

USING NONINVASIVE GENETIC SAMPLING METHODS TO ESTIMATE
DEMOGRAPHIC AND GENETIC PARAMETERS FOR LARGE CARNIVORE
POPULATIONS IN THE ROCKY MOUNTAINS

by

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of the requirements for the degree

of

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DEDICATION

I dedicate this dissertation to my son, Jesse Lewis Sawaya. It is my greatest hope that he is able to experience the same natural wonder that I've been fortunate to witness while studying carnivores in the ever-fragmenting Rocky Mountain wilderness. I remain optimistic that ingenuity can mitigate the growing human population problem on Earth and help to restore severed ecological connections. May the research contained within this dissertation be used to address the world's tragic and unforgiveable loss of biodiversity, thus preserving or improving the quality of life for the next generation.

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ABSTRACT

Healthy carnivore populations are important to maintaining ecosystem balance, but many species are declining globally at disturbing rates due to anthropogenic causes. To effectively manage and conserve carnivores, wildlife managers must be able to obtain reliable estimates of population parameters. Noninvasive genetic sampling (NGS) methods such as hair or scat collection offer new and exciting alternatives to traditional carnivore research methods involving capture, drugging, and handling of animals; however, the potential of NGS methods to answer applied ecological questions has not been fully realized. The main objective of my doctoral research was to develop and apply NGS methods to estimate demographic and genetic parameters for large carnivore populations in the Rocky Mountains. First, I evaluated two NGS methods, hair snares and snow tracking, for cougars (*Puma concolor*) in Yellowstone National Park. I developed a method to collect hair while following cougar tracks in snow to bed sites and natural hair snags (e.g. thorn bushes, branch tips) from which I demonstrated that samples collected using NGS can provide reliable information on cougar population abundance. Next, I compared the ability of two NGS methods, hair traps and bear rub surveys, to estimate population abundance and trend of grizzly (*Ursus arctos*) and black bears (*U. americanus*) in Banff National Park. I found that bear rubs performed better than hair traps for estimating grizzly bear abundance and population growth rates, whereas hair traps worked better than bear rubs for black bears. I then used NGS to examine demographic and genetic connectivity at wildlife crossing structures along the Trans-Canada Highway that bisects Banff National Park. I compared genetic data collected from the bear populations surrounding the highway to data collected at the crossing structures using a novel hair sampling system. This comparison allowed me to show that wildlife crossing structures provided demographic connectivity for bear populations and maintained sufficient gene flow across the highway to prevent genetic isolation. In short, I have demonstrated the power of using an array of NGS methods, alone or in combination, to estimate abundance, gene flow, genetic structure, migration, and population growth rates for large carnivores in the Rocky Mountains.

INTRODUCTION

Carnivore species are tremendously important to maintaining ecosystem balance, but their populations are declining at disturbing rates globally due to anthropogenic causes. Fragmentation and overexploitation have directly led to the extirpation of many apex consumers (i.e. carnivores). The removal of top predators has led to drastic changes in abundance and distribution of species from lower trophic levels, often with undesirable consequences. In order to counteract the ever-increasing pressures on Earth's biodiversity, humans must be able to apply our ingenuity in creative ways to help staunch the tragic loss of biological diversity.

Technology has played a major role in allowing the unprecedented population growth of humans, therefore technology should also play a major role in mitigating the negative impacts of having so many people on the planet (7 billion and counting). Technological advancements now allow the use of noninvasive survey methods to estimate population parameters for rare and elusive species without involving capture, drugging or handling. Noninvasive genetic sampling (NGS) methods such as hair and scat collection offer many advantages over conventional population monitoring methods, but their potential has yet to be fully realized for most species of concern in many geographic areas.

The primary objective of my doctoral research was to use NGS methods to estimate demographic and genetic parameters for large carnivore populations in the Rocky Mountains of the United States and Canada. I chose three large carnivore species for my investigation, grizzly bears (*Ursus arctos*), black bears (*U. americanus*), and

cougars (*Puma concolor*) based on their ecological prominence, sensitivity to human disturbance and importance to regional wildlife managers. I conducted research in two of the world's preeminent protected areas, Yellowstone National Park in the western United States and Banff National Park in Alberta, Canada. Both national parks provided ideal study sites with unhunted populations of carnivores to develop and apply NGS methods in relatively intact ecosystems.

The chapters in this dissertation represent a natural progression from simple to complex and reflect my growth as a scientist. In my second chapter, I evaluated two NGS methods, hair snares and snow tracking, for cougars in Yellowstone National Park. This evaluation was one of the first studies to assess the quality of NGS data using independent data from a radio-collared population. My time spent in the laboratory clipping follicles from cougar hair was challenging for me as a field scientist, but also provided me with a solid foundation from which to pursue more advanced uses of NGS.

In my third, fourth and fifth chapters, I used a combination of hair sampling methods for grizzly and black bears (i.e. hair traps, bear rubs, wildlife crossings) to evaluate demographic and genetic connectivity at wildlife crossing structures in Banff National Park. In Chapter 3, I compared the ability of hair traps and bear rubs to estimate abundance and population growth rates. In Chapter 4, I used NGS methods to evaluate the ability of crossing structures to provide spatial and temporal connectivity for bear populations. In Chapter 5, I used NGS to assess whether the Trans-Canada Highway affects genetic structure of bear populations and if crossing structures allow sufficient gene flow to prevent genetic isolation in two fragmentation-sensitive carnivore species.

CHAPTER TWO

EVALUATION OF NONINVASIVE GENETIC SAMPLING METHODS FOR COUGARS IN YELLOWSTONE NATIONAL PARK

Contribution of Author and Co-authors

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Research Article

Evaluation of Noninvasive Genetic Sampling Methods for Cougars in Yellowstone National Park

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ABSTRACT Conventional methods for monitoring cougar, *Puma concolor*, populations involve capture, tagging, and radio-collaring, but these methods are time-consuming, expensive, and logistically challenging. For difficult-to-study species such as cougars, noninvasive genetic sampling (NGS) may be a useful alternative. The ability to identify individuals from samples collected through NGS methods provides many opportunities for developing population-monitoring tools, but the utility of these survey methods is dependent upon collection of samples and accurate genotyping of those samples. In January 2003, we initiated a 3-yr evaluation of NGS methods for cougars using a radio-collared population in Yellowstone National Park (YNP), USA. Our goals were to: 1) determine which DNA collection method, hair snares or snow tracking, provided a better method for obtaining samples for genetic analysis, 2) evaluate reliability of the genetic data derived from hair samples collected in the field, and 3) evaluate the potential of NGS for demographic monitoring of cougar populations. Snow tracking yielded more hair samples and was more cost effective than snagging hair with rub pads. Samples collected from bed sites and natural hair snags (e.g., branch tips, thorn bushes) while snow tracking accurately identified and sexed 22 individuals (9 F, 13 M). The ratio of the count from snow tracking to the count from radio-telemetry was 15:24 in winter 2004, 13:12 in 2005, and 22:29 for both years combined. Annual capture probabilities for obtaining DNA from snow tracking varied considerably between years for females (0.42 in 2004 and 0.88 in 2005) but were more consistent for males (0.77 in 2004 and 0.88 in 2005). Our results indicate that snow tracking can be an efficient, reliable NGS method for cougars in YNP and has potential for estimating demographic and genetic parameters of other carnivore populations in similar climates. © 2011 The Wildlife Society.

KEY WORDS cougars, genotyping errors, hair snares, individual identification, noninvasive genetic sampling, *Puma concolor*, radio-collar, snow tracking, Yellowstone National Park.

Reliable information on populations is essential for successful conservation and management of many wildlife species. Carnivores such as cougars (*Puma concolor*) are particularly difficult to study due to their large home ranges, low densities, and secretive nature (Logan and Sweaner 2001). Conventional methods for monitoring cougar populations involve capture, tagging, and radio-collaring, but these methods are time-consuming, expensive, and logistically challenging. For difficult-to-study species such as cougars, noninvasive genetic sampling (NGS) may be a useful alternative. Extracted DNA from hair or scat can be used to identify

and sex individuals, estimate abundance, distribution, and population growth rates, and examine patterns of genetic population structure (Foran et al. 1997, Woods et al. 1999, Mowat and Paetkau 2002, Palomares et al. 2002, Frantz et al. 2004, Long et al. 2008, Kendall et al. 2009). The ability to identify individuals from genetic samples provides many opportunities for developing population-monitoring tools, but the utility of these survey methods is dependent upon the collection of a representative sample from the population and the accurate genotyping of those samples.

Although NGS methods are gaining popularity, problems with sample collection and genotyping errors may be difficult to overcome (Taberlet et al. 1999, Mills et al. 2000). Scat sampling has been proven to be a useful method for identifying individual cougars (Ernest et al. 2000), but the merits of hair sampling have not been fully evaluated. Hair samples are difficult to obtain from some wild felid species (Downey

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et al. 2007, Ruehl and Crooks 2007), but 2 collection methods show promise: hair snares and snow tracking (McDaniel et al. 2000, McKelvey et al. 2006, Ulizio et al. 2006). Neither hair snares nor snow tracking has been adequately tested for obtaining hair samples from cougars and little is known about the effectiveness and reliability of these methods.

Genotyping error is another problem that can exist with noninvasively collected samples. Noninvasive samples such as hair and scat contain low concentrations of DNA, which can lead to errors during polymerase chain reaction (PCR) and genotyping, particularly allelic dropout and false alleles (Taberlet et al. 1999). These errors can be difficult to track and quantify (Bonin et al. 2004, Broquet and Petit 2004). As individuals are usually identified on the basis of unique, multi-locus genotypes, genotyping errors can lead to generation of false DNA profiles and overestimation of population size (Waits and Leberg 2000, Creel et al. 2003). Numerous methods have been proposed to minimize and correct genotyping errors in population studies (e.g., Taberlet et al. 1996, Paetkau 2003, McKelvey and Schwartz 2004, Miquel et al. 2006, Scandura et al. 2006), but few wildlife studies have examined the reliability of genetic data by comparing genotypes from hair or scat samples collected in the field with genotypes from the same individuals produced from blood or tissue samples, which have much higher concentrations of DNA and therefore lower rates of genotyping errors (Bayes et al. 2000, Lathuilliere et al. 2001). These comparisons of NGS to independent field data are necessary to evaluate and validate the information derived from noninvasive sampling studies (Arrendal et al. 2007).

Long-term population research on cougars in Yellowstone National Park (YNP) provided an opportunity to evaluate NGS methods using a radio-marked population (Ruth et al. 2003). Our goals were to: 1) determine which DNA collection method, hair snares or snow tracking, provided a better method for obtaining hair samples for genetic analysis, 2) evaluate the reliability of genetic data derived from hair samples collected in the field, and 3) evaluate the potential of NGS for demographic and genetic monitoring of cougar populations.

STUDY AREA

We conducted NGS surveys on the Northern Range of Yellowstone National Park (NRYNP) during winter of 2003–2005. The NRYNP is characterized by steep, rocky slopes with primarily south and north facing aspects along the Yellowstone River corridor. Elevations ranged from 1,500 to 3,000 m although most surveys were limited to elevations below 2,400 m due to snow accumulation. Vegetation consisted primarily of grasslands interspersed with patches of Douglass fir (*Pseudotsuga menziesii*) and juniper (*Juniperus occidentalis*; Despain 1990). This region experienced cold, dry winters and provided habitat for many of the Park's ungulates, particularly elk (*Cervus elaphus*) and mule deer (*Odocoileus hemionus*; Frank and McNaughton 1992, Singer et al. 1994). This area supported a variety of

large carnivores, which fed on the seasonal abundance of ungulates, including cougars, grizzly bears (*Ursus arctos*), black bears (*U. americanus*), wolves (*Canis lupus*), and coyotes (*C. latrans*). The study area was bordered by the Absoraka-Beartooth Wilderness Area and the Gallatin National Forest to the north and 1 paved road along the south.

METHODS

Cougar Capture and Radio-Collaring

In 1998, Ruth (2004a) initiated a study to determine the effects of wolf reintroduction on the population of cougars on the NRYNP. Ruth et al. (2008, 2010) captured, tagged, and radio-collared 83 cougars to determine survival rates, habitat use, prey selection, and predation rates following wolf reintroduction. Cougar capture, handling, collaring, and sexing procedures are described in Ruth et al. (2010). December through March, 1998 through 2005, T. Ruth (Hornocker Wildlife Institute/Wildlife Conservation Society, unpublished data) estimated the minimum number of cougars present each winter by surveying for cougar tracks in snow while traversing nonoverlapping transects (approx. 1,500 km each winter) and through intensive efforts to capture cougars (average = 226 person days/4–5 months of winter) following Murphy (1998). Ruth et al. (2010) permanently marked cougars with numbered colored ear tags and tattooed them with the same number in the opposite ear (Logan and Sweaner 2001). They pre-punched a hole in the center of an ear using sterile procedures prior to affixing ear tags. They macerated the removed tissue and placed it into a tube of lysis buffer and stored it at room temperature for future genetic analyses. Ruth also collected blood samples for use in pathologic and genetic studies (see Biek et al. 2006a,b). When time allowed Ruth also plucked hair from individuals captured during the 3-yr DNA study to aid with optimization of DNA extraction and PCR protocols. Ruth dried hair samples and stored them in silica desiccant to prevent bacterial degradation (Roon et al. 2003). Ruth et al. (2010) fitted each cougar with a very high frequency (VHF) collar (Telonics, Inc., Mesa, Arizona) or Global Positioning System (GPS) collar (Televilt/TVP Positioning AB, Lindesberg, Sweden) to obtain location and mortality data (Ruth et al. 2010). In 2006 Ruth ceased monitoring and removed any remaining collars.

Collection Methods

We synchronized the timing of hair snaring and snow tracking so that we could make direct comparisons of the 2 hair collection methods. We accessed survey sites primarily by foot and snowshoe travel from the south and west due to the limited road access. We allocated effort differently between the 2 collection methods across years in response to poor performance of hair snares in year 1.

Hair snares.— We conducted hair snaring from January through March of 2003 and 2004. We sampled in winter to allow direct comparisons with snow tracking and because cougar home ranges were more concentrated during this time. We delineated a NGS study area boundary of

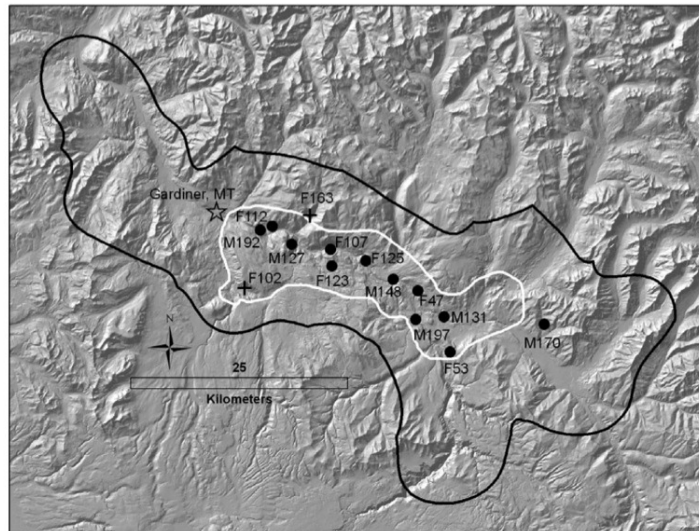


Figure 1. Map showing noninvasive genetic sampling (NGS) study area (white polygon) encompassed by the Yellowstone Cougar Project (YCP) winter study area (black polygon). Centroids for all adult cougars ($n = 14$) radio-located within the NGS study area during winter 2004 or 2005 are shown: closed circles represent adult cougars we detected from hair samples collected while snow tracking ($n = 12$), crosses represent adult cougars we did not detect with snow tracking ($n = 2$). Centroids are labeled with the animal's individual identity (ID) for the YCP and denoted F for females and M for males. We did not include on the map radio-collared cats not detected within the NGS study area during winter 2004 or 2005 ($n = 5$).

