81	3	0.000	0.000

ΔAIC_c = difference in AIC_c from top-ranked model, k = number model parameters, L = relative likelihood, w_i = AIC_c weight

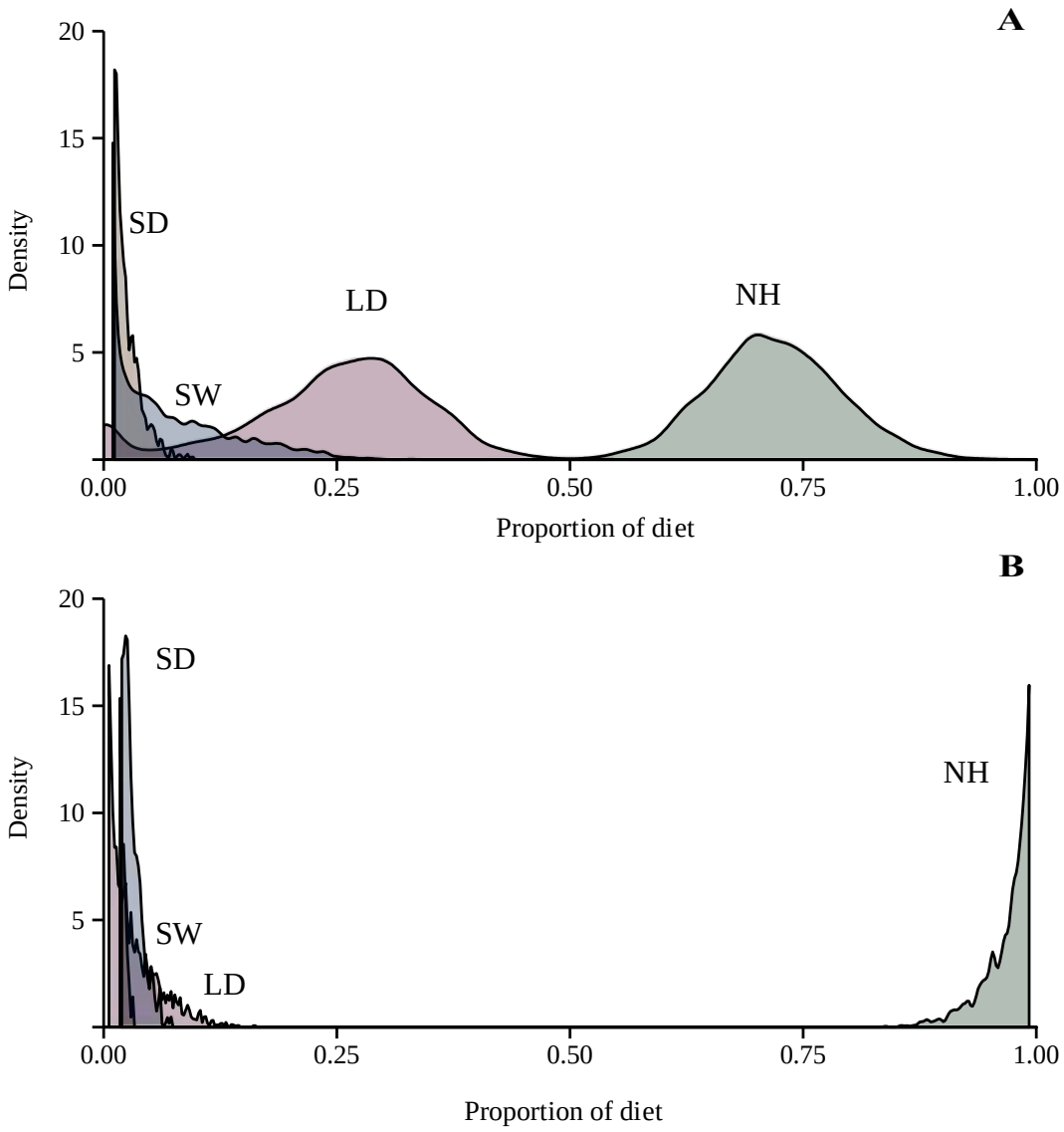

Figure 1. Relative contributions of diet items to the cougar populations in the Front Range (A) and Uncompahgre Plateau (B). Output from isotope mixing models are shown as density plots from simulations, or the relative likelihood of a diet item occurring in a given proportions. Native herbivores (NH) contribute the most to both populations' diet, followed by large domestics (LD), synanthropic wildlife (SW), and small domestics (SD). Cougars in the Uncompahgre Plateau rely much more heavily upon native herbivores, primarily elk and mule deer.

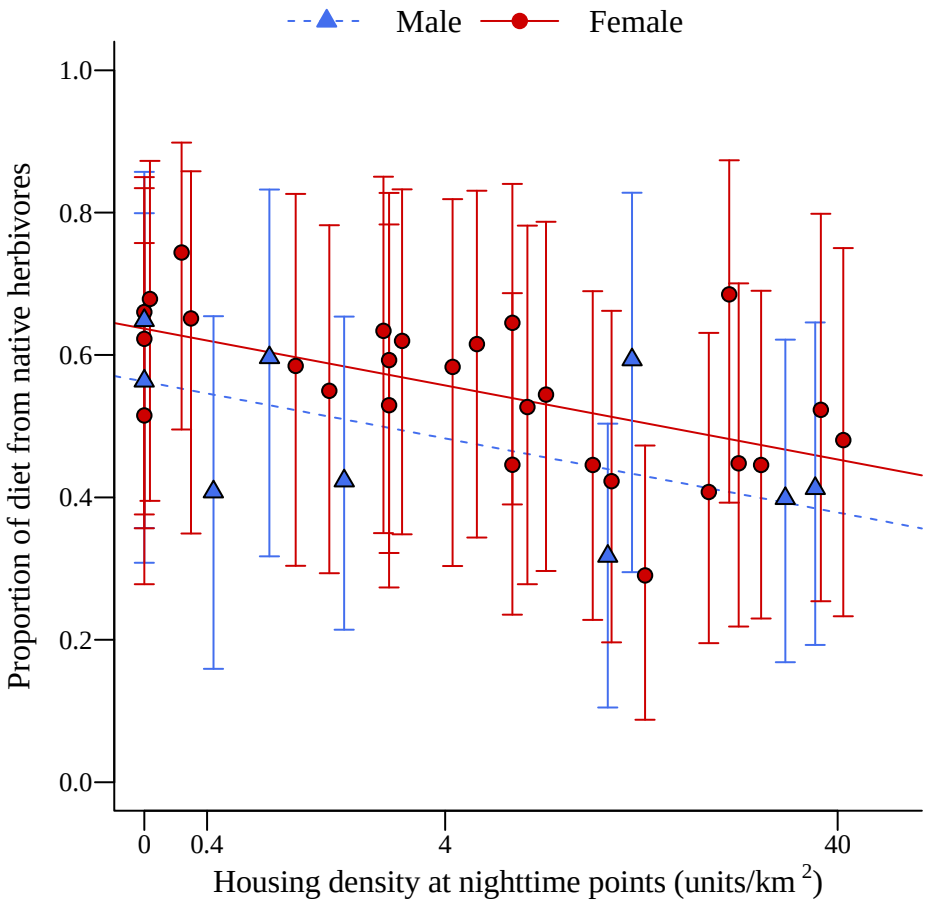

Figure 2. Effect of housing density and sex on proportional contribution of native herbivores to cougar diet. Housing density at foraging locations and sex were the two most important covariates in predicting isotopic signature. The percent of diet from native herbivores was estimated using mixing models and mean and 95% credibility intervals are plotted for each individual. As individuals foraged in more urban areas, where housing density is greater, their use of primary prey decreased. Overall, males utilized less primary prey than females, across all levels of housing density.