284 km² within the NRYNP study area based on detections of cougars from previous winters track surveys (Fig. 1). We deployed hair snares following the National Forest Lynx Detection Protocol (McKelvey et al. 1999). Hair snare sites consisted of carpet pads baited with a mixture of beaver castorium, catnip oil, and dried catnip along with an aluminum pie plate attached to a tree branch with wire and swivel. The 10-cm $\times$ 10-cm carpet pads had roofing nails driven through them to maximize collection of hair; we nailed pads to the base of a tree at a height of 30 cm. We used pie plates as visual attractants and carpet pads and bait to entice animals to rub their cheeks and deposit hair on the barbed pads. We established 5 of these stations per line transect oriented uphill to the point of origin. We overlaid a 3.2-km $\times$ 3.2-km point grid randomly over our study area to determine the point of origin for transects. We used the grid design to distribute effort across the study area and minimize capture variation. We chose grid size based on the Lynx Detection Protocol and the minimum winter home range size for female cougars in our study area (McKelvey et al. 1999).

In 2003, we deployed 73 hair snare transects consisting of 5 stations per transect. We set and maintained 365 stations from 9 January to 19 April 2003. We re-baited all stations at least once 10–14 days after installation and left them out for ≥ 20 trap nights. Station set-up required approximately 90 min/transect, re-baiting took 60 min/transect, and removal took 45 min/transect. During this first trial winter, we spent more time and money but collected fewer samples

with hair snaring than with snow tracking (Fig. 2). Hair pad results prompted us to allocate more time to snow tracking during winters 2004 and 2005. In 2004, we deployed 40 hair pad transects consisting of 5 stations per transect. We placed snares in 10 locations frequented by cougars based on >10 yr of radio-telemetry data (Murphy 1998, Ruth 2003). We

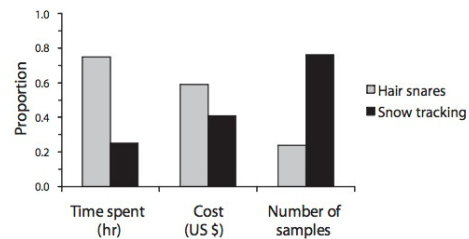


Figure 2. Hair snares versus snow tracking DNA collection methods: comparing proportions of time, cost, and sample yield for data collected during winter of 2003 in Yellowstone National Park, USA. We estimated time spent and costs due to the impossibility of differentiating between overlapping costs and time spent on the 2 methods. Expenses of \$13,971.14 included hair pad station materials and costs that overlapped between hair snares and snow tracking such as collection materials, fuel for vehicles, stipends for 2 volunteers, and 1 graduate student salary. During the winter 2002–2003 sampling period, we placed more effort on hair snaring and less effort on snow tracking, although we also spent more money to conduct hair snaring. Snow tracking also yielded many more DNA samples. We compared the estimated time spent, estimated cost, and sample yield of the 2 methods and decided to allocate most of our effort to snow tracking for the following 2 field seasons, as it took less time, was cheaper, and yielded more samples.

checked and re-baited hair pads once a week during 15 January to 17 February, then every other week from 17 February to 25 March. We removed hair pads from the field by 25 March 2004. We deployed no hair snares in 2005 due to 2 yr of poor DNA collection success.

Snow tracking.—We conducted snow-tracking surveys to locate hair samples from January to March of 2003 and 2004 and from December to March 2005. We forward or back-tracked cougar tracks until we located a hair sample, tracking snow degraded to a level where tracks were no longer visible, or until time limited following the track further. Initially we found hair samples at only bed and kill sites, but during the second sampling season we discovered that careful trackers often found cougar hair within tracks and on natural hair snags such as thorn bushes, branch tips, and rock edges. During the period from January to March 2003 we looked for cougar tracks opportunistically while hair snaring. In winters 2004 and 2005 we used the hair snaring grid to guide our survey efforts but we focused effort on snow tracking. We converted the 3.2-km $\times$ 3.2-km point grid to cells by connecting the points.

We created 16 survey routes that we could walk in one day based on logistical considerations such as coverage of cells, travel efficiency, and vehicle access. We designed these routes to maximize probability of encountering a cougar track so we targeted rocky outcrops and creek bottoms. We used track surveys from capture and monitoring efforts in previous years to determine likely places to encounter cougar tracks; we used this information to build the NGS survey routes. We walked ≥ 1 km within each grid cell every 2 weeks and surveyed every route ≥ 6 times during the 2004 and 2005 field seasons. We determined Universal Transverse Mercator (UTM) coordinates of track locations and hair samples using a hand-held Garmin 12 Global Positioning System (Olathe, KS). We recorded all locations, survey routes, and backtracking segments on 1:25,000 scale maps. We used a Scalex PlanWheel (Carlsbad, CA) to determine the distance of the survey route and to measure tracking distances.

In 2003, we conducted snow track surveys opportunistically while en route to hair snare transects. We surveyed >948 km from 9 January to 19 April 2003. In 2004 and 2005, we used the 3.2-km $\times$ 3.2-km hair snare grid to guide our snow-tracking surveys. We surveyed $>1,285$ km from 27 December 2003 to 25 March 2004 and $>1,140$ km from 27 December 2003 to 20 March 2004.

Reliability of Noninvasive Genetic Sampling Data

We performed DNA extraction and PCR set up in a room isolated from amplified DNA with minimal movement of people and materials between facilities to prevent contamination of genomic DNA with amplified DNA (Paetkau 2003). We extracted DNA from blood using the Qiagen Blood Kit (Qiagen, Valencia, CA). We extracted DNA from tissue using the Qiagen DNeasy Tissue Kit. Before DNA extraction, we rinsed hair follicles with distilled water and placed them in microcentrifuge tubes. We used 5–10 follicles when possible but we also extracted DNA from single hair follicles when necessary. We finished the extraction using the

user-developed protocol posted on the Qiagen website (Isolation of genomic DNA from nails and hair using the DNeasy Tissue Kit, unpublished protocol at <http://www1.qiagen.com>).

We evaluated 19 microsatellite loci developed for the domestic cat by Menott-Raymond et al. (1999). We chose a subset of 7 loci based on their heterozygosity and performance using blood or tissue samples taken from 42 individual cougars during capture. We genotyped all 42 individuals twice at all 7 loci to establish accurate genotypes for each individual. We also looked for Mendelian inheritance between mothers and litters as another way to validate genotypes. We used Program MM-dist (Kalinowski et al. 2006) to calculate allele frequencies and heterozygosities from blood and tissue samples. We used HW-QUICKCHECK to check for Hardy–Weinberg equilibrium (HWE, Kalinowski 2006). We chose loci Fca008, Fca057, Fca083, Fca096, Fca132, Fca205, and Fca293 for individual identification and sample quality screening based on their ability to amplify, heterozygosities, number of alleles, and fragment lengths (Buchan et al. 2005, Broquet et al. 2007). We used GENECAAP (Wilberg and Dreher 2004) to detect genotyping errors in our capture-mark-recapture (CMR) data set and to calculate probability of identity $P(\text{ID})$ and probability of identity siblings ($P(\text{ID})_{\text{sib}}$; Waits et al. 2001) for the 7 loci. We then used an error-checking and removal procedure developed by Paetkau (2003) for use on poor-quality samples. We removed hair samples from further analysis if they failed to amplify at ≥ 4 loci. We also removed samples from analysis if they produced multiple alleles at ≥ 1 loci, an indication that the sample is mixed or contains hair from >1 individual (Roon et al. 2005). Two independent observers determined consensus genotypes based on strength and confidence in the results. Our approach differed from Paetkau (2003) in that we amplified each locus at least twice and only accepted consensus genotypes if 2 heterozygotes and 3 homozygotes were replicated and confirmed through visual inspection by both observers. We used Amelogenin and Zn-finger regions to determine genders for all blood, tissue, and hair samples (Pilgrim et al. 2005).

We used GENECAAP to identify samples that produced multi-locus genotypes that differed by only 1 or 2 loci, a potential warning sign that a genotyping error occurred (Paetkau 2003). Once we had consensus genotypes for all 7 microsatellite loci, we ran the multi locus data set through GENECAAP. If multi locus genotypes differed by 1–2 alleles, we either reran the loci that mismatched or scrutinized the electropherograms from previous runs. Once we were satisfied that we had accurate genotypes, we ran the hair samples again along with the tissue samples through GENECAAP to see if any of our hair samples produced multi-locus genotypes that differed from the capture samples by only 1–2 loci. This last step provided a robust test of the reliability of the noninvasive genetic data, as it directly compared genotypes from hair samples to those from blood and tissue samples.

We used 2 tests in Program DROPOUT to identify problem loci and evaluate the reliability of our final set of con-

sensus genotypes (McKelvey and Schwartz 2004, 2005). We used the Examining Bimodality test to detect if our CMR data set contained genotyping errors. This test assumes that in a population sampled in a manner that generates recaptures, some of the samples will be genetically identical, whereas the rest will differ (McKelvey and Schwartz 2004). An ideal sample free of errors produces a unimodal distribution with individuals being less likely to differ at many loci or few loci. Multi locus genotypes that differ at only 1 or 2 loci are an indication that those samples came from the same individual and that ≥ 1 genotyping errors occurred (Mowat and Paetkau 2002, Paetkau 2003). We identified problem loci by examining the effect of including and excluding loci on the number of individuals using the Difference in Capture History test (McKelvey and Schwartz 2005).

Comparison of Snow-Tracking and Radio-Telemetry Data

We produced 2 abundance measures for winters of 2004 and 2005: 1 count from hair samples collected while snow tracking (snow-tracking count) and 1 count from radio-telemetry data (radio-telemetry count). We defined the snow-tracking count as the total number of males and females detected via DNA analysis during a winter sampling period. We defined the radio-telemetry count as the total number of radio-collared males and females located within the study area at least once during a winter sampling period. We evaluated the efficacy of using snow tracking as an index of cougar population abundance by examining the ratio of the snow-tracking count to the radio-telemetry count. We also estimated annual capture probabilities (proportion of the population sampled in a given year) by dividing the snow-tracking count by the total count. We calculated total counts as the sum total of all unique individuals detected on the study area from radio-telemetry and DNA sampling adjusted by adding the number of unmarked individuals known to be on the study area from snow track surveys used to estimate percent accuracy (PA) following Logan and Sweanor (2001).

RESULTS

Collection Methods

Hair snaring proved ineffective for collecting cougar hair. During winter 2003, we collected 24 hair samples from rub pads, 5 of which appeared through visual examination of the hair to be from cougars. We also collected samples from coyotes, wolves, bears, and possibly bobcat (*Lynx rufus*). In 2 instances we located cougar tracks within 1 m of hair pads without the cat investigating or rubbing on the pad. In 2 other instances, cougars made kills within 30 m and 40 m of hair pads but did not rub on them. During winter 2004, we collected 15 hair samples from hair pads, only 1 of which appeared to have come from a cougar. We also collected samples from coyotes, wolves, bears, and possibly bobcat. None of the 14 samples collected from the hair snares in 2004 amplified at any locus indicating those hairs were not from cougars.

Table 1. Snow tracking effort for cougars in Yellowstone National Park, USA (2004–2005). We defined individual identity (ID) as a hair sample that amplified at enough loci to produce a multi-locus genotype and unique DNA profile.

Effort	<i>n</i>	$\bar{x}$ (km)	SD
Length of survey	231	10.5	4.38
Distance backtracked	67	1.09	1.44
Distance to DNA sample	64	0.32	0.42
Distance to individual ID	33	0.81	2.24

Snow tracking was an efficient and cost-effective way to sample the study population and obtain individual identities (ID's) from free-ranging cougars in YNP. During winter 2003, we did not conduct any surveys specifically to locate cougar tracks. Although we were only looking for tracks opportunistically while hair snaring, we were able to collect 12 cougar hair samples from bed or kill sites. We determined species identification of hair samples through both visual inspection and track identification of species; therefore all samples collected had a high probability of coming from a cougar. We were able to successfully backtrack to a hair sample 80% of the time when tracking conditions were favorable. During winter, 2004, we collected 81 cougar hair samples; 79% of hair samples came from bed sites and we collected 21% from natural hair snags, mostly broken branch tips and thorn bushes. We determined species identification of the samples through accurate track identification of species then confirmed these identifications by microsatellite analysis. During winter 2005, we collected 128 cougar hair samples; we collected 38% of hair samples from bed sites and 62% of hair samples came from natural hair snags.

During the 2 yr we concentrated on backtracking, 2004 and 2005, average distance backtracked from the start of a track to a sample was 1.09 km (Table 1). Average distance to a sample that produced an individual ID was 0.81 km. We spent an average of 2.25 hr backtracking each time we were able to follow a track. We spent an average of 1.09 hr following a track to a hair sample that resulted in an individual ID (Table 1). Average survey length was 10.5 km and average survey duration was 6.10 hr. We encountered cougar tracks on 34% of survey days, were able to follow 92% of tracks we encountered, and collected DNA from 88% of those backtracks (Table 2). Although our overall success rate for obtaining an individual ID from a survey was only 15%, we were able to obtain an individual ID from 49% of the tracks that we followed (Table 2).

Table 2. Snow tracking success for cougars in Yellowstone National Park, USA (2004–2005). We defined individual identity (ID) as a hair sample that amplified at enough loci to produce a multi-locus genotype and unique DNA profile.

Success	<i>n</i> 1	<i>n</i> 2	Proportion
Survey routes with cougar tracks	85	253	0.34
Tracks able to backtrack	78	85	0.92
Survey routes with ≥ 1 DNA sample	69	253	0.27
Backtracks with ≥ 1 DNA sample	69	78	0.88
Backtracks with ≥ 1 individual ID	38	78	0.49
Survey routes with ≥ 1 individual ID	38	253	0.15

Reliability of Noninvasive Genetic Sampling Data

To evaluate the reliability of genetic data generated from hair samples, we first assessed accuracy of our blood and tissue genotypes. Running HWQuickCheck (Kalinowski 2006) with blood and tissue genotypes indicated that none of the 7 loci departed significantly from HWE. We calculated $P(\text{ID})$ as 0.000003 and $P(\text{ID})\text{Sibs}$ as 0.0041, which were low enough for individual identification (Mills et al. 2000). Once we optimized PCR conditions, we detected no genotyping errors in the blood and tissue sample data set using GENECAAP and DROPOUT. Results from these analyses were encouraging and validated our lab techniques. Additional evidence for accurate genotypes came from a test for Mendelian inheritance; all of the offspring from 11 marked litters shared ≥ 1 allele with their respective mothers. Both gender tests matched the genders we determined during capture of all 42 individuals.

After optimizing our protocols with blood and tissue samples, we genotyped 21 hair samples plucked from captured cougars. Nineteen (90%) of these samples produced IDs and 2 (10%) of them failed to amplify. Multi-locus genotypes from these hair samples matched the genotypes from blood or tissue samples from the same individual further validating our methods.

Of 112 samples collected from bed sites in 2004 and 2005, 112 (100%) were extracted, 44 (39.2%) produced IDs, 53 (47.3%) failed to amplify, and 15 (13.3%) were mixed (Fig. 3). Of 104 samples collected from natural hair snags, 97 (93.3%) were extracted and 7 (0.07%) contained inadequate material for extraction. Of the 97 samples that were extracted, 24 (25%) produced IDs, 68 (70.1%) failed to amplify, and 5 (0.05%) were mixed (Fig. 3). Of the 22 individuals identified from genetic samples obtained from snow tracking, we identified 13 from samples collected at bed sites, 2 from natural hair snags, and 7 from both bed sites and natural hair snags.

Differences in sample quality were apparent between bed site samples and natural hair snag samples. The number of follicles per sample, an indirect measure of sample quality (Goossens et al. 1998), was higher for bed site samples than for samples collected from natural hair snags. We placed samples into 3 subjective categories of low quality (0), fair to

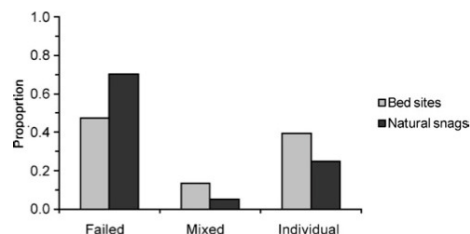


Figure 3. Results of DNA amplification of 209 hair samples collected during winters of 2004 and 2005 while snow tracking to bed sites or natural hair snags in Yellowstone National Park, USA. We defined individual identity (ID) as a hair sample that amplified at enough loci to produce a multi-locus genotype and unique DNA profile.

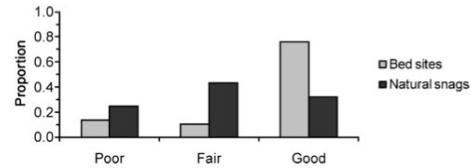


Figure 4. Proportion of cougar hair samples that were of poor, fair, or good condition collected from beds or natural snags in Yellowstone National Park, USA, during winters of 2004 and 2005.

moderate quality (1–5), and good to high quality (6–10). Bed site samples had a much higher proportion of samples in the good to high category than did the natural hair snag samples (Fig. 4). Error-checking procedures aided in identifying and removing genotyping errors before they were incorporated into multi-locus genotypes (DNA profiles). Program DROPOUT did not identify any suspicious multi-locus genotypes. The Examining Bimodality test produced a plot of the differences in genotypes between hair samples collected through snow tracking, which showed a relationship indicating the sample was error-free, with no 1—locus mismatches and few 2—locus mismatches (McKelvey and Schwartz 2004). The Difference in Capture History test produced another plot with a flat horizontal line, which indicated that none of the loci were particularly problematic or more prone to genotyping errors. In other words, no individuals would be added or removed if a locus was included or excluded from our multi-locus data set.

Once we removed genotyping errors, we compared our final hair genotypes to blood and tissue genotypes. We identified 22 cougars (9 F, 13 M) from hair samples, 20 of which matched genotypes from blood and tissue samples. Genders from all 20 of these individuals matched the genders we determined from live capture. Two individuals genotyped from hair that did not have matching blood or tissue genotypes were identified as kittens from separate litters that we observed but never captured. We collected one of the hair samples from the kittens as a mortality while backtracking. The kittens had multi-locus genotypes that were unique and differed by ≥ 2 loci with any other individual. Both the Amelogenin and Zn-finger tests produced results indicating these 2 individuals were female. A test of Mendelian inheritance confirmed their putative mothers.

Comparison of Snow-Tracking and Radio-Telemetry Data

We detected 22 individuals through genotyping hair samples collected from bed sites and natural hair snags while snow tracking during winters of 2004 and 2005 (Table 3). We detected 15 individuals in 2004 (5 F, 10 M), 13 individuals (6 F, 7 M) in 2005, and 22 individuals (9 F, 13 M) for both years combined. The number of individuals we detected on the study area with radio-telemetry was 24 (12 F, 12 M) in 2004, 12 (6 F, 6 M) in 2005, and 29 (15 F, 14 M) for both years combined.

Table 3. Counts from snow tracking and radio-telemetry sampling and annual capture probabilities, P , for snow tracking. We calculated snow-tracking count as the total number of unique DNA profiles generated from hair samples collected while following cougar tracks in snow. We calculated the radio-telemetry count as the total number of individuals radio-located on the DNA sampling area during a winter. We calculated total counts by summing the total number of individuals known to have been on the study area from snow tracking and radio-telemetry at least once during a winter. We adjusted total counts by adding the number of unmarked individuals estimated to be on the study area using PA following Logan and Sweanor (2001); we added 1 unmarked male individual to the count in 2004 and zero unmarked individuals in 2005. We calculated annual capture probabilities for snow tracking by dividing the snow-tracking count by the total count.

Parameter	2004			2005			2004–2005		
	F	M	Total	F	M	Total	F	M	Total
Snow-tracking count	5	10	15	6	7	13	9	13	22
Radio-telemetry count	12	12	24	6	6	12	14	15	29
Total count	12	13	25	8	7	15			
Annual P	0.42	0.77	0.60	0.88	0.88	0.87			

We backlogged cougars into the winter population and estimated proportion of adults marked each year using the PA method of Logan and Sweanor (2001). An estimated 68% and 88% of adult cougars present in our study were radio-marked by winters 2000–2001 and 2001–2002, respectively, with 88–93% radio-marked in all subsequent years until the final year of study (Ruth et al., Hornocker Wildlife Institute/Wildlife Conservation Society, unpublished data). The ratio of snow-tracking count to radio-telemetry count was 15:24 in winter 2004, 13:12 in 2005, and 22:29 for both years combined (Table 3). Total counts were 25 in 2004 (12 F, 13 M) and 15 in 2005 (8 F, 7 M; Table 3). Using total count as population size, estimates of annual capture probabilities for females with snow tracking ranged from 0.42 in 2004 to 0.88 in 2005 (Table 3). Annual capture probabilities for males were 0.77 in 2004 and 0.88 in 2005 (Table 3). Although DNA analysis cannot determine age, an examination of our radio-telemetry data revealed that our hair samples detected 6 adult females, 6 adult males, 2 juvenile females, and 7 juvenile males (we pooled kittens and independent subadults together as juveniles). Over the 2-yr sampling period, we detected 12 of 14 (86%) adult individuals that were radio-located on the study area. One of the adult females that were not detected, F163, had a home range centered on the north side of the Yellowstone River, which is primarily south-facing and snow-free for much of the winter (Fig. 1). The only other adult that was radio-located on the DNA study area but not detected with DNA sampling was F102, who was only alive for the first season of sampling and had a home range that had little overlap with the DNA sampling area (Fig. 1). Radio-collar data also revealed that we had collected samples from 2 individuals, F53 and M148, after they died. In the case of adult female F53, we lost a set of cougar tracks due to poor tracking conditions and collected a sample from a nearby snow-free bed site. This hair sample, collected on 14 March 2004, produced a multi-locus genotype which matched the genotype from F53's blood sample even though she had been killed by wolves on 2 February 2004. In the case of M148, we lost a set of cougar tracks due to lack of snow and collected a hair sample from a nearby bed site located under a protected rock outcrop. We collected that hair sample on 18 February 2005 and it produced a genotype that matched M148's tissue sample taken during capture even though he died on 16 January

2004 due to injuries sustained during a confrontation with another male cougar.

DISCUSSION

Our evaluation supports previous research (Boulanger et al. 2004, Kendall et al. 2008, Ruell et al. 2009, Williams et al. 2009, Stetz et al. 2010) indicating NGS can provide a low-cost, long-term, population-monitoring tool for YNP and other management agencies, thus reducing the need for capturing and collaring cougars depending on management needs and objectives. Methods to identify individual cougars from their tracks (Smallwood and Fitzhugh 1993, Grigione et al. 1999, Lewison et al. 2001) and using counts of cougar tracks as a measure of abundance (Van Dyke et al. 1986, Van Sickle and Lindzey 1991, Beier and Cunningham 1996, Anderson and Lindzey 2005, Choate et al. 2006) have limited application for monitoring cougars, as those methods typically cannot accurately identify and sex individuals. Ours is the first study to identify and sex individuals using non-invasive hair sampling methods and evaluate the genetic data by comparing genotypes from hair with genotypes from blood and tissue and relating the number of detections to the number of cougars known to be on the study area from radio-telemetry.

Collection Methods

Although hair snares have the potential to be an effective hair collection method (e.g., lynx [*Lynx Canadensis*], gray fox [*Urocyon cinereoargenteus*]), they were not cost effective and yielded few samples ($n = 5$) using our sampling design (Fig. 2). Other studies have reported a similar lack of success with hair snares targeting felids (Downey et al. 2007). We limited hair snaring to winter, when cougars were restricted to lower elevation winter range, the population was assumed to be closed, and bears were primarily nonactive while in winter dens. However, colder air temperatures during winter may inhibit dispersion of scent lures, which could lower capture probability. Testing multiple visual and scent lures may be necessary to optimize this procedure for cougars. Research on whether seasonal variation affects behavioral response of cougars to scent lures could also better determine the usefulness of hair snare stations to obtain DNA from cougars. Further, cougars may avoid hair pads that have been

visited by wolves or bears, species that are dominant competitors (Murphy 1998, Ruth 2004b).

Snow tracking was an efficient and cost-effective way to sample the study population and obtain IDs from free-ranging cougars in YNP. The effort required to follow tracks in snow to hair samples was reasonable (Table 1). Average distance and time spent backtracking to a sample and to obtain a sample yielding an individual ID was small compared to the total time spent on a backtracking survey route. We encountered cougar tracks on 1 out of every 3 days and were able to follow most tracks that we encountered. Although our overall success rate for obtaining an individual ID from a snow-tracking survey was low, we were able to obtain an individual ID for many of the tracks that we followed. Forty-one surveys produced an individual identification and 7 of 41 surveys identified >1 individual. These data demonstrate snow tracking can be an efficient and productive NGS method.

Advantages and disadvantages exist for both hair snares and snow tracking. Advantages of hair snaring include applicability to other felines, success is unrelated to snow availability and snow conditions, and hair samples may be of higher quality. Disadvantages of hair snares include tendency towards nontarget species detections (Downey et al. 2007), equipment costs, and dependency on a specific behavioral response (rubbing face on carpet pad with nails).

The advantages of snow tracking include greater sample yield during winter, high detection probability along with auxiliary information about age (track measurements), and locating kills where bed sites are easily located with large quantities of hair. Another benefit of snow tracking is that >1 sample type can be collected; individuals have been successfully identified from blood, urine, and scat samples found while following tracks in snow (Valiere and Taberlet 2000, Flagstad et al. 2004, Scandura 2005). A disadvantage of snow tracking is that sampling is strongly influenced by snow conditions to the extent that individuals may be missed or, conversely, DNA from dead individuals may be collected. These omissions and inclusions may limit the accuracy of the method, yet even in light of the limitations, snow tracking was a better, more efficient method than hair snares at obtaining hair samples for genetic analysis in our study area.

Reliability of Noninvasive Genetic Sampling Data

Our results show that reliable genetic data can be derived from a population of free-ranging cougars using NGS if care is taken to remove problematic samples and genotyping errors. Schwartz et al. (2006) highlight the importance of removing genotyping errors from data sets to avoid an artificial increase in abundance estimates. The results of the Examining Bimodality and Difference in Capture History tests indicate that our final data set is free of genotyping errors and hence reliable for CMR purposes. Our comparison of the data derived from the hair samples with the data from the blood and tissue samples was a robust test of whether we had genotyping errors and false DNA profiles in our data set. Our use of 2 independent sex ID tests validated our methods since the results of the 2 tests agreed

with each other for all samples. We also compared these results to the known genders from capture and those genders matched for every individual for which we had a blood or tissue sample ($n = 20$) further validating our methods. Both bed sites and natural hair snag samples detected unique individuals but bed sites appeared to be more productive, as they produced larger samples and had better amplification rates (Fig. 3). Samples collected from bed sites were higher quality than those taken from natural hair snags (Fig. 4) because when we located a bed site, there was usually ample hair for collection and extraction. The number of hairs found on a natural hair snag was limited by the short duration of exposure to the cougar, whereas cougars will often spend hours in one bed site and 3–6 days bedded near kills in between feeding bouts (Murphy 1998, Murphy and Ruth 2010). Even though bed site samples had higher amplification rates, they also had a higher proportion of mixed samples than did natural hair snag samples (Fig. 3). We speculate that this is due to the tendency of family groups to bed together and the affinity for certain bed sites by multiple individuals. Regardless of the limitations, our research has shown that snow tracking can be used to obtain cougar hair samples for genetic analysis and that those hair samples can produce reliable genetic data for individual identification and gender determination. Our results also suggest that hair samples collected from snow tracking could even be used to examine genetic structure in cougar populations similar to studies done with tissue such as Sinclair et al. (2001), Anderson et al. (2004), and McRae et al. (2005).

Comparison of Snow-Tracking and Radio-Telemetry Data

Snow tracking was an effective and reliable method for collecting genetic information on the minimum abundance and sex ratio of the population of cougars on the NRYNP. When designing the study, we focused our efforts on a small area that we intensively studied rather than a larger area with animals that we did not know as well but with a sufficient number of individuals for a CMR estimate. Because we did not design our study for abundance estimation, we correctly predicted that our data would be too sparse to reliably estimate abundance and capture probabilities using CMR methods. However, if our methods of collection are subsequently applied to a larger area, we expect that CMR estimates will be more precise than what would have been achievable previously.

We accurately identified 86% of the total count of male and female cougars from hair samples collected while snow tracking (Table 3). Yet, we also identified some limitations, in that we missed an individual overlapping the DNA study area, F163, due to lack of snow for tracking (Fig. 1). We included other individuals in the snow-tracking count after they had died. Optimizing sampling after heavy snows, at times when snow persists on all areas to be sampled, and only collecting hair in beds associated with fresh tracks should help minimize omission and inclusion. Yet, bed sites still have the potential to yield hair samples from multiple indi-

viduals, including those that may have died or emigrated prior to sampling.

We also supplemented our radio-telemetry data with DNA data by identifying and sexing 2 female kittens, 1 of which we removed from our counts as we collected it as a mortality. Although we had 2 false detections of cougars that were dead, we are confident that these situations could be avoided with careful sample collection and meticulous record keeping. Regardless, it is both remarkable and concerning that we were able to genotype a sample from M148 that was in the field for >13 months.

We documented greater heterogeneity in annual capture probabilities for female than male cougars, which could possibly be explained by improvements to our methods over time. However, we had surprisingly little variation for males, possibly a function of lower male densities and greater male movements, which are typical in a primarily nonhunted cougar social system (Logan and Sweaner 2001). Although our data were too sparse to use CMR modeling, the high capture probabilities we obtained from these methods suggest that a larger study area would yield adequate detections to provide precise abundance estimates. Further, these data are well suited for estimating population growth rates using open models such as those developed by Pradel (1996) and recently explored for grizzly bears in the northern Rocky Mountains of Montana (Stetz et al. 2010). Combining snow tracking with other methods such as biopsy darts, which were successfully used in Northwest Washington, USA to obtain tissue samples from cougars for individual identification and population enumeration (Beausoleil et al. 2008), may result in greater sampling coverage yielding more precise and less biased abundance estimates (Boulanger et al. 2008).

MANAGEMENT IMPLICATIONS

Successful conservation and management of cougars, and many other carnivore species, depends on the ability to reliably estimate demographic and genetic parameters for populations of interest. The results of our research have global applicability for managers interested in using NGS to monitor elusive carnivore species, though our findings may not be directly applicable everywhere and results may vary for different tracking substrates and conditions. We have shown that important parameters such as population size and sex ratio could be estimated by analyzing the DNA found in hair samples collected while following tracks in snow. We make the following recommendations to wildlife managers considering the use of snow tracking to monitor cougar or other carnivore populations: 1) conduct preliminary track surveys to determine the most likely locations and optimum snow conditions for finding tracks in a study area, 2) be sure to match a track with a corresponding DNA sample to avoid false detections of dead individuals, 3) take detailed measurements of tracks so that individuals identified from hair samples can be put into age classes, 4) follow tracks to bed sites whenever possible to maximize hair sample collection, but try to avoid collecting mixed samples from family groups, and 5) collect samples from natural hair snags oppor-

tunistically along the way to bed sites to increase sample yield and the number of individual identifications.

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CHAPTER THREE

ESTIMATING GRIZZLY AND BLACK BEAR POPULATION ABUNDANCE AND TREND IN BANFF NATIONAL PARK USING NONINVASIVE GENETIC SAMPLING

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**Estimating Grizzly and Black Bear Population Abundance and Trend in Banff
National Park Using Noninvasive Genetic Sampling.**

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Abstract

We evaluated the potential of two noninvasive genetic sampling methods, hair traps and bear rubs, to estimate population abundance and trend of grizzly (*Ursus arctos*) and black bear (*U. americanus*) populations in Banff National Park, Alberta, Canada. Using Huggins mark-recapture models, we obtained the first precise abundance estimates for grizzly bears ($\hat{N} = 73.5$, 95% CI=64-94 in 2006; $\hat{N} = 50.4$, 95% CI=49-59 in 2008) and black bears ($\hat{N} = 62.6$, 95% CI=51-89 in 2006; $\hat{N} = 81.8$, 95% CI=72-102 in 2008) in the Bow Valley. Hair traps had high detection rates for female grizzlies, and female and male black bears, but extremely low detection rates for male grizzlies. Conversely, bear rubs had high detection rates for male and female grizzlies, but low rates for black bears. We estimated realized population growth rates, lambda, for grizzly bear males ($\lambda = 0.94$, 95% CI=0.75-1.19) and females ($\lambda = 0.89$, 95% CI=0.67-1.20) using Pradel open population models with 3 years of bear rub data. Lambda estimates are supported by abundance estimates from combined hair trap/ bear rub Huggins closed models and are consistent with a system that is likely driven by documented high levels of human caused mortality. Our results suggest that noninvasive genetic sampling would provide an efficient and powerful means to monitor the effectiveness of bear management in the Canadian Rocky Mountains.