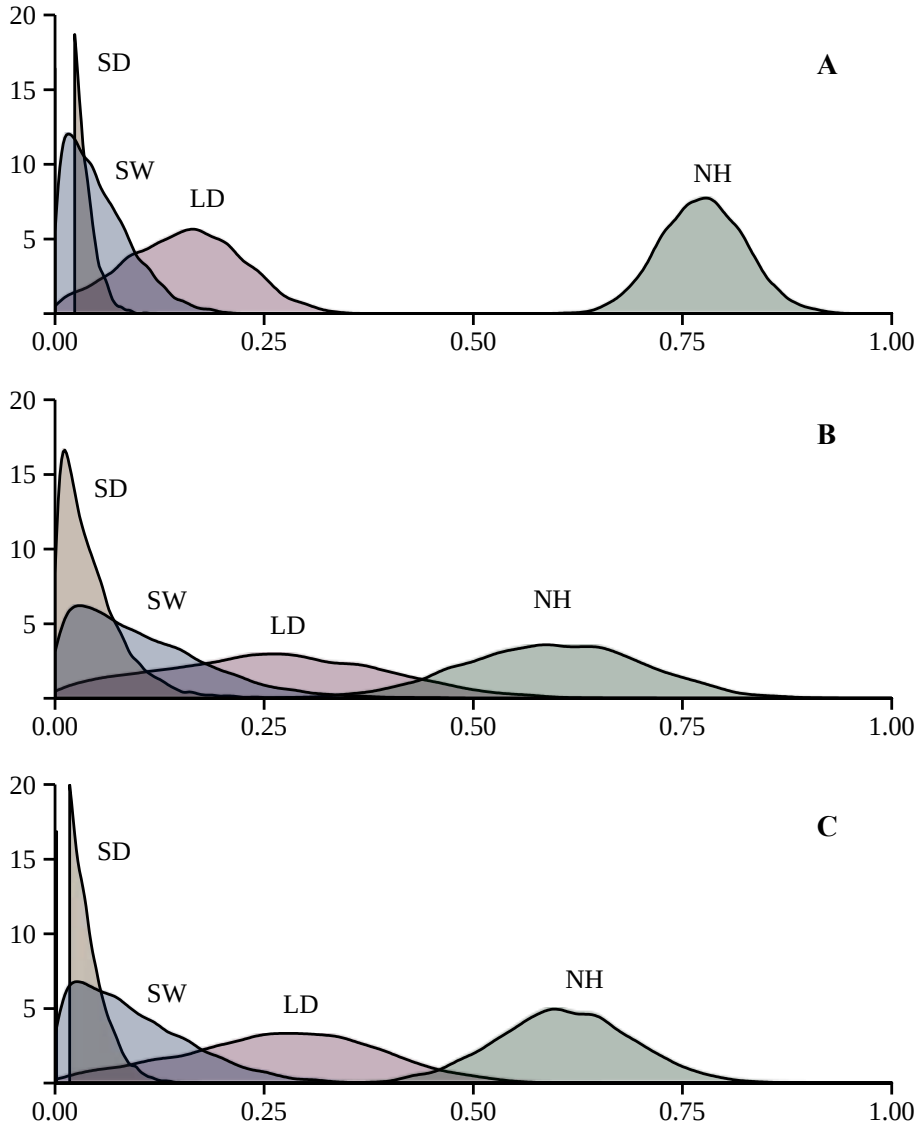

Figure 3. Relative contributions of diet items to different age-sex classes of cougars in the Front Range study area, 2007-2013. Output from isotope mixing models are shown as density plots from simulations, or the relative likelihood of a diet item occurring in a given proportions. Age-sex classes are adult females (A), adult males (B), and subadults (C). Prey groups are native herbivores (NH), large domestics (LD), synanthropic wildlife (SW), and small domestics (SD). Adult females consume the highest proportions of native herbivores, which are principally elk and deer. Adult males and subadults have almost identical diets, and obtain nearly a third of their diet from alternative prey species.

Appendix IV

Front Range Cougar Research

Winters, 2011–2012 & 2012–2013

PUMA FORAGING IN AN URBAN TO RURAL LANDSCAPE

CSU & Colorado Parks and Wildlife

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Research Summary

June 30, 2014

PUMA FORAGING IN AN URBAN TO RURAL LANDSCAPE

RISK-REWARD TRADEOFFS IN THE FORAGING STRATEGY OF COUGAR (*PUMA CONCOLOR*): PREY DISTRIBUTION, ANTHROPOGENIC DEVELOPMENT, AND PATCH-SELECTION

Empirical efforts for understanding whether the space utilization patterns of large elusive carnivores foraging on highly mobile prey are sparse. Having an understanding of the patch

choices made by a large carnivore while engaged in foraging behaviors is of particular importance to understanding their conflicts with humans. The over-arching goal of this thesis is to test whether the foraging strategies carried out by a large carnivore inhabiting an area marked by human housing development can be explained by classic optimal foraging theory (OFT). My research takes place in a portion of the Colorado Front Range, which is a foothill-montane system characterized by the urban-wildland interface of the greater Denver metropolitan area and surrounding cities (Boulder, Golden, Evergreen). A matrix of varying levels of rural, exurban, and suburban development are expected to drive the patch-choices made by cougar, a large obligate carnivore that can conflict with human interests when it conducts foraging behaviors.

Before answering questions involving patch choice foraging behaviors, several pieces of information must be acquired. Specifically, Chapter 1 and Chapter 2 take an Eulerian approach to understanding the space utilization patterns of wild prey commonly sought by cougar in this area. Predicted utilization by these prey species is mapped for the study area on a fine (30 m) scale, with the premise that cougar may be attracted to localities where the opportunity of encountering a potential prey item is greater. Appendix 2 provides details on methods used to determine the distribution of housing development, a patch characteristic that cougars may have fear toward. This appendix also provides some discussion on the anthropogenic development experienced in the study area. Appendix 4 provides details on the construction of various "natural" landscape variables from readily available data sources.

Chapter 1 shows that simple encounter measures collected from camera traps can provide a measure of landscape utilization for an animal population at extremely fine scale patch size. I demonstrate that the amount of utilization at a patch, whether by one or many animals, is a function of the abundance of animals within some area around the camera and the micro-habitat utilization patterns of the individuals in that population. However, I show that biases will exist in many situations if certain protocols are not adhered to.

Chapter 2 applies the principle from Chapter 1 to produce a landscape utilization map of common cougar prey species at a fine scale. This was done using a count measure of the amount of time spent by animals within the field-of-view of a sample of 131 camera trap sites monitored over a one year period. While doing so, I accounted for the probability of detection within the camera's field-of-view in the count response. Probability of detection was found to be influenced by several environmental and animal specific variables. A secondary focus was to understand the associations between animal utilization and housing development. The associations found were generally supportive of those found in previous studies using habitat selection, occupancy, and abundance as response variables.

Finally in Chapter 3, using cougar as a model species, I test whether a large carnivore's foraging strategy can be explained by optimal foraging theory, which says that an animal makes decisions while foraging that balances the process of acquiring energy with the process of avoiding risks. In seminal optimal foraging works, authors proposed that an animal will be less cautious in avoiding risks when energetically stressed. I demonstrate that cougars make a tradeoff between choosing locations that would yield a higher encounter rate of prey with choosing safer patches. Cougars were found to show avoidance of higher housing densities, but also shown to be attracted to higher primary prey (mule deer) availability. Support for this tradeoff was shown by demonstrating that hunting success increased as cougars hunted in higher housing densities. Furthermore, the strength of the housing avoidance behavior declined as cougar hunger levels increased. A similar behavior was observed during temporal periods

associated with assumingly decreased availability of primary prey; cougars became less cautious when imposed with energetic constraints.

Appendix IV

Front Range Cougar Research

Winters, 2011–2012 & 2012–2013

Predator-Prey Dynamics in Relation to Chronic Wasting Disease and Scavenging Interactions at Cougar Kill Sites

Colorado Parks and Wildlife

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June 30, 2014

Predator-Prey Dynamics in Relation to Chronic Wasting Disease and Scavenging Interactions at Cougar Kill Sites

2014 Progress Report Submitted by:
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Need:

The current Colorado Parks and Wildlife (CPW) cougar (*Puma concolor*) research on the Front-range is utilizing GPS radio collar technology allowing researchers to track cougar movements on a real time basis. With up to seven uploads a day, the roughly 20 current active project collars give researchers the ability to identify possible kill sites quickly, sometimes as soon as 6 to 12 hours after a kill is made. This provides the opportunity to explore previously un-researched facets of cougar behavior during the relatively short time interval from the point a cougar makes a kill, to the point at which it abandons the carcass. Feeding behavior, intraspecific kill site interaction, and scavenger competition can now be investigated.

Similar data to that collected in Krumm et al.'s (2005) and Miller et al.'s (2008) cougar studies, which examined cougar selection of Chronic Wasting Disease (CWD) positive mule deer (Odocoileus hemionus), can now be collected with a greater degree of efficiency. The study areas of each of the two prior CWD cougar projects lie within the more broad boundaries of the current Front-range cougar project, and a larger number of known cougars will increase sample sizes of CWD tissues from cougar killed mule deer. Additionally, much of the field work from the two previous studies is nearly a decade old which justifies another project to compare to past results. The ability to collect a potentially larger sample size will yield more accurate findings, identify gaps in need of further study, and/or detect developing trends in regards to possible temporal patterns.

The ongoing cougar project's available technology and resources, and the relatively minor additional project costs, provide the opportunity to initiate a camera study to explore cougar feeding behavior and scavenger interaction in the period immediately following a cougar kill. Site visitation of fresh cougar kills also allows for the collection of adequate tissue samples to test for CWD, in order to further explore if cougars are selecting for CWD positive mule deer or other ungulates.

Background:

Cougar behavior and scavenger interaction:

Although there have been significant cougar research projects in the U.S. and Canada, only recent GIS advancements have allowed researchers the ability to monitor cougar movements and locations with dependable accuracy on a real-time basis. With GPS collar technology, researchers can collect data on kill sites, prey items, home ranges, den locations, preferred habitats, and a variety of other previously under-explored areas of cougar ecology and behavior.

This new technology initiated many projects that examined cougar feeding behavior. These projects collected extraordinary data documenting duration of kill site occupation, prey analysis, biomass consumption, and feeding patterns (Anderson and Lindzey 2003, Bauer et al 2005, Knopff et al 2010, Blecha and Alldredge unpublished data). However, actual behavior, feeding activity, consumption rates, and scavenger interactions has yet to be thoroughly documented. Placing cameras on fresh kill sites will identify any patterns of behavior that exist during the progression of feeding on a prey item and document interaction with competing scavengers and conspecifics. A goal of this proposed project is to document how often scavengers challenge cougars on fresh kills and how successful these competing scavenging species are at stealing the food item. Using the time stamped photos from cameras, we will be able to determine the average time it takes for competing scavengers to arrive on site after a kill and the rate in which the scavenger species successfully displaces the cougar. Seasonal variation in scavenging rates of fresh carcasses will be analyzed, especially with regard to bear activity and changes in diet competition.

Basic cougar ecology suggests that with the exception of family groups and mating interaction, cougars are largely solitary animals (Seidensticker et al. 1973). On numerous occasions throughout the course of the ongoing lion project, researchers have documented two cougars on the same kill site. One can only speculate on their interaction. This proposed project also seeks to document behavior in such situations to observe if cougars are sharing kills or challenging one another for feeding opportunities.

CWD component:

Ongoing cougar research on the northern Front-range (Alldredge, unpublished data) as well as other significant cougar research (Logan and Sweanor 2001, Anderson and Lindzey 2003, Hornocker 1970) has shown that cougars, while predating on a wide diversity of prey species, select for deer and elk in higher proportions. Additionally, the northern Front-range has been identified as the epicenter of the Chronic Wasting Disease (CWD) epidemic, possessing the highest infection rates in the state (Miller et al. 2000). CWD is a naturally occurring prion disease effecting deer, elk and moose. Early stages of infection are difficult to recognize but advanced signs of CWD infected deer are more readily identified by humans, with symptoms including poor body condition, reduced coordination, excessive salivation, and increased isolation from other deer (Williams and Young 1980). Basic predation theories suggest that predators prey upon young, sick, and older individuals in greater proportion than fit, mature individuals. Optimal foraging theory predicts that predators ought to choose the most "profitable" prey (MacArthur and Pianka 1966, Schoener 1971, Pulliam 1974), which should be the largest prey available that can safely be killed. Thus, we might assume cougars can identify a deer in the later stages of CWD infection. Miller et al. (2008) speculated that cougars could have the ability to identify the most subtle changes in behavior or body condition in early stage CWD positive deer, causing them to be more vulnerable to predation.

While it is known that cougars prey on deer or other ungulates as a primary food source, only two studies have explored whether cougars are selecting for CWD positive deer (Krumm et al. 2005, Miller et al. 2008.) Krumm et al. (2005) found the percentage of CWD infected mule deer killed by cougars was significantly higher than hunter harvested deer in the same area. Miller et al. (2008) found infected deer were much more likely to be killed by cougars than

uninfected ones. There is little information on cougar selection of CWD infected elk but this proposed study will document any CWD occurrence in cougar killed elk.

It is the responsibility of CPW to utilize the best science when managing Colorado's wildlife resources. Exploring cougar kill site behavior will determine loss rates from scavenging/competition of fresh carcasses. This could provide insight on actual prey consumption and clarify an important variable in estimating the frequency of cougar deer and elk kills. Documenting feeding behavior has not previously been done in this proposed fashion and will provide invaluable information on basic cougar ecology and behavior. Collecting samples for CWD testing will provide a welcome opportunity to compare new data to the two previous studies and to existing (and evolving) CPW CWD data. Furthering our understanding of the relationships between predator/prey and disease dynamics will afford biologists better information in managing Front-range wildlife populations.

Objectives:

1. Document sharing and/or abandonment rates of cougars occupying kill sites in response to presence of other cougars and/or scavengers
2. Document time from kill until presence of competing scavengers
3. Document feeding patterns and length of individual feeding sessions.
4. Compare CWD infection rates from cougar killed deer and elk to existing CPW CWD infection rates to determine if cougars are selecting for CWD positive deer and elk.