Key-words: abundance, Banff National Park, bear rub, hair trap, mark-recapture, noninvasive genetic sampling, population growth rate, *Ursus americanus*, *Ursus arctos*

Introduction

Carnivore populations are disappearing globally at an incredible rate due to anthropogenic causes [1], but dedicated monitoring programs rarely exist due to technical, financial, and logistical constraints [2]. Conservationists have long predicted that major shifts in ecosystems can follow changes in the abundance and distribution of apex consumers (e.g. carnivores), but a recent review of empirical evidence sheds new light on the critical role that consumers play to ensure the maintenance of top-down ecological processes at every trophic level [1]. Climate change further affects the removal of top-down selective pressures [3] and makes it increasingly important for conservation managers to be able to monitor changes in carnivore populations and respond to biodiversity threats accordingly [4]. Reliable estimates of population parameters such as abundance and realized population growth rate, λ , are needed for successful adaptive management of carnivore species, but these data are difficult to collect for secretive and wide-ranging species that often occur at low densities such as grizzly (*Ursus arctos*) and black bears (*U. americanus*) [5].

Advancements in molecular analysis techniques provide new cost-effective and reliable monitoring methods that do not require capturing or handling animals [2, 6]. DNA extracted from hair and scat samples collected noninvasively can be used to identify individuals and their gender. Noninvasive genetic sampling (NGS) provides an alternative to conventional bear population monitoring methods such as biomarking, and radio-telemetry [7]. Genetic monitoring can provide information on abundance, distribution, vital rates, and genetic interchange, but wildlife managers have at times been

reluctant to embrace NGS methods because they are relatively new [8]. Understandably, there has been concern that genotyping errors might inflate DNA censuses, but recent studies show that NGS methods can provide reliable and accurate information on carnivore populations [9]. Barbed wire hair traps developed by Woods et al. [10] have become the standard for collecting genetic data to estimate bear population parameters trend in North America [11, 12]. However, the relative strengths and weaknesses of alternative NGS monitoring methods such as surveys using bear rubs (i.e. trees, power poles, etc) must be evaluated before one method becomes established.

In the past 15 years, there has been a dramatic increase in research on North American bear populations using NGS [12], but other than one study which used scat detection dogs with limited success [13], research has almost exclusively focused on hair traps as the single DNA collection method. Numerous studies throughout North America (e.g. [14-16]) have used barbed wire hair traps in a mark-recapture framework to estimate population abundance and density for grizzly and black bears. Research conducted in Glacier National Park, MT has shown that bear rubs detect many grizzly bears not detected in hair traps and the use of bear rubs significantly improves precision of grizzly bear abundance estimates compared to estimates with hair traps alone [14, 15, 17]. Stetz et al. [18] used simulations based on empirical data from Glacier to demonstrate that bear rub surveys can produce precise estimates of grizzly population growth rate with just several years of sampling. However, there has yet to be a published study that has used bear rub surveys to directly estimate abundance or λ .

Precise and unbiased estimates of carnivore population abundance and λ are especially important to adaptive management in areas with low densities and limited reproductive capacity such as Banff National Park (BNP) in Alberta, Canada which is home to one of lowest density and slowest reproducing populations of grizzly bears in North America [19]. Differences in density and population growth rates could greatly affect the performance of population monitoring methods; therefore sampling methods must be evaluated in different geographic areas in order to gain a comprehensive understanding of their ability to monitor wildlife populations under variable environmental and demographic conditions. BNP provides a unique contrast to the Northern Continental Divide Ecosystem in MT, the central area of focus for past research using bear rubs [15].

Population monitoring is particularly important in national parks such as BNP because protected areas often serve as source populations for much larger geographic areas [20]. BNP along with Yoho and Kootenay National Parks comprise the UNESCO Rocky Mountain World Heritage Site and make up the core of the Central Rockies Mountains. With over 4 million visitors per year, however, BNP is also one of the world's most heavily visited national parks and this high level of human visitation acts as a major stressor on the ecosystem [21]. BNP has identified grizzly bears as one of the park's indicators of biological integrity [22], but despite the protections afforded by the national park system and provincial parks in the region, much of the habitat within the ecosystem, including BNP, is insecure for grizzly bears [23]. Studies have shown that human-caused mortality from highways and railways significantly affects grizzly and

black bear survival in the Bow Valley of BNP [19, 24]. In June 2010, the Alberta Provincial government listed the grizzly bear as “threatened” under the Species At Risk Act. This move increased the prominence of BNP for bear populations in Alberta and it added urgency to bear managers’ need for efficient and affordable NGS methods.

To successfully manage sympatric grizzly and black bear populations in the face of increasing human pressures, wildlife managers should understand the specific performance of hair traps and bear rubs to detect individuals and estimate demographic parameters for both species. In many areas of North America, grizzly and black bear diets completely overlap which may lead to direct competition between species [25]. Intraspecific competition could strongly influence the effectiveness of different survey methods, yet few studies (i.e. [26]) have compared NGS results from both species. NGS studies conducted in areas with sympatric populations readily collect hair from both grizzly and black bears and can produce demographic estimates for both species even though only one species, grizzly, is typically targeted. However, black bear hair is rarely analyzed in conjunction with grizzly bear hair due to financial constraints and less conservation concern for black bears in western North America [26].

The goal of this successful investigation was to evaluate the relative ability of two NGS methods, hair traps and bear rubs, to detect individuals and estimate population parameters for sympatric grizzly and black bear populations in the Bow Valley of BNP. Our specific objectives were to 1) estimate the abundance of grizzly and black bears in the Bow Valley of BNP using hair traps and bear rubs, 2) compare detection rates of hair traps and bear rubs for grizzly and black bears, and 3) evaluate the potential for using hair

traps and bear rubs for long-term monitoring of grizzly and black bear population growth rates. This is the first study to directly estimate abundance and population growth rates using bear rub surveys.

Study Area

The flagship of Canada's extensive national park system, Banff National Park (6848 km²) in the Central Rocky Mountains of Alberta was established in 1885 making it the third national park created in the world. Our 2246 km² Bow Valley study area (BVSA) was located approximately 120 km west of Calgary, Alberta, east of the Continental Divide in the central Rocky Mountains (Figure 1). The study area was mostly contained within BNP, but also extended slightly into Kootenay National Park and Alberta Provincial lands. The lower Bow Valley is a human-dominated landscape with the Trans-Canada Highway, the Town of Banff with 8000 residents, a golf course, 3 ski areas, a railway, and a secondary highway. Between 1980 and 1998, 45 km of the Trans-Canada Highway were widened for safety reasons [27] and 25 wildlife crossing structures were constructed to reduce wildlife–vehicle collisions and facilitate wildlife movement across the roadway [28].

We initially defined our study area (Figure 1) as a 14 km buffer around the 45 km length of mitigated highway in BNP in order to assess the performance of wildlife crossing structures for grizzly and black bears in the Bow Valley [29]. We overlaid a 7 x 7 km grid, two cells deep, across our study area, for a total of 42 cells. Studies using hair traps for grizzly bear population estimates often employ a 7 x 7 km grid [30] to distribute

effort across the region of interest. The orientation of our grid was chosen to be continuous with a larger grid used to inventory grizzly bears in Alberta [31]. We modified the study area boundary slightly to the north and south of the sampling grid to account for geographic features and minimize geographic closure violation.

Detailed ecological descriptions of the study area can be found in Holroyd & Van Tighem [32] and Holland & Coen [33]. The lower slopes on both sides of the BVSA were predominated by lodgepole pine (*Pinus contorta*) forests on glacial till terraces while the dry open south facing slopes were dominated by Douglas fir (*Pseudotsuga menziesii*). Fluvial bottomlands were a mix of mature white spruce (*Picea glauca*), dry, open spruce forest, and moist shrubland [34]. Important foods for grizzly and black bears are bearberry (*Arctostaphylos uva-ursi*), horsetails (*Equisetum arvense*), graminoids, and buffaloberry (*Shepherdia canadensis*) [35, 36].

Methods

Ethics Statement

Our research did not involve capture or handling of animals, therefore did not require approval of animal care and use procedures. Permissions for field studies in Banff National Park were given by Parks Canada Agency under research permit #BAN-2007-999. Permissions for field studies in Alberta provincial lands were given by the Alberta Minister of Community Development under research permit #RC-06-22.

Sampling

We used two methods, hair traps and bear rubs, concurrently to collect hair from black

and grizzly bears for genetic analysis. We surveyed bear rubs in 2006, 2007, and 2008. We deployed hair traps in 2006 and 2008, but chose not to set and maintain any hair traps during our second field season due to the high cost and effort associated with hair trapping and the greater potential with repeated sampling for bears to show a negative trap response over time due to a waning interest in the scent lure [17, 37].

We placed 1 hair trap in each grid cell for each of five, 14 day sampling periods from mid-May to mid-August in 2006 and 2008. Hair traps consisted of a 25 m length of barbed wire nailed at a height of 50 cm to a series of trees to form an enclosure [10]. We lured bears into the enclosure by placing 3 L of liquid scent lure poured on rotten wood and other debris piled in the center of the enclosure [15]. The scent lure consisted of a 2:1 mix of aged cattle blood and decomposed fish oil. We moved traps each session to minimize behavioral response to the lure [30]. For sessions 1-4 in 2006, we also dragged a burlap sack splashed with lure towards game trails in order to increase the attractiveness of the hair trap. For session 5 in 2006 and sessions 1-5 in 2008, we hung a small cloth doused in lure 4-5 m above the center of the trap in order to increase scent dispersion [15]. During session 5 in 2008 we also placed a splash of anise oil on trees within each trap site to attract bears and elicit a rubbing response. We chose hair trap locations before the field season using criteria based on Geographic Information System layers and expert opinion. We selected hair trap locations that were ≥ 1 km apart and in proximity to seasonal food sources or natural travel corridors. We located each trap ≥ 100 m from maintained trails, and ≥ 500 m from heavy human use areas such as campsites. We collected hair samples from each barb separately and also collected discrete clumps of

hair below the wire and from the lure pile. We placed samples in paper envelopes labeled with a uniquely numbered barcode and burned every barb after collection to prevent contamination between sessions [15].

We conducted bear rub surveys throughout the study area from mid-August to mid-October 2006, and mid-May to mid-October 2007 and 2008. We focused our efforts on maintained or recently unmaintained trails (<20 years) in the BVSA (Figure 1). We identified 321 bear rubs based on characteristics such as smoothed bark, claw marks, and bear paths [14]. We nailed short pieces of barbed wire to the tree in a zigzag pattern, placed unique numbered ID tags at the back of the base of the tree, and recorded locations with a GPS. We conducted initial surveys on all trails to inspect bear rubs and remove any hair. We then systematically surveyed all trails within the BVSA ≥ 2 times. We collected hair samples from each barb and each wire end separately; we also collected discrete clumps of fresh (i.e. not brittle or bleached) hair from the bark of the tree.

We collected hair from 20 of 25 wildlife crossing structures along the TCH by stretching two lengths of barbed wire perpendicular to the line of movement in order to snag hair from passing bears [38]. Finally, we collected hair opportunistically from various bear management actions, such as during radio collaring efforts and necropsies.

Genetic Analysis

We stored hair samples at room temperature on silica desiccant. Samples were analyzed at Wildlife Genetics International (Nelson, British Columbia), a lab that specializes in analysis of noninvasive genetic samples. The lab used protocols for DNA extraction and

microsatellite analysis of samples detailed in Paetkau [39] and validated in Kendall et al.[15]. All DNA extraction was performed using QIAGEN's DNeasy Tissue Kit (Qiagen, Valencia, CA). We extracted DNA from all samples with ≥ 1 guard hair follicle or 5 underfur hairs, using up to 10 guard hairs with underfur when available. We used seven microsatellite loci developed by Paetkau et al. [40] that have become standard for individual identification of grizzly bears in the Rocky Mountains: G10J, G1A, G10B, G1D, G10H, G10M, and G10P.

Mark-recapture estimates can be biased by low power to distinguish individuals (i.e. shadow effect) and genotyping errors such as allelic dropout and false alleles that result in the creation of false individuals [41-43]. We therefore used variable markers and genotyping error and removal procedures developed by Paetkau [39]. Because we were interested in both species, we chose a procedure that allowed us to obtain black bear results in addition to grizzly without adding any substantial costs. For most bear projects in western North America, samples that were extracted are first analyzed at the G10J locus, which has a high amplification success rate and is diagnostic between grizzly and black bears in the region [15]. Instead, we attempted all 7 microsatellite loci for all hair samples for which we were able to extract DNA. We excluded samples from further analysis that produced high confidence results for < 3 markers. For the samples that produced 3-5 locus genotypes on the first pass, we re-analyzed the markers, but excluded samples from further analysis if they failed on the second pass. Once we had complete 7-locus genotypes, we began the error-checking and removal process by identifying pairs of genotypes that mismatched by one or two loci, a potential warning sign that a genotyping

error occurred [39, 44]. We scrutinized the results from any pairs of samples that differed by one or two loci and re-analyzed loci until we had consensus genotypes. Once we had complete multi-locus genotypes, we analyzed 1-5 samples from each individual for gender using amelogenin [45, 46]. We used GENALEX [47] to calculate marker power. We calculated expected allele frequencies, expected heterozygosity (H_e), observed heterozygosity (H_o), the probability of identity (P_{ID}) that two randomly drawn individuals would share the same multi-locus genotype and the probability that full siblings would have identical multi-locus genotypes (P_{SIB}).

We analyzed all hair samples with adequate genetic material in 2006, but due to budget constraints, we subsampled bear rub samples in 2007 and both bear rub and hair trap samples in 2008. In 2007 and 2008 we genotyped one sample from each bear rub per sampling event (~50% of samples). For hair traps in 2008, we tried to fit the subselection rules to the budget, with a goal of extracting 50% of the samples.

Population Abundance

We used data from our three sampling methods to estimate superpopulation abundance of grizzly and black bears in the BVSA during 2006 and 2008. We used hair samples collected from multiple methods (i.e. hair traps, bear rubs, and wildlife crossing structures) to increase sampling coverage and improve precision of abundance estimates [17]. We compiled individual bear encounter histories for each species for each year and analyzed them using Huggins [48] closed population models in program MARK [49, 50].

Our candidate models included gender as a group covariate and hair trap effort (HTE), bear rub effort (BRE), crossing structure effort (CSE), and distance to edge

(DTE) as individual-level covariates to model capture probability heterogeneity [37]. We created minimum convex polygons (MCP) for each individual using all available location data including data points not used in our abundance estimates from 176 bear rubs outside the study area. We used centroids of MCPs to approximate bear detection centers for 45% of black bears and 78% of grizzly bears, but when <3 points were available; we calculated the average of 2 points or used 1 point. We calculated DTE as the distance from the edge of the grid to each individual's representative home range center. We calculated the gender-specific mean maximum distance moved (MMDM) by taking the average of the furthest distance between any 2 points for each individual (black bear F=8.2 km, M=13.5 km; grizzly bear F=14.3 km, M=23.1 km). We then buffered each individual's detection center by the MMDM and $\frac{1}{2}$ MMDM. We calculated method-specific sampling effort covariates by summing the number of hair traps (HTE), bear rub days (BRE), and crossing structures (CSE) that were located within each bear's idealized home range for each session. We used Akaike Information Criteria for small samples sizes (AICc) to evaluate relative support for candidate models. We used model averaged estimates of derived parameters to account for model selection uncertainty [51].