Methods:

Researchers will monitor cougar movements using GPS data on a GIS to detect possible kill sites as early as possible. After a location is deemed permissible and realistic to access, researchers will travel to the kill site area and navigate to the potential kill site location. Personnel will use a VHF signal to monitor cougar location during the approach to avoid contact. While some disturbance to cougars may be unavoidable if the animal is alerted upon researcher approach, precautions will be taken to avoid frequently forcing cougars off a kill. Past experiences, especially those associated with capture activities, on the Front-range cougar project have shown that a cougar is not likely to be affected if briefly disturbed at their kill. Ideally, the potential kill site will be approached between feeding sessions when the cougar is day bedded offsite. Initial kill site investigations are currently being conducted in the parent cougar project to establish the probability a kill site is detected by technicians at a later date. There have been no instances of abandonment. Additionally, many bait sites occupied by cougars are visited daily by technicians to switch memory cards in cameras, adjust location of placed bait carcasses, and/or refresh bait as needed to keep a cougar in the immediate area. Often times this is done for a series of days until researchers can attempt to conduct a capture. Even with these daily visits, patterns of bait site abandonment have not been observed. However, if these kill site visits and camera placements prove to disturb the cougar, and a pattern of kill site abandonment is observed, site visits and camera placement will cease

In the event a kill is found, a maximum of two cameras will be placed to document feeding activity and scavenger interaction. Multiple cameras will be used in the event the cached prey item is slightly moved and to monitor activity within a larger area. Cameras will be affixed

to adequate stationary objects and camouflaged with vegetation to minimize sight manipulation and detection. The reconyx cameras currently used in the parent cougar project are 4x6 inches and emit a low glow instead of a flash during nighttime photographs. Cameras will be left in place up to two weeks after the cougar has left the kill site.

If the prey item is a mule deer or other ungulate, retropharyngeal lymph nodes and/or the medulla oblongata at the obex will be collected for CWD testing. Additionally a lower incisor will be obtained for accurate age analysis. Krumm et al. (2005) collected 54 testable samples from cougar killed mule deer in 42 months. Miller et al. (2008) observed 11 CWD positive collared deer succumbed to cougar predation at a rate nearly four times that of uninfected collared deer. With the large number of collared cougars in the current Front-range cougar project (n≈25), we predict the ability to collect a target sample size of 4-5 tissue samples per month. A large sample is necessary to determine if cougars are selecting for CWD positive deer, as the power to detect a 10% difference using binomial proportions is only 0.75 (n=200).

2013-2014 Progress:

This past spring we completed data collection looking at scavenging and other interactions at cougar kill sites and in determining CWD infection rates of cougar killed ungulates. We continue to analyze the GIS data, photo database and CWD results. Overall, our methods worked well and we were fortunate to have success in our data collection over the past 2 ½ years.

Scavenging and Kill Site Interactions

Placing cameras at kill sites was completed in January 2014 wrapping up 25 months of data collection. Over the course of the study we placed cameras on 225 kill sites recording over 400,000 photos. Pictures have been identified once and are currently in the process of a second round of identification.

Timely approaches to kill sites continued to be successful in 2013 and early 2014, usually occurring within 24 hours of a cougars first GPS location at a kill site. This allowed technicians to evaluate the prey item to ensure the estimated time of death matched the carcass condition in order to rule out other possible causes of death (road kill, hunting loss, etc). Cougars were often onsite at the kill site upon approach but usually retreated as the researcher neared the site. There were several situations where a cougar had been unwilling to move from a kill. In these situations technicians left the area and if time allowed, returned at a later time.

There were no more situations of carcass abandonment in 2013 by cougars after a carcass had been visited and cameras placed and only six total instances throughout the study. Four of these abandonments were due to the cougar occupying a second kill site and never returning to the first, and not likely a result of human visitation and camera placement on the first carcass. Cameras continued to document bear visitation in both scavenging and direct competition situations and photo sequences continue to be analyzed to determine frequency of these scenarios.

Red fox (Vulpes *vulpes)* continued to be observed scavenging at cougar kill sites in high frequency. Other scavengers documented include striped skunk (*Mephitis mephitis)*, spotted skunk (*Spilogale gracilis)*, raccoon (*Procyon lotor*), ringtail cat (*Bassariscus astutus)*, grey fox

(*Urocyon cinereoargenteus)*, coyote (*Canis latrans*), domestic dog (*Canis lupus familiaris)*, bobcat (*Lynx rufus),* golden eagle (*Aquila chrysaetos)*, red-tailed hawk (*Buteo jamaicensis*), great-horned owl (*Bubo virginianus)* and a variety of *Corvidae* bird species.

Over the course of the study there have been at least 12 camera sites where we have identified multiple cougars simultaneously occupying a kill site. These observations include two 'sharing' situations involving two cougar family groups and multiple sharing situations involving an adult male and female. Other interactions include two instances of female cougars stealing food items from other female, three known unrelated adult females, and one instance of an adult male feeding on a prey item occupied by a female and three young kittens. There have also been several instances where non-focal cougars scavenge on the remains of prey items already consumed and abandoned by the focal cougar.

CWD component:

Collecting CWD samples from cougar killed ungulates was completed in April 2014 wrapping up 30 months of data collection. In 2013 and 2014, there continued to be no problems with obtaining tissue samples to test for CWD from cougar killed ungulates except in rare situations where the testable tissues have been consumed by the cougar. Samples collected in the field were issued a head tag and transferred to the CPW Wildlife Health Lab in Fort Collins for testing. Two samples in early 2014 were determined untestable after a refrigerated cooler failed at the Fort Collins office and the samples decayed before they could be processed. Throughout the course of the study, we collected 192 samples from cougar killed ungulates of which 190 were testable. Of these, 163 were adult mule deer (65M, 98F), 11 were adult elk and rest comprised fawn mule deer (n=14), elk calves (n=1), and adult white tailed deer (n=1).

Table 1 shows the breakdown of species, age and test results within each deer DAU from adult mule deer sampled within the broad boundary of the front-range cougar project. Tables 2 and 3 show mule deer sampling by sex and figure 1 shows the sampling breakdown by month throughout the entire study.

DAU	GMU	Total Sampled	Total Positive	% Positive
D-10	20	28	4	14.29%
D-27	29	78	17	21.79%
D-27	38	45	13	28.89%
D-17	39	2	0	0.00%
D-17	391	10	3	30.00%
	Total	163	37	22.70%

Table 1. Total CWD results

DAU	GMU	Males Sampled	Males Positive	% Positive
D-10	20	8	1	12.50%
D-27	29	32	10	31.25%
D-27	38	18	8	44.44%
D-17	39	2	0	0.00%
D-17	391	5	1	20.00%
	Total	65	20	30.77%

Table 2. Male mule deer CWD results

DAU	GMU	Females Sampled	Females Positive	% Positive
D-10	20	20	3	15.00%
D-27	29	46	7	15.22%
D-27	38	27	5	18.52%
D-17	39	0	0	0.00%
D-17	391	5	2	40.00%
	Total	98	17	17.35%

Table 3. Female mule deer CWD results

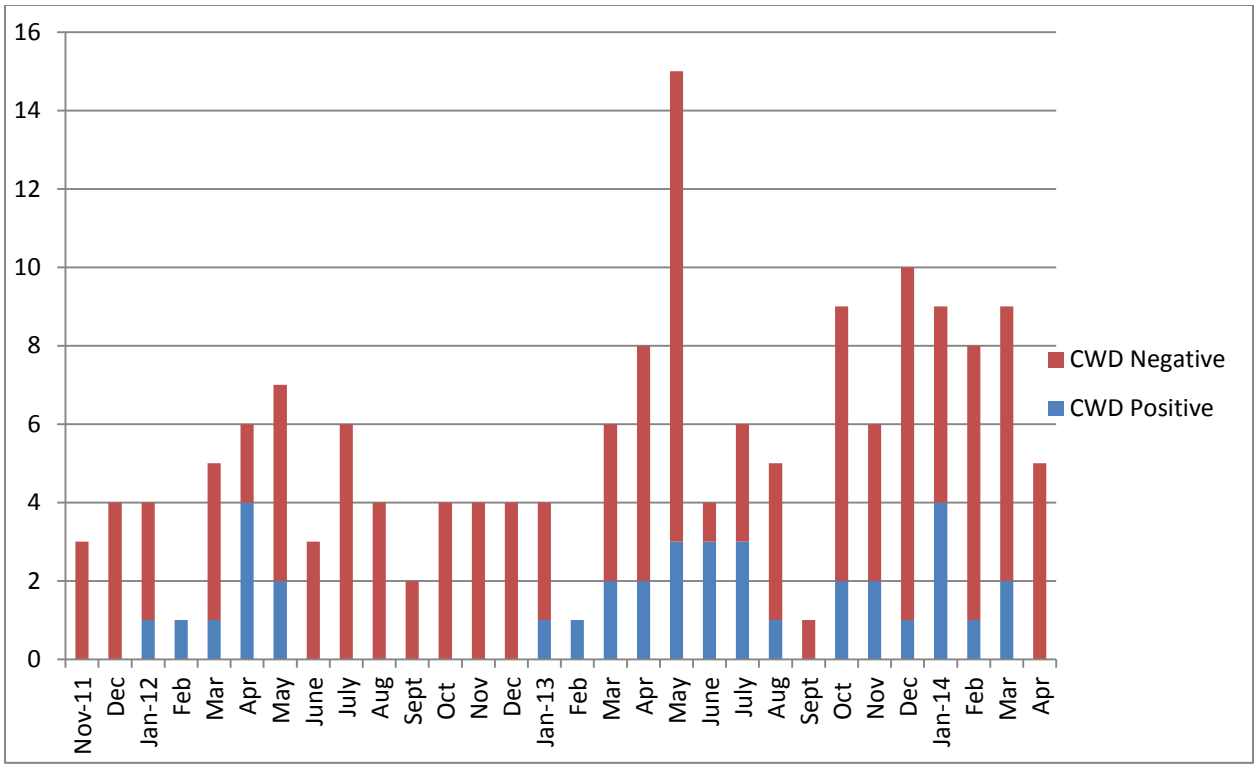

Figure 1. Mule deer CWD results by month.

Appendix VI

Front-Range Cougar Research

Winters, 2011–2012 & 2012–2013

The Use of Lures, Hair Snares, and Snow Tracking as Non-Invasive Sampling Techniques to Detect and Identify Cougars

CSU - Colorado Cooperative Fish and Wildlife Research Unit & Colorado Parks and Wildlife

Kirstie Yeager

Bill Kendall

Mat Alldredge

Research Proposal

June 30, 2014

Development of a Noninvasive Method to 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. I established 16 – 20 sites over four ≈ 30-day sampling periods. In general, my results suggest calls are more effective attractants than scents. At sites with auditory calls, photographs documented 40 visits by ≥ 13 individual cougars, and I obtained 14 hair samples. Only 2 hair samples were collected using scented scratch pads and no samples were acquired via a novel hair snare. I conclude that given the ability to successfully genotype the hair samples collected, auditory calls and hair snares may be an effective way to assess the various population demographics that are needed to inform management decisions.

Assessing the Probability of Identifying Cougars by Noninvasive-Genetic Sampling with Auditory Predator Calls and Hair Snares

Detecting all individuals in a population equally and with certainty will yield unbiased population estimates; however, many current sampling techniques for cougars have inherent variation, such as a trap response or individual heterogeneity in detection probability. From November, 2012 – April, 2013, I applied a noninvasive method to sample cougars and assessed variation in detection in 2 study areas in Colorado, one on the Front Range (FR; 1,270 km^2) and one on the Uncompahgre Plateau (UP; 540 km^2). In total, I established 148 lure sites with auditory predator calls and hair snares over 3 (UP) and 4 (FR) sampling periods. 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, 2 sibling groups, and ≥ 16 unique unmarked animals. On the UP, I documented 18 detections by 7 independent marked cougars and no unmarked animals. 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 estimated detection probabilities. The probability of detecting an independent marked cougar at least once during the study adjusted for partial availability was 0.83 ± 0.10 (FR) and 1.00 (UP). I collected 59 hair samples. Thirty-two were genotyped at ≥ 8 loci identifying 26 unique cougars. I concluded that a noninvasive-sampling technique using auditory calls and hair snares can be a useful tool in assessing population demographics of cougar populations.

Appendix VI

Front-Range Cougar Research
Winters, 2014–2015

NONIVASIVE GENETIC SAMPLING TO ESTIMATE COUGAR AND BOBCAT ABUNDANCE, AGE STRUCTURE, AND DIET COMPOSITION

Colorado Parks and Wildlife

Mat Alldredge

June 30, 2015

PROGRAM NARRATIVE STUDY PLAN
FOR MAMMALS RESEARCH
FY 2013-14 – FY 20016-17

State of:	Colorado	:	Colorado Parks and Wildlife
Cost Center:	3430	:	Mammals Research
Work Package:	3003	:	Predatory Mammals Conservation
Task No.:		:	Noninvasive Genetic Sampling to
		:	Estimate Cougar and Bobcat Abundance,
		:	Age Structure, and Diet Composition
Federal Aid Project No.			

NONINVASIVE GENETIC SAMPLING TO ESTIMATE COUGAR AND BOBCAT ABUNDANCE, AGE STRUCTURE, AND DIET COMPOSITION

Principal Investigators
Mathew W. Alldredge, Wildlife Researcher, Mammals Research

Cooperators
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NE Area District Wildlife Managers
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STUDY PLAN APPROVAL

Prepared by:	Mathew W. Alldredge	Date:	Dec. 2013
Submitted by;	Mathew W. Alldredge	Date:	Dec. 2013
Reviewed by:		Date:	
		Date:	
		Date:	
Biometrician Review		Date:	

Approved by: Charles R. Anderson, Jr. Date: Nov. 29, 2013
Mammals Research Leader

PROGRAM NARRATIVE STUDY PLAN
FY 2013-14 – 2016-17

NONIVASIVE GENETIC SAMPLING TO ESTIMATE COUGAR AND BOBCAT ABUNDANCE, AGE STRUCTURE, AND DIET COMPOSITION

A study proposal submitted by:

Mathew W. Alldredge, Wildlife Researcher, Mammals Research

Program Overview:

Cougar and bobcat populations are actively hunted throughout the state of Colorado and management is applied using the best available information. Unfortunately, reliable information on cougar and bobcat populations is nascent. The best information available comes from long-term studies on relatively small populations where animals have been repeatedly captured. However, to better manage these populations, broad-scale information for these species is necessary.