We explored whether we could use hair trap-only or bear rub-only data to produce CMR estimates of abundance by comparing them to our models using the combined data set using hair traps, bear rubs, and other methods. We compared abundance estimates for grizzly bears from models using bear rub-only, hair trap-only and combined data. We compared hair trap-only abundance estimates for black bears with estimates from models using the combined data set.

Population Trend

We used Pradel [52] open population models to estimate realized population growth rates (λ) and apparent survival (ϕ) for grizzly bears in the BVSA. We compiled encounter histories using bear rub data collected in 2006, 2007 and 2008. We analyzed encounter histories using robust design models in program MARK by treating years as primary occasions and sampling sessions as secondary occasions [53]. The robust design uses Huggins [48] closed capture models within an open model to estimate abundance for each year as well as annual population growth rates across primary time intervals [53]. We used gender as a group and BRE and DTE as individual covariates. We assessed the fit of our models with the bootstrap goodness-of-fit test in MARK.

Results

Sampling

We established hair traps from 25 May - 9 August 2006 and 28 May - August 2008. During five 14-day sessions ($\bar{x}$ = 13.8 days, SD = 1.0 in 2006, $\bar{x}$ = 14.0 days, SD = 0.7 in 2008), we collected 884 hair samples from 47% of 210 traps in 2006 and 1125 samples from 53% of 210 traps in 2008 (Table 1; Figure 1). We surveyed bear rubs from 10 September - 21 October 2006, 3 June - 27 October 2007, and 28 May - 27 October 2008. We monitored 284 bear rubs in 2006 and 321 bear rubs in 2007 and 313 in 2008.

We collected 2910 hair samples from 38.4.0% of rubs in 2006, 59.5% of rubs in 2007, and 556.2% of rubs in 2008 (Table 2). We collected hair from 78% of all bear rubs monitored (n=321) over the 3 year period (Figure 1). We obtained DNA samples (tissue

or hair) from bear management actions and wildlife crossings in the BVSA between 15 May – 18 October 2006, 22 April – 29 October 2007, and 22 April–18 October 2008.

We collected 348 samples in 2006, 416 in 2007, and 553 in 2008.

Genetic Analysis

We calculated marker power using data from 80 grizzly bears and 85 black bears identified from hair and tissue samples collected in BNP, including individuals detected outside the study area. Marker power was high for grizzly and black bears using the same seven loci for individual identification (Table S1). Mean H_o across loci was higher for black bears (0.85) than for grizzly bears (0.77). The mean number of alleles per locus was also higher for black bears (10.3) than for grizzlies (7.3). $P_{(ID)}$ was 2.3×10^{-8} for grizzly bears and 1×10^{-9} for black bears. $P_{(SIB)}$ was 0.0013 for grizzly bears and 0.0007 for black bears (Table S1). We assumed our final multilocus dataset was error free based on our mismatch distributions and lack of any 1 of 2 locus mismatches. As part of another study which is not reported here for the sake of brevity, we ran 13 additional microsatellite loci on each individual and these additional loci concurred with our individual identifications from our original 7 locus genotypes providing further evidence of an error free dataset.

Of the 6236 samples we collected over the 3 year study, we extracted DNA and attempted genetic analysis on 76.0% ($n=4741$). When we analyzed all of the bear rub samples in 2006, we found that we detected >1 individual on a bear rub during a sampling period <5% of the time. Based on these results, in 2007 and 2008, we genotyped 1 sample from each bear rub per sampling event (~50% of samples). For hair

traps in 2008, we extracted 51.0% of 1125 samples. We obtained individual identities (ID) for 46.4% of all hair trap samples (n=2009; 51.6% in 2006 and 42.2% in 2008). Of the 2910 bear rub samples, we obtained IDs for 26.8% (n=779, 62.5% in 2006, 23.0% in 2007, and 19.0% in 2008). Of the 932 samples that produced multi-locus genotypes from hair traps, 43.6% came from black bears (n=400) and 56.4% came from grizzlies (n=517). Of the 779 bear rub samples that produced multi-locus genotypes, 94.5% came from grizzly bears (n=736) and 5.5% came from black bears (n=43).

We identified a total of 80 unique grizzly bears and 85 black bears from multi-locus genotypes across all methods and years. Genetic analysis of samples collected at hair traps identified 31 grizzly bears and 40 black bears in 2006 and 29 grizzlies and 57 black bears in 2008 (Table 1). From bear rub samples, we identified 40 grizzlies and 2 black bears in 2006, 46 grizzlies and 11 black bears in 2007 and 43 grizzlies and 6 black bears in 2008 (Table 2). During the two years that we conducted hair trap and bear rub sampling simultaneously, we identified the majority of individual grizzly bears with bear rubs trees, 75.5% in 2006 and 91.5% in 2008, and the majority of black bear individuals with hair traps, 100% in 2006 and 96.6% in 2008 (Table 3). We identified 58% of grizzly bears at hair traps in 2006 and 61.7 in 2008. Only 5% of black bears were detected with bear rubs in 2006 and only 10.2% in 2008. We identified 11 grizzlies (6 M, 5 F) at wildlife crossings in 2006, 12 (6 M, 6F) in 2007, and 10 (5 M, 5F) in 2008. We also identified 11 black bears (7 M, 4 F) in 2006, 8 (4 M, 4F) in 2007, and 9 (4 M, 5, F) in 2008. Of the bears detected at wildlife crossings, we identified some bears that were undetected with hair traps or bear rubs, including: 4 grizzlies (1 M, 3 F) in 2006, 3 (1 M,

2 F) in 2007 and 1 (1 M, 0 F) in 2008, and 3 black bears (3 M, 0 F) in 2006, 7 (3 M, 4 F) in 2007, and 4 (2 M, 2 F) in 2008.

Population Abundance

Our best supported models for grizzly bear abundance varied by sex and time in both years and contained covariates for DTE, CSE and BRE in 2006 and DTE, CSE, BRE, and HTE in 2008 (Table S2). We estimated grizzly bear abundance in the BVSA to be 73.5 (95% CI=64-94) in 2006 and 50.4 (95% CI=49-59) in 2008 (Table 4). Our estimated per-session capture probabilities for males were higher than females at bear rubs, but lower at hair traps (Figure 2A,C). High detection rates of grizzly bears at bear rubs allowed us to produce precise mark-recapture estimates of abundance using bear rub data alone.

Abundance estimates for grizzly bears derived from bear rub-only models were more similar to models using combined data from rub trees, hair traps, and other methods than were hair trap-only estimates (Figure 3A).

Our best supported models for black bears varied by sex and time, and contained covariates for DTE and HTE in 2006 and 2008 (Table S3). We estimated black bear abundance at 62.6 (95% CI=51-89) in 2006 and 81.8 (95% CI=72-102) in 2008 (Table 5). Our estimated capture probabilities for female black bears were higher than males at hair traps (Figure 2B), but lower at bear rubs. Per-session model averaged capture probability estimates for black bears at bear rubs were extremely low so we collapsed sessions into one per year ($\hat{p}=0.02$, SE=0.02 for males and females in 2006; $\hat{p}=0.07$, SE=0.03 for males and females in 2008) in our joint dataset models. Estimates for black bears derived from hair trap-only models were comparable to models using combined data from bear

rub trees, hair traps, and other methods (Figure 3B).

Population Trend

The most supported models for λ and ϕ varied by sex and contained individual covariates for DTE and BRE (Table S4). Time varying models for λ were not considered due to grizzly bear life history and the short study duration. Time-invariant models were constrained to produce a single λ estimate for males and females between the time intervals 2006-2007 and 2007-2008, therefore we only present one lambda estimate for each sex. Our model averaged λ estimates for grizzly bears with three years of bear rub data in the BVSA were 0.94 for males and 0.89 for females (Table 6) and both confidence intervals for males and females overlapped 1.0 (CI=0.75-1.19 and 0.67-1.20, respectively). Per session model averaged capture probability estimates from the Pradel [52] model were generally lower for females than for males, except during sessions 2 and 5 in 2007, and 4, 5 and 6 in 2008 (Figure S1). We derived precise abundance estimates (CV<20%, [54]) from robust design models for 2006, 2007, and 2008 (Figure 4). Results of the bootstrap goodness-of-fit test suggested no lack of fit for the general model to our data (200 simulations; P>0.4). We therefore assumed no overdispersion in our data.

Discussion

Estimating demographic parameters for carnivore populations in protected areas such as BNP is essential for effective management and conservation. We conducted the first detailed evaluation of hair traps and bear rubs for long-term monitoring of the sympatric grizzly and black bear populations in BNP. Although studies have reported abundance

measures of grizzly and black bears in the Bow Valley of BNP [34,55,56], we produced the first rigorous abundance estimates with confidence intervals for grizzly and black bear populations based on DNA-based mark-recapture methods. We demonstrated that bear rubs have higher detection rates than hair traps for grizzly bears and low detection rates for black bears in BNP. We also provided empirical evidence ($\lambda < 1$) that the grizzly bear population may be declining in the Bow Valley.

Sampling

Detection rates from the two NGS methods were drastically different for grizzly and black bears in the BVSA (Table 3). Hair traps had high detection rates for grizzly bear females as well as both male and female black bears, but extremely low capture probabilities for male grizzlies (Figure 2A, B). Bear rubs detected many grizzly bears that were not detected at hair traps, but detected very few black bears (Figure 2C).

Competition has been well-documented between grizzly and black bears in North America and is the result of almost complete dietary overlap in some regions [25]. Black bears may avoid rub trees in BNP due to interspecific competition or because rubbing behavior is less advantageous to them compared to grizzlies in the region for some other ecological reason (e.g., fewer ecto-parasites). Limited food resources in BNP resulting in some of the lowest grizzly bear densities in North America [19] could explain the relatively high detection rates of grizzlies on rub trees if rubbing behavior is density dependent. Where grizzly bear density is low and home ranges are large such as in BNP, individual bears could be more detectable with bear rub surveys than in areas with much

higher grizzly bear densities and smaller home ranges such as Glacier National Park, MT, USA [14, 15].

Genetic Analysis

Well-thought sub-sampling can greatly reduce analysis costs while maintaining data integrity by selectively targeting the best samples for extraction and thus the most likely samples to produce individual identities. When we analyzed all of the bear rub samples in 2006, we found that we detected >1 individual on a rub tree during a sampling period <5% of the time so in 2007 and 2008 we subsampled, greatly reducing the number of duplicate samples from the same individuals in time and space while likely missing only a few individuals. For hair traps in 2008, we came very close to our subsampling criteria (50.0%) with an extraction rate of 51.0%.

Genetic analysis was facilitated and expedited by simultaneously analyzing grizzly and black bear samples to obtain individual IDs. The seven microsatellite markers we used to determine individual IDs had high power to distinguish individuals for both grizzly and black bears (Table S1). The proportional cost of obtaining black bear data in addition to grizzly bear data is minor with our analysis approach, especially considering that all time, labor, and equipment costs associated with field collection of hair samples will be paid for (along with DNA extraction) regardless of whether or not individual ID analysis for black bears is performed.

Population Abundance

Combining bear rubs and hair traps with other DNA sampling methods allowed us to produce precise abundance estimates for grizzly bears (Table 4, CVs<16%) in a relatively

small study area with little geographic closure. Our issues with geographic closure were most likely minimized due to our high sampling intensity, and the combination of NGS methods providing greater sampling coverage [17, 57]. Our use of DTE and other individual covariates allowed us to model individual capture heterogeneity, resulting in robust estimates of superpopulation abundance [37, 58]. Because our objectives were not to produce density estimates or to correct population estimates for closure, we chose to present superpopulation size estimates to allow for more direct comparisons between sampling methods. Not surprisingly, our estimates of grizzly bear density based on $\hat{D} = \hat{N} / \hat{A}$ were similar to density estimates based on radio-telemetry data (1.2-1.6 bears/100 km²; [55]), but meaningful comparisons are difficult due to differences in study area and methodology.

The higher precision of estimates produced for grizzlies in 2008 compared to 2006 (Table 4) can most likely be explained by a longer bear rub survey season with more sampling sessions coupled with higher capture probabilities at hair traps (Figure 2A). We believe that capture probabilities were higher for grizzly bears at hair traps in 2008 than 2006 due to our greater knowledge of the area based on hair trap success in the first year and a more adaptive approach to site selection. In addition to the measures we described above to increase capture probabilities, during session 5 in 2008 we also used anise oil to further entice bears to enter hair traps. We detected noticeably more female grizzlies in Session 5 than in any other session in 2006 or 2008 (Table 1), which leads us to conclude the anise oil may have been an attractant to female grizzly bears and initiated

a rubbing response. As suggested by Stetz et al. [18], liquid scent lures used with bear rub surveys could increase capture probabilities and their use should be explored.

Our efforts to maximize detection rates appear to have been successful, but it is remarkable that we achieved such high capture probabilities for female grizzly bears in 2008 and that they were so much higher than in 2006 (Figure 2A). Kendall et al. [15] showed that subadults and dependent offspring are detected with hair traps and bear rubs. In an area like BNP, where the age to independence is 2.5-5.5 years [19] and the seasonal food availability so variable, it is possible that there were more dependent juveniles traveling with their mothers in 2006 than in 2008. Changes in the proportion of females with dependent offspring and/or annual fluctuations in food availability could explain variation in detection probabilities such as those seen in our study.

Capture probabilities for male grizzlies at hair traps were very low relative to females in 2006 and 2008 (Figure 2a). Other studies have documented higher capture probabilities for females than males at hair traps, but this discrepancy is surprising because research has shown that males typically encounter more hair traps than females [37]. Hair trap sampling was conducted in large grids to the north and south of the BVSA in 2005 and 2006, respectively [31], so it is plausible that a high proportion of male grizzly bears had already encountered hair traps (or been captured) in previous years and were therefore less likely to enter the enclosure to investigate the lure [37, 59].

Per session bear rub capture probabilities of grizzly bears were lower for females than males early in the year, but became higher than males as the season progressed (Figure 2C). The same pattern of increasing female capture probabilities has been

documented by Kendall et al. [14, 15], but they documented that male capture probabilities also increased over time. Surprisingly, we were able to obtain abundance estimates from bear rub data alone that were comparable in magnitude and precision to our best estimates using the combined hair methods data set (Figure 3A). We compared hair trap-only estimates with bear rub-only estimates because bear population estimates based on hair traps alone have become the convention in much of North America [11, 12]. In general our estimates using bear rub-only data were more accurate and precise than hair trap-only estimates as judged by comparing them to the estimates from the combined data set. The single bear rub-only estimate which was imprecise ($CV > 20\%$) was for females in 2006. Based on the capture probabilities we achieved with bear rubs in 2006, we attribute the imprecision of the 2006 estimate for females to a late sampling season and only two sampling sessions as compared to the subsequent years. Our hair trap-only estimates were imprecise for males, especially in 2006 due to extremely low capture probabilities ($\hat{p} < 10\%$). The results from our comparison of estimates using different data types support the findings of other researchers that combining sampling methods increases precision [14, 15, 17, 60]. Our results also suggest that bear rub-only abundance estimates can be comparable to those obtained using both hair traps and bear rubs combined in some populations.

Even though our grid cell size was tailored to the average home range size of grizzly bear females [30], we were able to obtain precise estimates ($CV < 20\%$) for black bears in both 2006 and 2008. Bear rubs did not add significantly to the number of black bears we detected in 2006 or 2008 (Table 3). As bear rub capture probabilities were so

low for black bears, we attribute the precision of our estimates to the relatively high capture probabilities at hair traps (Figure 2B). Per session capture probabilities at hair traps were higher for females than for males in both years. It appears that there is a decline in capture probabilities through time for both years, but a distinct increase for both males and females in session 5 of 2006. This anomaly could be attributed to our use of the hanging lure-soaked rag as session 5 was the only session where it was used in 2006 and the pattern holds true for male and female black bears during this last session. Combining bear rubs and hair traps did not improve our precision much over estimates for black bears using hair trap-only datasets (Figure 3B). Our hair trap-only point estimates and measures of precision are comparable to our estimates using a combined dataset, suggesting that wildlife managers interested in monitoring black bears in BNP should consider the use of hair traps, rather than bear rub surveys since the latter provides little additional precision while increasing labor and costs.