We have begun developing noninvasive genetic sampling (NGS) techniques to provide better, less expensive data for cougars and bobcats that can be implemented at broad geographic scales with state-wide application. The methods being developed should provide information on population size, sex structure, age structure, and diet composition. This information is valuable to the future management of these species and for the justification of harvest levels imposed on them.

Over the next few years we intend to further refine these NGS techniques for cougars and bobcats so that they can be reliably implemented to inform management decisions. We also intend to perform at least one full survey over multiple years so that we can assess the reliability and repeatability of this approach. Following these efforts our hope is that we will have a fully developed NGS approach for cougars and bobcats that can be implemented at a state-wide level for future monitoring of these species.

Need:

In order to set harvest quotas, evaluate management practices and understand the dynamics of predator-prey systems, it is desirable to have reliable estimates of population size. Unfortunately, with many predators, it can be very difficult and expensive to obtain these estimates. This is especially true with cougars because of their low densities, secretive nature, and unpredictable response to lures. Most reliable estimates of population size for cougars have come from intensive capture and monitoring studies, which were expensive and time consuming (Logan 1983, Lindzey et al. 1994, Murphy 1998, Logan and Sweanor 2001).

One approach that is used to estimate cougar population size is the two-sample Lincoln Petersen estimator in conjunction with an ongoing marking study (Anderson and Lindzey 2005). However, this method does require a marked population and is subject to all of the Lincoln-Petersen model assumptions, which include constant probability of capture among all individuals and time periods and population closure (Williams et al. 2002).

Because of the difficulty and expense associated with typical mark-recapture techniques for estimating carnivore abundance, alternate techniques have been developed. Many of these techniques involve noninvasive genetic sampling, which is a type of mark-recapture sampling. Noninvasive genetic sampling (NGS) (Hoss et al. 1992, Taberlet and Bouvet 1992) has the potential to provide a realistic method for sampling a population of interest. Noninvasive sampling techniques include the use of hair

snares and scat collections (Ernest et al. 2000, Harrison et al. 2004, Smith et al. 2005). The use of scats for sampling cougar populations may be particularly useful and provide a representative sample of the population. Scat collections can either be done by searching transects with human observers (Harrison et al. 2004) or with trained dogs (Smith et al. 2005). Scats could also be collected from kill sites.

Although the use of scats for noninvasive genetic sampling may sound appealing, based on personal experience, the actual encounter rate of scats may be prohibitively low to make this a viable option. The alternative approach would be to collect hair or tissue from cougars that are lured into a site. Although the use of hair snags and lures have proved effective on many species, such as bears, the technique has not been rigorously evaluated for cougars. Typical lures have been found relatively ineffective at luring cougars to a specific site, even when cougars are known to be in close proximity (Long et al. 2003, Choate et al. 2006). The types of lures that have been tried are various scents, food sources, and animal calls. Having a significant number of cougars GPS collared in an area provides a unique opportunity to evaluate the effectiveness of a variety of lures, because we will be able to map the location of known individuals in relation to various lures and assess detection rates based on documented availability.

Track counts have also been used to assess cougar population trends (Smallwood and Fitzhugh 1991, 1995, Smallwood 1994, Cunningham et al. 1995), but actual relationships to population size are generally weak (Van Dyke et al. 1986, Van Sickle and Lindzey 1992). For example, Cunningham et al. (1995) failed to detect an estimated 33% decline in cougar abundance using track surveys. Based on computer simulations, sampling effort required to detect a change in cougar populations is very high (Beier and Cunningham 1996). Difficulty detecting tracks in dense vegetation or rocky slopes in conjunction with access limitations to some areas may limit the utility of this approach (Anderson 2003).

Researchers have tested several noninvasive techniques, some quite creative, on a variety of carnivores to detect and count individuals. Track surveys have been used with success in occupancy studies but fall short in their ability to produce accurate and precise abundance estimates (Diefenbach et al. 1994, Sargeant et al. 1998, Wilson and Delahay, 2001, Hayward et al. 2002, Choate et al. 2006, Gompper et al. 2006). However, when track surveys are combined with the collection of genetic material, species identification can be confirmed (McKelvey et al. 2006) and/or individuals identified, allowing for abundance estimates using mark-recapture analysis (Ulizio et al. 2006). Cameras, lures, and/or hair snares have also been used to survey cougars (Long et al. 2003, Choate et al. 2006, Sawaya et al. 2011), lynx (McDaniel et al. 2000, Schmidt and Kowalczyk 2006), bobcats (Harrison 2006), ocelots (Weaver et al. 2005), multiple felids (Harrison 1997, Downey et al. 2007), and carnivore communities (Sargeant et al. 1998, Long et al. 2007, Ruell and Crooks 2007, Castro-Arellano et al. 2008, Crooks et al. 2008). Though dozens of lures have been tested along with several novel hair-snaring devices, results have been variable, suggesting no single method superior above all others.

With regard to cougars, the potential of NGS has not been realized. Inconsistent results have left the techniques needing further testing and refinement. In past studies involving attractants, almost all have primarily used scents. Few surveys have incorporated auditory calls despite the fact that felids may exhibit a greater response to auditory cues than to olfactory stimulus (Chamberlain et al. 1999). Further testing of this component is needed to assess whether calls will attract cougars to sites. Furthermore, McDaniel et al. (2000) described a hair-snaring device that consisted of a board with a scent-lure-covered carpet pad and nails protruding through it nailed to a tree. Harrison (2006), McKelvey et al. (2006), Schmidt and Kowalczyk (2006), Long et al. (2007), and Sawaya et al. (2011) tested similar mechanisms on a variety of felids. These designs snagged hair part of the time though the quality of the hair and whether or not the hair was from the target species was inconsistent. Modifications in snare designs are needed to improve the reliability of the hair snagged, thus increasing the likelihood of obtaining a usable sample.

Barbed wire is an alternative hair-snaring mechanism to traditional scratch-pad designs. Barbed wire has long been used to collect hair samples 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). Ebert and Schulz (2009) used barbed wire to snag hair from wild boar; and Belant et al. (2007) obtained hair from white-tailed deer. We could not find a study that used barbed wire in an attempt to snag hair from a felid species.

Recent efforts by Yeager et al. to develop NGS as part of the Front-Range Cougar study have shown a great deal of promise (unpublished data). The use of an auditory call placed in a cubby with a barbed wire snag effectively sampled the majority of collared cougars on the Front-Range study and all of the collared cougars on the Uncompahgre Plateau cougar study. A significant proportion of the cougars sampled also provided hair that has yielded good quality DNA for genotyping.

Although this study has demonstrated positive results, there remain details on sampling design and field logistics that still need to be examined. Cubby sets can be extremely time consuming to build and cougars do not always enter the sets. Therefore it is important to investigate alternative designs to snag hair that still utilize the auditory call. It is also necessary to further investigate the detection process in order to develop the optimum site density and placement for snags. Finally, a full survey needs to be implemented and replicated to evaluate the technique, logistical constraints and long-term cost.

In addition to detecting cougars, the NGS design that we developed also provided a significant number of bobcat detections. With the increasing pelt prices for bobcats it is likely that harvest pressure will continue for bobcats. In order to better manage bobcats and justify harvest levels an estimate of bobcat density would also be useful. Preliminary data suggest that it could be possible to sample bobcats as well as cougars in the same NGS survey.

We have also been developing techniques to obtain population age structure and diet composition from hair samples. The use of telomeres to estimate population age structure for cougars has shown promising results. As this continues, it may be possible to obtain age structure information from NGS procedures as well as density. We have also successfully determined diet composition of cougars using stable isotope information from hair samples. Combining these data we hope to demonstrate the ability to obtain density, sex structure, age structure, and diet composition from a single NGS survey.

Objectives:

1. Continue to evaluate the use of auditory calls for NGS sampling of cougars.
2. Implement a NGS survey for cougars over multiple years to evaluate the consistency of the approach.
3. Use collared cougars to evaluate trap response of cougars and assess potential biases in the NGS approach.
4. Evaluate the potential to sample bobcats using the same NGS approach.
5. Test alternative hair snaring devices for felids.
6. Assess a simultaneous sampling approach for bobcats and cougars relative to differences in home-range size.
7. Implement an NGS survey over multiple years for bobcats and cougars to determine the logistics, cost and feasibility of sampling to obtain estimates of density, sex structure, age structure and diet composition.

Expected Benefits:

The ability to estimate population size, sex structure, age structure and diet composition or track population changes is critical to the management of a species, especially when harvest quotas are being set for that species. This study is designed to develop tools that can be implemented in areas where

bobcats and cougars are not actively being studied and marked that will allow biologists/managers to gain a better idea of population size and population response to management prescriptions. Such estimates, in conjunction with harvest data will allow managers to better understand bobcat and cougar populations they manage, set appropriate harvest quotas and defend our management actions to the public.

Approach:

Our primary objective is to continue to fully develop the NGS technique for cougars; therefore the base sampling approach will be designed for cougars. The secondary objective is to develop and evaluate this approach for bobcats, so a secondary grid will be overlaid on the primary grid that is more appropriately sized to their home-range. The intent is to effectively sample the area of interest for cougars, with a clustered sampling approach for bobcats. This should also provide a more efficient method for sampling the large number of sites that will be required to survey both species.

A typical grid cell size used for population surveys is one that is equal to a quarter of the average home-range size for the species of interest (Otis et al. 1978, White et al. 1982, Williams et al. 2002). The average home-range size for female cougars on the Front-Range is about 100 km^2 (Alldredge, unpublished data), so we will use a 5 km by 5 km grid cell size as our primary grid. A secondary 1 km by 1 km grid will then be overlaid on the primary grid. A cell from the secondary grid will then be randomly selected within each primary grid cell, omitting all cells on the edge. The four adjacent diagonal cells will also be selected, for a total of 165 sites (Figure 1). Within each selected cell, specific sites will be selected based on likely areas to attract a cougar, property access, and field logistics.

There will be 3 main sampling periods during the study, each 4 weeks in duration. Within each primary cell we will have 2 primary sites and 3 secondary sites during each sampling period. Primary sites will have a call, a camera, a scent, a visual lure and 1 to 2 hair snaring devices. Secondary sites will not have calls or cameras due to logistical and budgetary constraints. Primary sites will be randomly selected without replacement from the 5 available sites in each grid for each sampling period, such that all 5 sites will be primary sites once during the season and one will be a primary site twice. All sites will be checked at approximately weekly intervals for signs of visitation and hair, and batteries will be checked in cameras and calls.

All primary sites will be similar in design, containing the same elements. The primary attractant will be a predator call (Wasatch Wildlife Products® Custom FurFindR®) programmed to play a 5 second distressed fawn sound on 30 second intervals. These calls are also equipped with light sensors rendering them inactive during daylight hours. We will cable the calls ~ 1 m up from the base of a tree. To incorporate a natural prey scent and visual attractant, we will hang deer hide inside each tree. We will then build a perimeter around the tree with thick brush leaving an obvious entry way to the call and bait. We will configure lines of barbed wire (vertical or horizontal) within the entrance. Terrain and vegetation features will determine the height of the wire and consequently whether we desire a cougar to step over, under, or through 2 strands. In addition, we will attempt to conceal the wire with sticks and other natural materials. A sticky roller will also be used as a secondary hair snag at each site. A rub pad will also be placed within each site, specifically to target bobcats. Additional hair snag devices may be tested where a target animal has to reach for bait over a hair snag. At each site, we will position an infrared motion-sensor camera (Reconyx® PC85 Rapidfire® or PC800 Hyperfire®) set to rapidly take 5 photos when triggered. Secondary sites will be similar but without the call or camera.

To minimize the possibility of sample contamination (multiple animals leaving hair) and degradation, we will check the sites for activity every week. We will consider hair on a single barb as one sample and denote quantity with a score of 1 – 3 (1 equals < 5 hairs, 2 equals 6 – 15 hairs, and 3 equals > 15 hairs). We will remove hair using sterile tweezers and re-sterilize the barb by passing a flame under it (Kendall et al. 2008, Settlage et al. 2008). We will place the hair in a small paper envelope. Paper

envelopes will then be put in a plastic bag with a desiccant and stored at room temperature (Taberlet and Luikart 1999). If hair is on the sticky rollers the entire roller will be collected, wrapped in wax paper and placed in a plastic bag.

We will tally detections as one per night per cougar based on photographic confirmation. Dependent kittens will not be counted. Though we expect all animals visiting the sites to be detected by camera, hair samples may also provide proof of cougar presence as well as identifying unmarked animals.

Assessing the response of cougars to NGS and the lures is key to the development of this technique. To do this will require maintaining a sample of collared cougars throughout the study area. Currently there are approximately 20 GPS collared cougars within this study area and we will maintain a sample of up to 20 GPS collared cougars throughout this NGS study. Collars will be programmed to take 7 locations per day but this frequency may be increased periodically to obtain detailed locations to examine an individual's response to the NGS site. All capture and handling will follow the same procedures being used for the Front-Range Cougar Study parent project and the ACUC approved capture and handling guidelines (ACUC 03-2007), see attached. Capture of cougars will include the use of hounds, cage traps, and snares. To date we have had no capture related mortalities of cougars in the ongoing projects and injuries have been minimal with an occasional abrasion or broken claw.

The main variable of interest is the probability of detection given that an individual cougar was in the area. GPS information from collared cougars will be used to verify that a cougar was within the sampling grid (5 km), available for detection. Location data will also be used to approximate distance between a cougar and a lure, which will be used as a covariate in estimating the detection rate. Non-detection rates will also be of interest, especially with regard to distance from the lure, as this will provide information on the ability of a lure to attract an individual. For example, an individual cougar may travel very close to a lure but never approach the lure. A repeated measures analysis will also be used to determine if there is any behavioral effect associated with lures. This is important because if cougars begin to avoid lures (calls or scents) over time, estimates of population density will be biased. Maintaining these collared cougars will allow us to assess the magnitude of this bias.

The use of 20 collared cougars represents a balance between logistical constraints of capture and an adequate sample size to assess behavioral response. From past experience, maintaining 20 collared cougars will require considerable effort but is possible. To do this cougars will have to be recaptured every 1.5 to 2 years to replace batteries. Given mortality rates that we have seen over the last 5 years, it is likely that we will capture 5 to 8 new cougars each year to maintain this sample. Data on behavioral responses to the NGS sites is minimal so there is no way to assess the variability in potential responses. Males and females, subadults and adults, may all respond differently. The use of 20 collared animals representative of the sex and age classes should give us baseline data on the range of responses and potential differences between age and sex classes.