Population Trend

Using a Pradel [52] open population model with 3 years of bear rub data, we were able to obtain precise λ estimates for male and female grizzly bears in the BVSA (Table 6). Our estimates of lambda ($\lambda = 0.94$, $SE = 0.11$ for males and $\lambda = 0.89$, $SE = 0.13$ for females) and our decreasing annual abundance estimates suggest that the grizzly bear population was declining in the BVSA between 2006 and 2008.

We recognize that inferences from our results can only be taken so far with such a small study area and limited time frame. For example, our lambda estimates could be driven solely by annual fluctuations in food availability or patterns of reproduction.

McCall [61] found that black bear population dynamics in northern Idaho were driven by changes in berry productivity. Similarly, extensive movements of grizzly bears into the Bow Valley in years of good buffaloberry production have been documented [55].

Although annual fluctuations in berry production could influence our results, an examination of berry productivity data collected in BNP between 2006-2010 [62] shows no correlation between good berry years and the number of bears detected in the BVSA. Regardless of the limitations to identifying drivers for lambda estimates, an undeniable downward population trend exists in bear rub-only abundance estimates for male and female grizzlies across the 3 years of our study (Figure 4). This trend was also apparent in the abundance estimates for 2006 and 2008 from Huggins closed models (Table 4).

Although our objective of estimating lambda using open models was to examine the feasibility of using bear rub data for long-term grizzly bear population monitoring in BNP, we were able to obtain empirical evidence suggesting that the grizzly bear population was declining in the Bow Valley during our study. Garshelis et al. [19] determined that the grizzly bear population in BNP was slightly increasing while also being the slowest reproducing grizzly bear population yet studied at the time of their research. They estimated lambda for grizzly bears ($\lambda=1.04$, 95% CI=0.99-1.09) using Leslie matrices, but concluded that if even 1 more radiomarked female on average per year had been killed during their study that the population would have declined. The decline that we detected was not surprising as adult mortality remains high for both black and grizzly bears in BNP [63, 64] and studies have shown that grizzly bear population growth rates are most sensitive to changes in adult female mortality [55, 65]. Our lambda

estimates show concordance with previous research suggesting that the Bow Valley may act as an attractive sink for grizzly bears in the Central Rocky Mountains [66-68], but these findings should be confirmed with long-term monitoring across a much larger geographic area.

Our results suggest that bear rubs can provide an efficient, reliable method for long-term monitoring of grizzly bear populations the Central Rocky Mountains that may provide insights into drivers of population dynamics. Our results support previous research [18, 69] showing that DNA mark-recapture methods are able to precisely estimate grizzly bear population growth rates. We have shown that bear rubs surveys in BNP can be efficient through subsampling to reduce analysis costs and by partnering with existing Parks Canada staff and Citizen Scientist volunteer program to reduce labor costs. Bear rub surveys also require no lure, therefore have no trap response and far less safety concerns associated with them compared to hair traps. These characteristics make bear rub surveys particularly well-suited for long-term grizzly bear monitoring programs in human-dominated landscapes such as BNP. We recommend that the spatial and temporal scale of grizzly bear monitoring be extended as much as possible to avoid issues of closure and to be able to link changes in demographics to ultimate drivers of population growth in the region [58]. Use of abundance data to determine extinction risk for wildlife populations often fails to detect initial declines or is prone to high rates of error [70]. However, our results demonstrate that noninvasive genetic sampling can produce precise estimates of population abundance and trend, therefore reducing some of the uncertainty when classifying extinction risk for carnivore species.

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Figure Legends

Figure 1. Noninvasive genetic sampling locations for grizzly and black bears in Banff National Park, Alberta, Canada. Locations of 420 hair traps, 321 bear rubs and 20 wildlife crossings, monitored in the Bow Valley Study Area (BVSA) between 21 April and 31 October of 2006-2008.

Figure 2: Gender-specific per session grizzly and black bear capture probability estimates. Capture probabilities from (A) grizzly bears at hair traps, (B) black bears at hair traps, and (C) grizzly bears at bear rubs in the Bow Valley Study Area of Banff National Park. We derived model-averaged estimates from population models for grizzly bears (Table S2) and black bears (Table S3). Error bars represent model averaged estimates of Standard Error.

Figure 3: Comparison of Huggins closed population models of abundance. Estimates for (A) grizzly bears and (B) black bears in the Bow Valley Study Area of Banff National Park using data from two noninvasive genetic sampling methods, hair traps and bear rubs. Models were created with different datasets, (1) hair trap-only, (2) combined bear rub and hair trap, and (3) bear rub-only. We constructed hair trap-only and bear rub-only models with the same structure and covariates (DTE, HTE, and BRE) as models using the combined sampling methods. The combined models included data from a third DNA sampling method (i.e. bear management actions, wildlife crossings). We did not create

bear rub-only models for black bears due to the low capture probability estimates of black bears at bear rubs. Error bars represent model averaged estimates of Standard Error.

Figure 4: Estimates of abundance for male and female grizzly bears derived from Pradel Robust design open population models. Estimates were obtained using three years of bear rub data collected in the Bow Valley Study Area of Banff National Park, Alberta, Canada, from 2006 - 2008. We derived model averaged estimates from most supported models in Table S4. Error bars represent model averaged estimates of Standard Error.

Tables

Table 1. Bear hair trap results from the Bow Valley Study Area in Banff National Park, Alberta, Canada; we conducted hair trapping 25 May – 16 August 2006 and 28 May – 18 August 2008 for 5, 14-day sessions^a per year.

Year	Session	Install dates	No. traps	% traps with ≥1 hair sample	<u>Hair samples/ trap^b</u>		Total no. hair samples	<u>Grizzly bears</u>				<u>Black bears</u>			
					Mean	SD		No. new		No. unique		No. new		No. unique	
								F	M	F	M	F	M	F	M
2006	1	25 May - 3 Jun	42	59.5	9.7	8.9	243	4	4	4	4	11	9	11	9
	2	7 - 17 Jun	42	52.4	8.0	7.6	177	3	2	4	4	5	0	6	4
	3	21 Jun - 1 Jul	43	37.2	9.1	6.9	146	6	2	9	2	2	1	4	2
	4	5 - 18 Jul	43	41.9	8.9	9.4	160	0	6	3	7	1	1	3	1
	5	19 Jul - 2 Aug	40	45.0	8.8	6.7	158	2	2	3	3	5	5	9	6
Total			210				884			15	16			24	16
Mean			42.0	47.2	8.9	7.9	176.8	3.0	3.2	4.6	4.0	4.8	3.2	6.6	4.4
2008	1	28 May - 6 Jun	42	45.2	6.3	5.6	120	3	3	3	3	10	5	10	5
	2	10 - 23 Jun	42	59.5	12.2	12.8	305	2	3	5	3	18	9	21	10
	3	24 Jun - 8 Jul	42	59.5	9.5	8.7	238	10	3	14	5	4	3	10	9
	4	8 Jul - 22 Jul	42	45.2	9.3	9.9	177	2	1	6	4	3	2	11	3
	5	22 Jul - 5 Aug	42	59.5	11.4	10.4	285	0	2	16	4	3	0	8	5
Total			210				1125			17	12			38	19
Mean			42.0	53.8	9.8	9.5	225.0	3.4	2.4	8.8	3.8	7.6	3.8	12.0	6.4

^a $\bar{x}$ = 13.8 days, SD = 1.0 in 2006 and $\bar{x}$ = 14.0 days, SD = 0.7 in 2007.

^b Of those hair traps that had ≥ 1 bear hair sample.

Table 2. Bear rub survey results from the Bow Valley Study Area in Banff National Park, Alberta, Canada; we conducted bear rub surveys from 10 Sept – 21 Oct 2006, 3 Jun – 27 Oct 2007 and 28 May – 27 Oct 2008.

Year	Session	Survey dates ^a	No. rubs ^b	No. hair samples/ rub/ session ^c				BRE ^d	Total no. hair samples	Grizzly bears				Black bears			
				No. rubs with ≥1 sample	Mean	SD	No. new			No. unique		No. new		No. unique			
									F	M	F	M	F	M	F	M	
2006	1	10 - 30 Sept	190	67	3.7	2.4	5938	249	12	20	12	20	0	1	0	1	
	2	1 - 21 Oct	276	51	3.1	2.1	7652	159	3	5	6	14	1	0	1	0	
	Total		284				13590	408			15	25			1	1	
	Mean		233.0	59.0	3.4	2.3	6795.0	204.0	7.5	12.5	9.0	17.0	0.5	0.5	0.5	0.5	
2007	1	3 - 23 Jun	190	76	3.5	2.5	3392	265	0	14	0	14	0	0	0	0	
	2	24 Jun - 14 Jul	238	82	3.1	2.0	4783	253	7	5	7	11	1	2	1	2	
	3	15 Jul - 4 Aug	281	67	3.4	2.5	6068	231	4	4	5	14	0	4	1	4	
	4	5 - 25 Aug	305	58	2.7	1.8	6479	154	2	3	4	14	1	0	1	0	
	5	26 Aug - 15 Sep	311	42	2.4	1.6	6710	102	2	2	7	7	0	2	0	2	
	6	16 Sep - 6 Oct	304	53	2.3	1.5	6248	122	1	1	5	11	0	1	0	2	
	7	7 - 27 Oct	244	31	3.0	2.3	5387	94	0	1	2	10	0	0	0	0	
	Total		321				39067	1221			16	30			2	9	
Mean		267.6	58.4	2.9	2.0	5581.0	174.4	2.3	4.3	4.3	11.6	0.3	1.3	0.4	1.4		
2008	1	28 May - 22 Jun	116	37	5.7	4.1	1676	212	0	7	0	7	0	1	0	1	
	2	23 Jun - 22 Jul	213	84	4.0	2.8	6718	333	4	7	4	13	2	0	2	1	
	3	23 Jul - 23 Aug	259	74	3.8	2.4	8181	282	4	4	6	13	0	0	1	1	
	4	24 Aug - 13 Sep	285	72	3.5	2.4	7186	249	6	6	11	14	2	1	2	2	
	5	14 Sep - 4 Oct	281	60	2.6	1.6	6125	156	4	0	10	6	0	0	2	2	
	6	5 - 27 Oct	221	20	2.5	1.5	4959	49	1	0	7	5	0	0	0	0	
	Total		313				34845	1281			19	24			4	2	
Mean		229.2	57.8	3.7	2.5	5807.5	213.5	3.2	4.0	6.3	9.7	0.7	0.3	1.2	1.2		

^a Bear rubs were surveyed every 14-21 days.

^b Bear rubs were surveyed multiple times per year, therefore the totals do not sum as each bear rub was only counted once per session or year.

^c Of those bear rub visits that had ≥1 bear hair sample.

^d Bear rub effort (BRE) is the cumulative no. of days between hair collections for each bear rub tree sampled/ session.

Table 3. Number and proportion of individual grizzly and black bears identified per noninvasive genetic sampling method during 2006 and 2008 in the Bow Valley Study Area of Banff National Park, Alberta, Canada.

Sampling Method	2006				2008			
	M		F		M		F	
	n	%	n	%	n	%	n	%
Grizzly bears								
Hair traps-only	6	19.4	7	31.8	1	4.0	3	13.6
Bear rubs-only	15	48.4	7	31.8	13	52.0	5	22.7
Both methods	10	32.3	8	36.4	11	44.0	14	63.6
Total	31		22		25		22	
Black bears								
Hair traps-only	15	93.8	23	95.8	18	90.0	35	89.7
Bear rubs-only	0	0.0	0	0.0	1	5.0	1	2.6
Both methods	1	6.3	1	4.2	1	5.0	3	7.7
Total	16		24		20		39	

* Total counts only include bears detected with hair traps or bear rubs, therefore they are <min counts which include bears detected with other methods.

Table 4. Total minimum counts and model-averaged estimates of grizzly bear abundance in the Bow Valley Study Area of Banff National Park, Alberta, Canada, in 2006 and 2008.

Parameter	Min. Count	Estimate	SE	CV (%)	95% CI	
					Lower	Upper
2006						
M	32	39.9	4.7	11.9	35	55
F	25	33.6	5.3	15.9	28	51
Pooled	57	73.5	7.2	9.7	64	94
2008						
M	26	28.1	2.1	7.6	26	37
F	22	22.3	0.6	2.7	22	26
Pooled	48	50.4	2.2	4.4	49	59

Table 5. Total minimum counts and model-averaged estimates of black bear abundance in the Bow Valley Study Area of Banff National Park, Alberta, Canada, in 2006 and 2008.

Parameter	Min. Count	Estimate	SE	CV (%)	95% CI	
					Lower	Upper
2006 ^a						
M	19	30.2	7.3	24.1	23	55
F	24	32.3	4.9	15.2	27	48
Pooled	43	62.6	9.0	14.4	51	89
2008						
M	22	28.3	4.1	14.5	24	42
F	41	53.5	5.7	10.7	46	70
Pooled	63	81.8	7.2	8.8	72	102

^a 13 black bear samples from 6 hair traps were not analyzed in 2006.

Table 6. Estimates of realized population growth rate, λ , and apparent survival, ϕ , from Pradel open population models for grizzly bears in the Bow Valley Study Area of Banff National Park, Alberta, Canada between 2006 and 2008, sampled using bear rub tree surveys.

Parameter	Estimate	SE	CV (%)	95% CI	
				Lower	Upper
λ					
M	0.94	0.11	11.8	0.75	1.19
F	0.89	0.13	14.9	0.67	1.20
ϕ					
M	0.69	0.07	10.7	0.53	0.81
F	0.76	0.09	11.8	0.55	0.89

* Our models did not vary with time therefore they produced identical lambda estimates for 2006-2007 and 2007-2008.

Figure 1

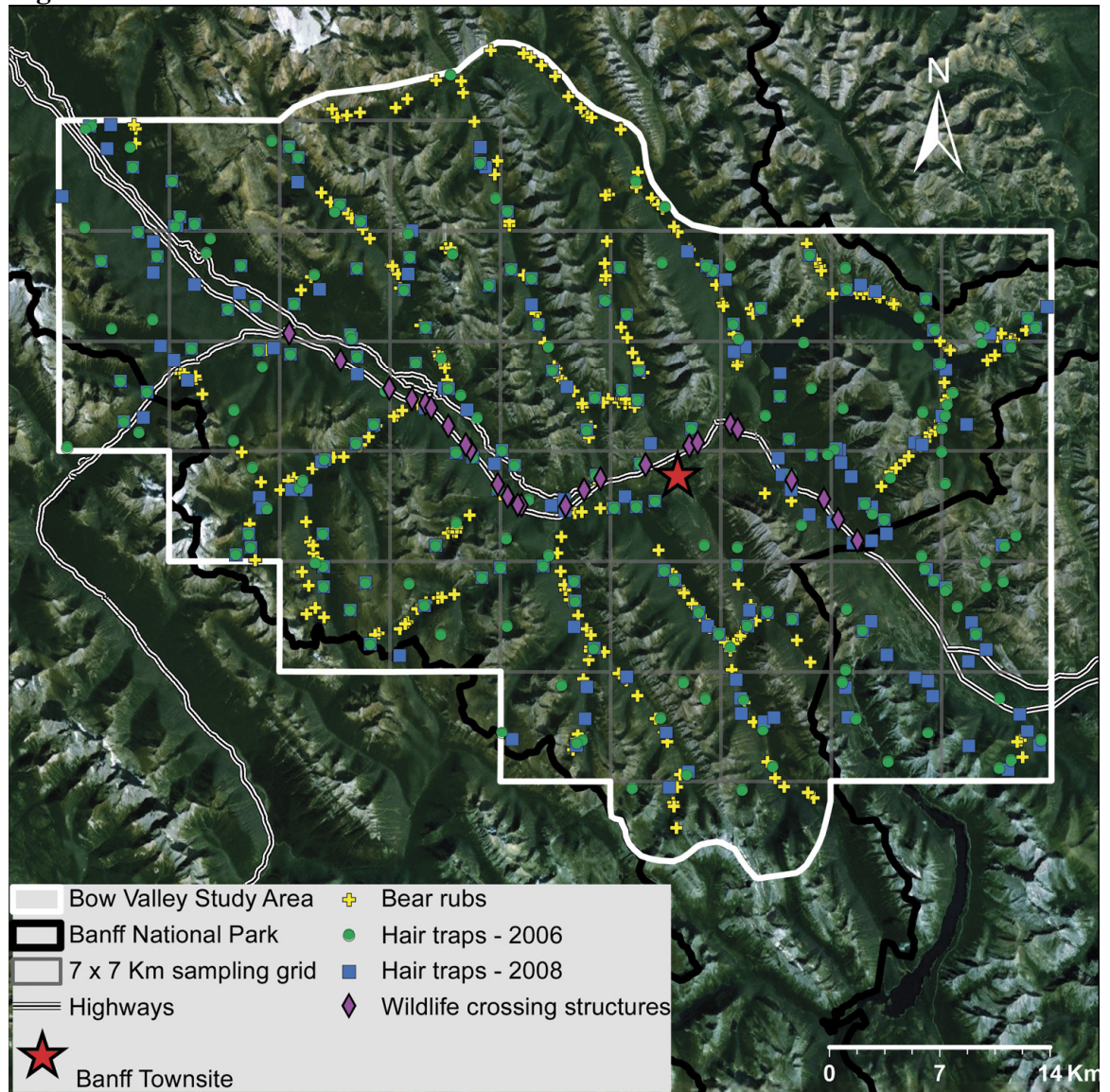


Figure 2

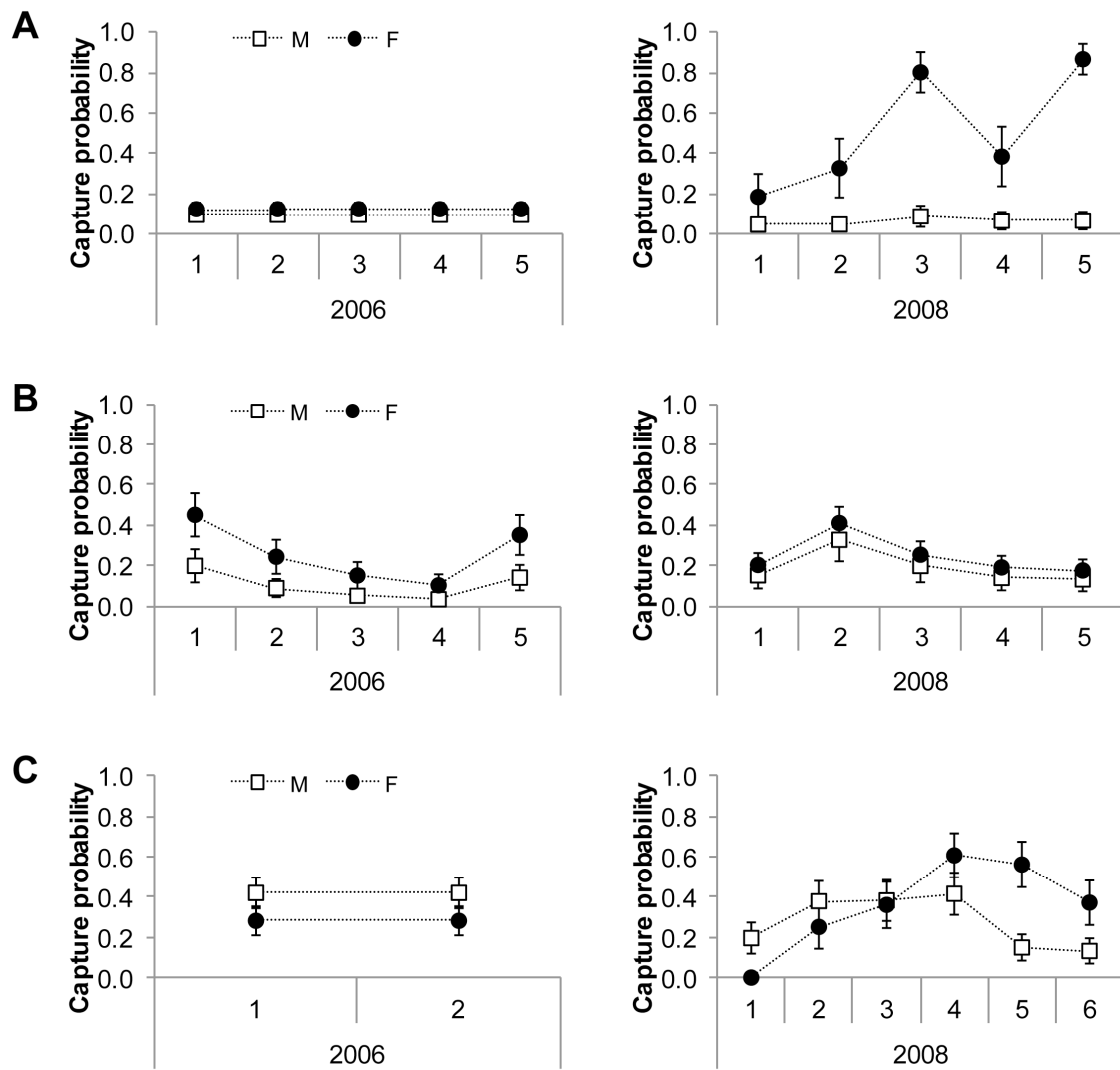


Figure 3

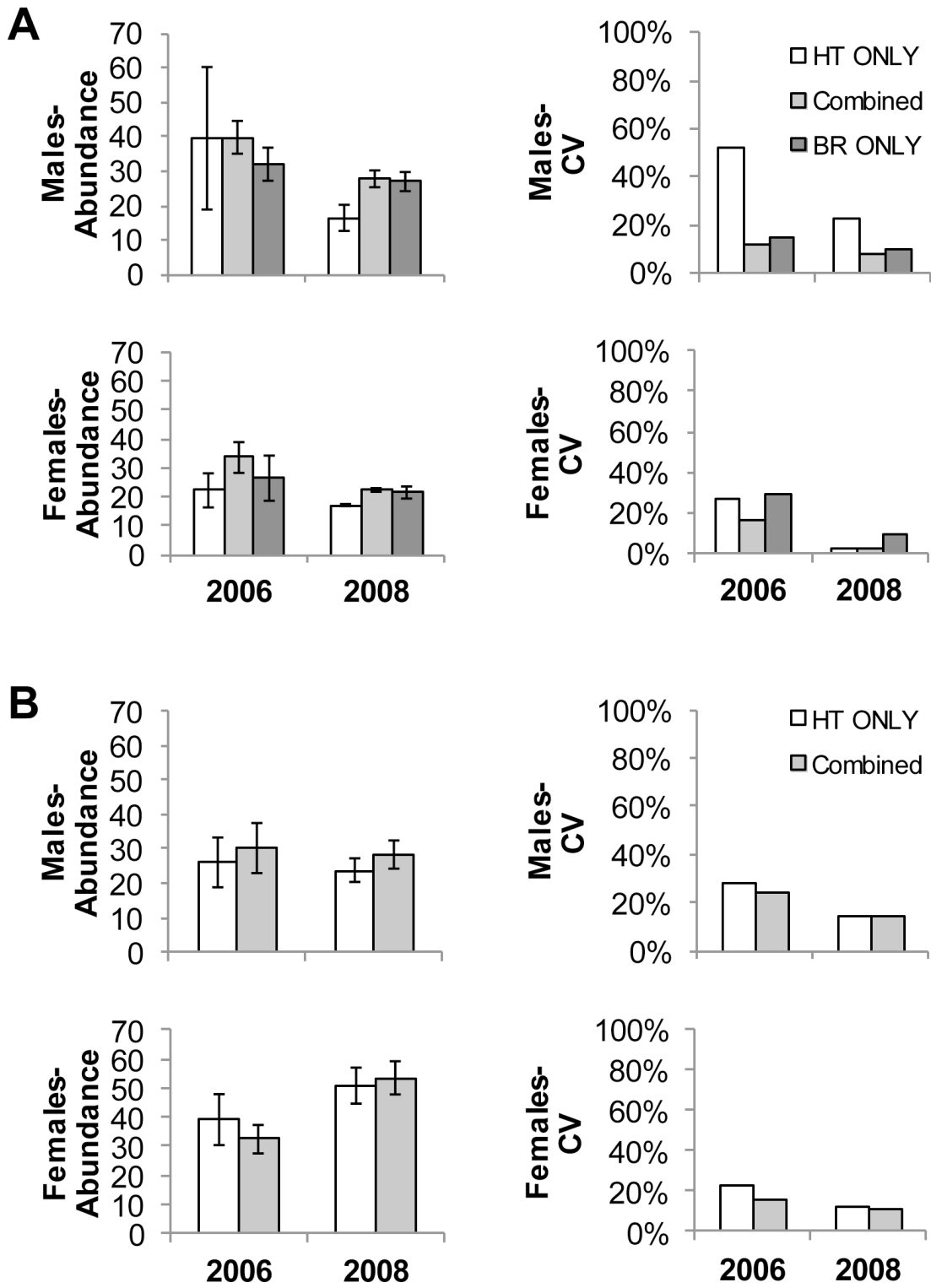
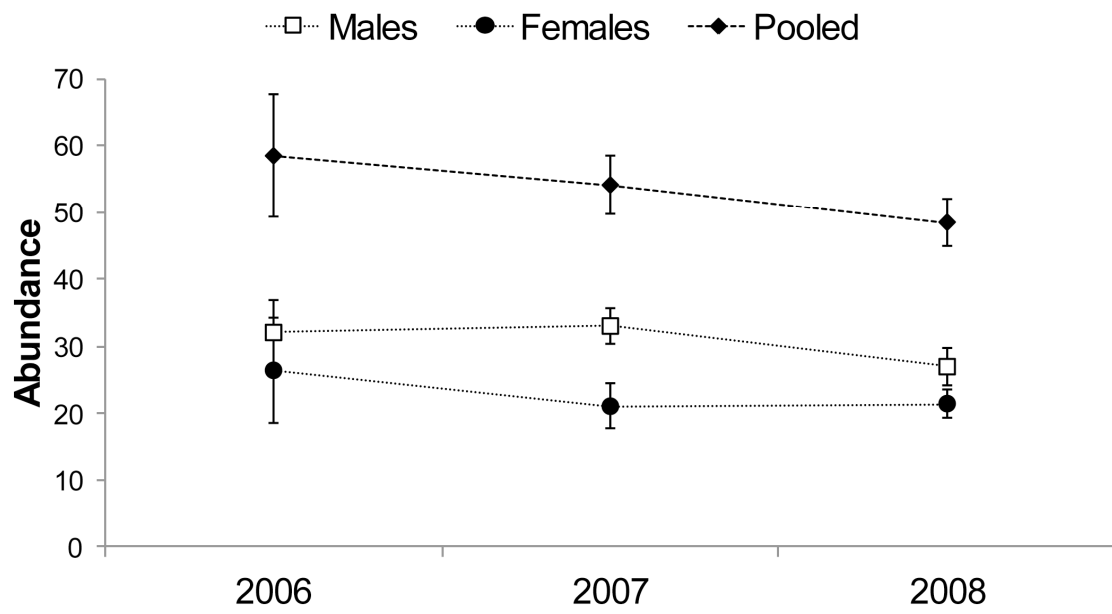


Figure 4

Supporting Information

Figure Legends

Figure S1: Gender-specific per session capture probability estimates from Pradel Robust design open population models. Estimates obtained using bear rub data collected in the Bow Valley Study Area of Banff National Park, Alberta, Canada, from 2006 - 2008. We derived model averaged capture probability estimates from most supported models in Table S4. Bear rub effort (BRE) was the cumulative number of days between successive hair collections summed over all bear rubs sampled per session: values were divided by 10,000 to standardize to scale of y axis. Error bars represent model averaged estimates of Standard Error.

Tables

Table S1. Microsatellite marker variability for determining individual identity of grizzly (n=80) and black bears (n=85) from DNA samples collected from hair traps, bear rubs, wildlife crossings and management actions throughout Banff National Park, Alberta, Canada from May 2006 – October 2008.

Marker	Grizzly bears					Black bears				
	H _E	H _O	A	P _{ID}	P _{SIB}	H _E	H _O	A	P _{ID}	P _{SIB}
G1A	0.70	0.69	6	0.08	0.38	0.76	0.73	12	0.04	0.33
G1D	0.82	0.85	10	0.13	0.43	0.81	0.82	9	0.09	0.39
G10B	0.83	0.81	9	0.05	0.35	0.80	0.78	7	0.07	0.37
G10H	0.80	0.80	8	0.06	0.35	0.88	0.94	10	0.06	0.36
G10J	0.79	0.80	6	0.06	0.37	0.85	0.87	14	0.02	0.32
G10M	0.75	0.78	6	0.10	0.40	0.82	0.94	11	0.06	0.36
G10P	0.70	0.65	6	0.13	0.43	0.84	0.84	9	0.05	0.34
Mean	0.77	0.77	7.3			0.82	0.85	10.3		
SE	0.02	0.03	0.6			0.02	0.03	0.9		
Overall probability of identity				2.30E-08	0.0013				1.00E-09	0.0007

* H_E=expected heterozygosity; H_O=observed heterozygosity; A=no. of alleles;
P_{ID}=probability of identity; P_{SIB}=probability of sibling identity.

Table S2. Model selection results from Huggins closed population models of grizzly bear abundance in the Bow Valley of Banff National Park, Alberta, Canada in 2006 and 2008; results from Program MARK, April 2011 build.

Model	AIC _c ^a	ΔAIC _c ^b	w _i ^c	Model Likelihood	No. parameters	Deviance
2006						
CS: p(CSE _{half} × DTE) HT: p(sex × DTE) BR: p(sex + BRE _{half}); DTE _{5km}	395.11	0.00	0.39	1.00	9	376.70
CS: p(CSE _{half} × DTE) HT: p(sex × DTE) BR: p(sex); DTE _{M10km, F5km}	395.74	0.63	0.28	0.73	8	379.42
CS: p(CSE _{half} × DTE) HT: p(sex × DTE) BR: p(sex); DTE _{5km}	395.74	0.63	0.28	0.73	8	379.42
CS: p(CSE _{half}) HT: p(sex) BR: p(sex) × DTE	400.97	5.86	0.02	0.05	8	384.65
CS: p(CSE _{half}) HT: p(sex) BR: p(sex) + DTE	401.72	6.62	0.01	0.04	7	387.47
CS: p(CSE _{half}) HT: p(sex) BR: p(sex) × DTE; DTE _{M10km, F5km}	403.77	8.67	0.01	0.01	8	387.45
CS: p(CSE _{half}) HT: p(sex) BR: p(sex) × DTE; DTE _{5km}	403.77	8.67	0.01	0.01	8	387.45
CS: p(CSE _{half}) HT: p(sex) BR: p(sex)	406.25	11.15	0.00	0.00	6	394.07
CS: p(CSE _{full}) HT: p(sex) BR: p(sex) + DTE	418.62	23.51	0.00	0.00	7	404.37
CS: p(DTE) HT: p(sex × DTE) BR: p(sex); DTE _{M10km, F5km}	419.06	23.95	0.00	0.00	7	404.81
2008						
CS: p(CSE _{half} × DTE) HT: p(sex × time + HTE _{half} × DTE) BR: p(sex × time + BRE _{half}); DTE _{5km}	599.93	0.00	0.17	1.00	27	543.17
CS: p(CSE _{half} × DTE) HT: p(sex × time + HTE _{half} × DTE) BR: p(sex × time + BRE _{half}); DTE _{M10km, F5km}	599.93	0.00	0.17	1.00	27	543.17
CS: p(CSE _{half} × DTE) HT: p(sex × time + HTE _{half} × DTE) BR: p(sex × time + BRE _{half}); DTE _{10km}	599.93	0.00	0.17	1.00	27	543.17
CS: p(CSE _{half} × DTE) HT: p(sex × time + HTE _{half} × DTE) BR: p(sex × time + BRE _{half})	599.93	0.00	0.17	1.00	27	543.17
CS: p(CSE _{half}) HT: p(sex × time + HTE _{full}) BR: p(sex × time + BRE _{full}) + DTE	601.63	1.70	0.07	0.43	26	547.07
CS: p(CSE _{half}) HT: p(sex × time + HTE _{half}) BR: p(sex × time + BRE _{full}) + DTE	602.16	2.23	0.06	0.33	26	547.60
CS: p(CSE _{half}) HT: p(sex × time + HTE _{half}) BR: p(sex × time + BRE _{half})	603.98	4.04	0.02	0.13	25	551.61
CS: p(CSE _{half} × DTE) HT: p(sex × time × DTE) BR: p(sex × time + BRE _{half}); DTE _{5km}	604.19	4.26	0.02	0.12	26	549.63
CS: p(CSE _{half}) HT: p(sex × time + HTE _{half}) BR: p(sex × time + BRE _{half}) × DTE; DTE _{5km}	604.30	4.37	0.02	0.11	27	547.54
CS: p(CSE _{half}) HT: p(sex × time + HTE _{half}) BR: p(sex × time + BRE _{half}) × DTE; DTE _{M10km, F5km}	604.30	4.37	0.02	0.11	27	547.54

^a Akaike's Information Criterion for small sample sizes.