Hair samples will be processed at the USGS Fort Collins Science Center, FORT Molecular Ecology Lab. Taberlet et al. (1996) suggested that to achieve a correct genotype at a 99% confidence level, 8 U template DNA is needed (1 U is equivalent to the DNA content of 1 diploid cell). Therefore when possible, we will extract DNA from 10 hairs (Goossens et al. 1998, Boersen et al. 2003) using Qiagen DNeasy® Tissue Kits (Qiagen Inc., Valencia, CA). Samples will be genotyped using 9 – 12 microsatellite primers shown to have high variability in cougars (Ernest et al. 2000, Sinclair et al. 2001, Anderson et al. 2004), which should work for bobcats. We will amplify the DNA by polymerase chain reaction (PCR) using a M13-tailed forward primer as described by Boutin-Ganache et al. (2001). Each locus will be analyzed via GeneMapper®. To assess error, the results from hair genotyping will be compared with archived blood and tissue samples collected during capture. If possible, we will re-process hair samples shown to contain error at ≥ 1 allele.

Cougar location data will be analyzed with regard to site response. Understanding the detection process is key to understanding potential biases in population parameters. We will examine cougar response to NGS sites as a function of distance from the site, site specific characteristics, and individual characteristics. We will also examine behavioral response with regard to the same individual repeatedly visiting the same site on different occasions and the same individual visiting multiple novel sites.

We will also examine different NGS site setups to determine if specific site designs attract cougars better or if some setups yield better or more reliable hair snags. A great deal of time can be spent locating the "ideal" site location and setting up an intricate snare design. However, constructing all sites in this manner will limit the number of sites that can be placed within the study area. Examining site specific differences will provide information on how to balance the trade-offs between few complex sites versus many simple sites.

Capture-recapture models (Williams et al. 2002) will be used to estimate population size or density for both bobcats and cougars. A robust design framework (Kendall 1999) will be used initially to assess temporary emigration. Given the sampling design we will also be able to use spatially explicit capture-recapture models (Borchers and Efford 2008, Royle et al. 2009) or models that incorporate auxiliary telemetry data (Ivan et al. 2013) that provide information on the effective area sampled. Estimates will be compared across years for consistency.

Similar assessments of capture efficiency and detection probability will be made for bobcats. We are also interested in the efficacy of using a clustered sampling approach for bobcats within the cougar sampling design to obtain reliable estimates of density. Given the importance of both of these species and the expense of surveys it would be beneficial to have a design that effectively samples them both.

Finally, we will analyze a sub-sample of collected hair to determine sex structure, age structure, and diet composition to demonstrate the amount of relevant management data that can be collected from NGS surveys. These techniques are currently being developed as part of the ongoing Front Range Cougar project and will be utilized here. This will also allow us to examine if potential genetic degradation related to the timing of collections will impact these techniques.

Location of Work:

This work will be conducted along Colorado's front-range, in Boulder, Jefferson, Gilpin and Larimer counties. The study area is essentially defined by the boundary of Hwy 36, Hwy 72, Hwy 93, and I-70.

Schedule of Work

Time	Activity
Fall, 2013, ongoing	Field work—primarily during the winter (December-March)
August 2017, ongoing	Summary report of findings

Estimated Costs

Salaries of permanent employees, will be covered by existing project funds in the CDOW carnivore research and terrestrial management programs. Other expenditures include technician time ($112,000) field supplies ($5000) and lab time and supplies ($15,000).

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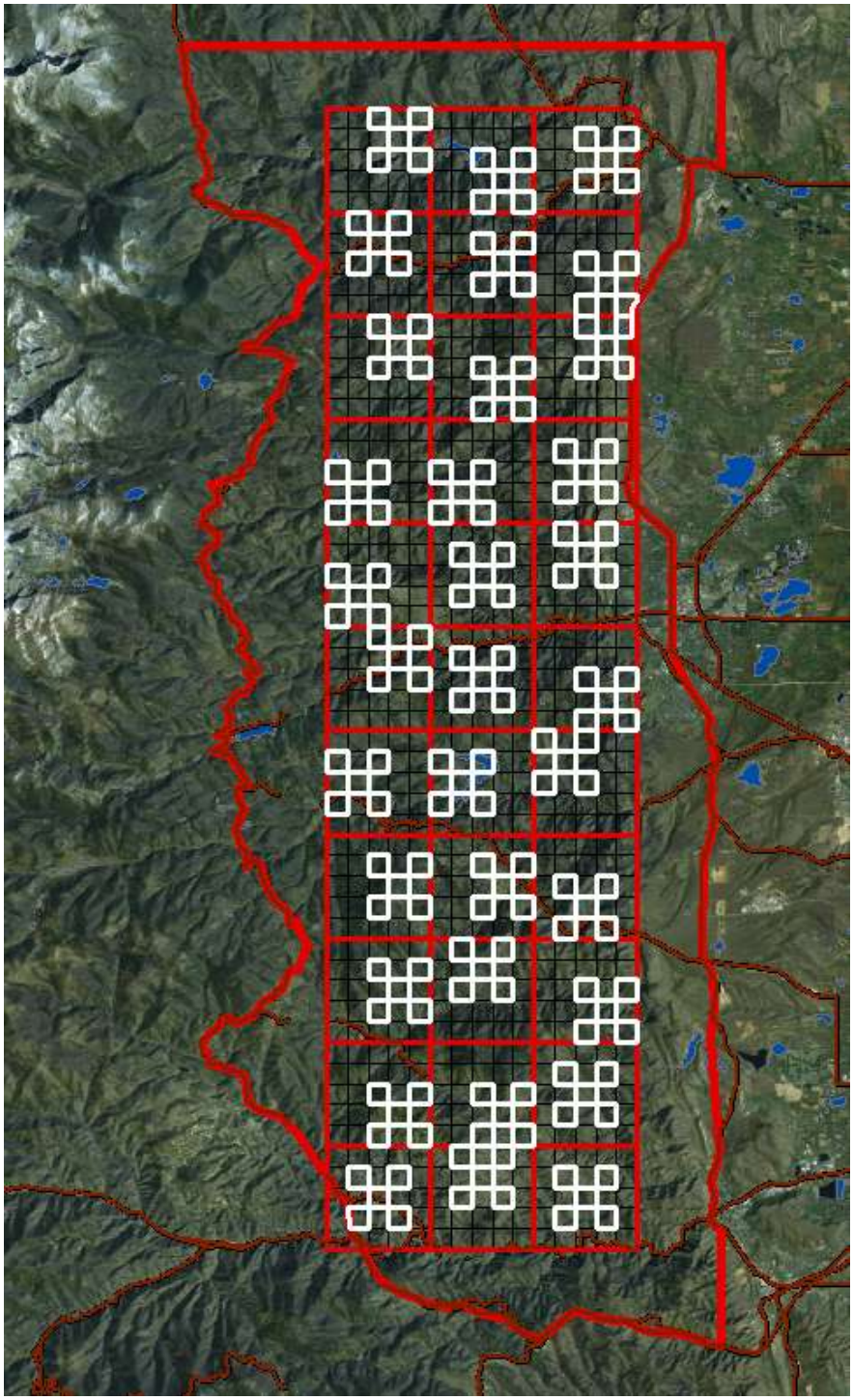

Figure 1: Study area boundary and grid layout for NGS cougar and bobcat sites. Larger squares represent the 5 km^2 grid overlaid with a 1 km^2 grid. White 1 km^2 cells represent the randomly selected cells where actual lure sites will be placed.