^b The difference in AIC_c value between the *i*th model and the model with the lowest AIC_c value.

^c Akaike wt used in model averaging.

^d DTE subscripts indicate threshold values in kilometers (M=male-only, F=female-only).

Table S3. Model selection results from Huggins closed population models of black bear abundance in the Bow Valley of Banff National Park, Alberta, Canada in 2006 and 2008; results from Program MARK, April 2011 build.

Model	AIC _c ^a	ΔAIC _c ^b	w _i ^c	Model Likelihood	No. parameters	Deviance
2006						
CS: p(CSE _{half}) HT: p(t + sex + HTE _{full}) BR: p(DTE)	271.66	0.00	0.55	1.00	11	248.75
CS: p(DTE) HT: p(t + sex + HTE _{half}) BR: p(.)	275.14	3.48	0.10	0.18	10	254.38
CS: p(.) HT: p(t + sex + HTE _{half}) BR: p(.)	275.20	3.54	0.09	0.17	9	256.58
CS: p(.) HT: p(t + sex + HTE _{half} + DTE) BR: p(.)	276.21	4.55	0.06	0.10	10	255.45
CS: p(DTE) HT: p(t + sex + HTE _{full}) BR: p(.)	276.72	5.05	0.04	0.08	10	255.96
CS: p(DTE) HT: p(t + sex + HTE _{half}) BR: p(DTE)	277.20	5.54	0.03	0.06	11	254.29
CS: p(.) HT: p(t + sex + HTE _{full}) BR: p(.) + DTE; DTE _{M10km² F5km}	278.37	6.71	0.02	0.03	11	255.46
CS: p(.) HT: p(t + sex + HTE _{full}) BR: p(.) + DTE; DTE _{5km}	278.37	6.71	0.02	0.03	11	255.46
CS: p(.) HT: p(t + sex + HTE _{full}) BR: p(.) + DTE	278.37	6.71	0.02	0.03	11	255.46
CS: p(.) HT: p(t + sex + DTE) BR: p(.)	278.54	6.88	0.02	0.03	9	259.92
2008						
CS: p(DTE) HT: p(t + sex + HTE _{full}) BR: p(DTE); DTE _{5km}	437.77	0.00	0.16	1.00	11	415.16
CS: p(DTE) HT: p(t + sex + HTE _{full}) BR: p(DTE); DTE _{M10km² F5km}	437.77	0.00	0.16	1.00	11	415.16
CS: p(DTE) HT: p(t + sex + HTE _{full}) BR: p(DTE)	437.77	0.00	0.16	1.00	11	415.16
CS: p(DTE) HT: p(t + sex + HTE _{full}) BR: p(.)	438.13	0.36	0.14	0.84	10	417.62
CS: p(DTE) HT: p(t + sex + HTE _{half}) BR: p(DTE)	438.15	0.38	0.14	0.83	11	415.53
CS: p(DTE) HT: p(t + sex + HTE _{half}) BR: p(.)	438.33	0.56	0.12	0.76	10	417.82
CS: p(CSE _{full} + DTE) HT: p(t + sex + HTE _{full}) BR: p(DTE)	439.51	1.74	0.07	0.42	12	414.78
CS: p(DTE) HT: p(t × sex + HTE _{full}) BR: p(DTE)	442.89	5.12	0.01	0.08	15	411.76
CS: p(DTE) HT: p(t × sex + DTE) BR: p(DTE)	443.20	5.43	0.01	0.07	15	412.07
CS: p(DTE) HT: p(t × sex + HTE _{half}) BR: p(DTE)	443.32	5.55	0.01	0.06	15	412.19

^a Akaike's Information Criterion for small sample sizes.

^b The difference in AIC_c value between the *i*th model and the model with the lowest AIC_c value.

^c Akaike wt used in model averaging.

^d DTE subscripts indicate threshold values in kilometers (M=male-only, F=female-only).

Table S4. Model selection results from Pradel open population models of realized population growth rate, λ , and apparent survival, ϕ , for grizzly bears in the Bow Valley of Banff National Park, Alberta, Canada between 2006 and 2008; results from Program MARK, April 2011 build.

Model	AIC _c ^a	Δ AIC _c ^b	w _i ^c	Model Likelihood	No. parameters	Deviance
$\phi(\text{sex} + \text{DTE}) \lambda(\text{sex}) p(t \times \text{sex} + \text{BRE}_{\text{full}} + \text{DTE})$	1077.98	0.00	0.31	1.00	36	993.98
$\phi(\text{sex}) \lambda(\text{sex}) p(t \times \text{sex} + \text{BRE}_{\text{full}}) + \text{DTE}$	1078.96	0.98	0.19	0.61	36	994.96
$\phi(\text{sex}) \lambda(\text{sex}) p(t \times \text{sex} + \text{BRE}_{\text{full}} + \text{DTE})$	1079.53	1.55	0.15	0.46	36	995.53
$\phi(\text{sex}) \lambda(\text{sex}) p(t \times \text{sex} + \text{BRE}_{\text{full}} + \text{DTE}_{10\text{km}})$	1079.53	1.55	0.15	0.46	36	995.53
$\phi(\text{sex} + \text{DTE}) \lambda(\text{sex}) p(t \times \text{sex} + \text{BRE}_{\text{half}} + \text{DTE})$	1081.09	3.10	0.07	0.21	36	997.09
$\phi(\text{sex}) \lambda(\text{sex}) p(t \times \text{sex} + \text{BRE}_{\text{half}} + \text{DTE})$	1083.12	5.14	0.02	0.08	36	999.12
$\phi(\text{sex}) \lambda(\text{sex}) p(t \times \text{sex} + \text{DTE})$	1083.46	5.48	0.02	0.06	33	1007.49
$\phi(\text{sex}) \lambda(\text{sex}) p(t \times \text{sex} + \text{DTE}); \text{DTE}_{10\text{km}}$	1083.46	5.48	0.02	0.06	33	1007.49

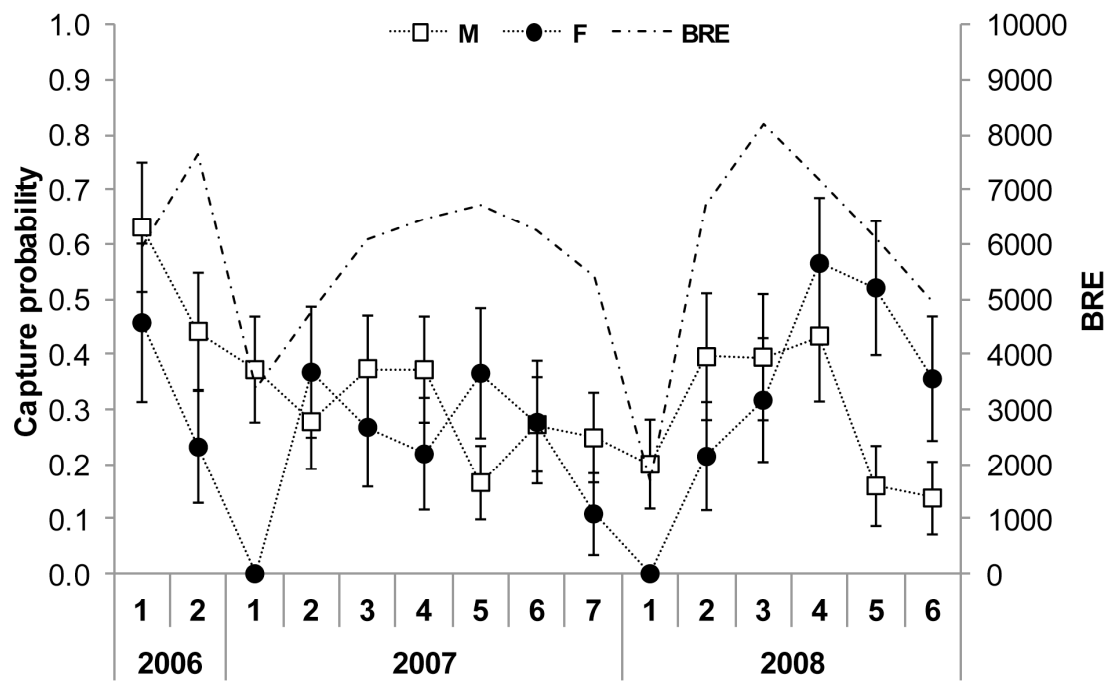
^a Akaike's Information Criterion for small sample sizes.

^b The difference in AIC_c value between the *i*th model and the model with the lowest AIC_c value.

^c Akaike wt used in model averaging.

^d DTE subscripts indicate threshold values in kilometers.

Figure S1



CHAPTER FOUR

WILDLIFE CROSSING STRUCTURES CONNECT URSID POPULATIONS IN
BANFF NATIONAL PARK

Contribution of Author and Co-authors

Manuscript in Chapter 4

Chapter 4:

Author: Michael A. Sawaya

Contributions: conceived the study and figures, designed methods, collected data, and wrote the manuscript.

Co-author: Dr. Anthony P. Clevenger

Contributions: helped conceive the study, designed methods, collected data, and wrote the manuscript.

Co-author: Dr. Steven T. Kalinowski

Contributions: discussed the results and implications, and commented on the manuscript at all stages.

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RH: Wildlife crossing structures

Wildlife Crossing Structures Connect Ursid Populations in Banff National Park.

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ABSTRACT

Wildlife crossing structures are one solution to mitigating the fragmentation of wildlife populations caused by roads, but the effectiveness of wildlife crossings to provide connectivity has only been superficially evaluated. Hundreds of grizzly and black bear passages have been recorded at Banff National Park's iconic system of underpasses and overpasses. However, the ability of wildlife crossings to allow individual and population-level movements across road networks remains unknown. In April 2006, we initiated a three-year investigation to determine whether wildlife crossings provide connectivity for grizzly (*Ursus arctos*) and black bears (*U. americanus*) in the Bow Valley of Banff National Park. We used multiple noninvasive hair collection methods to sample the grizzly and black bear populations around the Bow Valley. Our main objectives were to: 1) determine the number of individual male and female grizzly and black bears using wildlife crossings structures, 2) examine the spatial and temporal patterns of individual bear crossings, and 3) estimate the proportions of grizzly and black bear populations in the Bow Valley using wildlife crossing structures. We identified a total of 15 grizzly (7 F and 8 M) and 17 black bears (8 F and 9 M) using wildlife crossings. The number of individuals detected at wildlife crossings was highly correlated with the number of passages in space and time. Grizzly bears showed strong preference for more open crossing structure types (i.e. overpasses and open-span underpasses). Peak use for both bear species occurred in July when high rates of foraging activity coincide with mating season. We detected significant proportions of grizzly (15.0% in 2006 and 19.8% in 2008) and black bear (17.6% in 2006 and 11.0% in 2008) populations using

crossing structures. We conclude wildlife crossing structures provide spatial and temporal connectivity for grizzly and black bear populations in Banff National Park.

INTRODUCTION

Road networks are one of the most ubiquitous anthropogenic features on the planet and pose a major threat to Earth's biodiversity (Forman et al. 2003). The presence of roads is highly correlated with changes in species composition, population sizes, and ecological processes (Trombulak & Frissell 2000). Roadways, particularly ones with higher traffic volumes, can negatively affect wildlife populations through direct mortality and habitat fragmentation (Fahrig & Rytwinski 2009). Roadkills are a substantial source of mortality for many vertebrate populations, but road-caused mortality rates rarely limit population abundance. Barrier effects caused by road avoidance are thought to be a much bigger ecological problem (Forman & Alexander 1998). Barrier effects constrain wildlife movements thus inhibiting landscape connectivity and disrupting ecosystem functions such as individual survival and reproduction, population persistence and evolution, predator-prey dynamics and resource competition. Wildlife crossing structures combined with exclusion fencing are one solution to keeping animals off of roadways while maintaining or restoring animal movements, but the ability of wildlife crossings to provide connectivity for populations fragmented by roads has yet to be properly evaluated (Corlatti et al. 2009; Kaplan 2009).

According to the theory of island biogeography, patches of habitat that are connected will have greater species richness, diversity, and persistence than isolated

patches (MacArthur & Wilson 1967). Corridors are designed to link patches of fragmented habitat, thus stabilizing population dynamics and community processes. Wildlife crossing structures are “in essence site-specific movement corridors strategically placed over a deadly matrix habitat of pavement and high-speed vehicles” (Clevenger & Wierzychowski 2006). Ecological connectivity is becoming increasingly more important in the face of a changing climate (Krosby et al. 2010). Wildlife crossings may play an important role in allowing animals to change movements (i.e. latitude, elevation) in response to rising temperatures and by allowing the flow of genes so that populations can adapt and evolve to changing environmental conditions. Despite these potential benefits, conservation biologists have hotly debated the conservation value of corridors, including engineered wildlife crossing structures, for the last two decades (Hobbs 1992; Simberloff & Cox 1987; Noss 1987; Beier & Noss 1998). The debate has understandably focused on whether wildlife crossings and other landscape corridors designed to increase population viability are worth the relatively high costs (Simberloff et al. 1992; Dixon et al. 2006).

The ability of wildlife crossing structures to connect wildlife populations remains surprisingly unknown considering an aggressive global campaign currently underway to incorporate wildlife crossings into construction projects (Forman 2000; Ament et al. 2008; Mata et al. 2008). Research has shown that exclusion fencing can reduce wildlife-vehicle collisions for some species (Clevenger et al. 2001; Jaeger & Fahrig 2004). It has also been well-documented that a variety of species will use wildlife crossings to traverse busy highways (Clevenger & Waltho 2000; Clevenger & Waltho 2005). Nonetheless, few studies have evaluated the effectiveness of wildlife crossing structures beyond

documenting the number of species using crossings and their frequency of use (Cain et al. 2003; Ng et al. 2004; Clevenger & Waltho 2005; Gagnon et al. 2011).

One of the world's most developed and well-known systems of wildlife crossings is located within Banff National Park (BNP) in the Central Rocky Mountains of Alberta, Canada. With over 4 million visitors per year, BNP is one of the world's most visited national parks and this high level of human visitation acts as a major stressor on the ecosystem (Ford et al. 2010). Some of the seminal publications in road ecology have come from studying the two overpasses (costing over \$2 million US per structure) and 23 underpasses that were built in the 1980s and 1990s to reduce wildlife-vehicle collisions and restore or maintain wildlife movement across the four-lane section of the Trans-Canada Highway (TCH), (Clevenger & Waltho 2000; Clevenger et al. 2001; Clevenger & Waltho 2005). The TCH is Canada's busiest highway (17,970 vehicles per day on average in BNP) and serves as the nation's primary east-west transportation route (Ford et al. 2010). Given the extent and duration of road mitigation efforts in BNP along with its protected status and biodiversity, it is viewed the world over as the quintessential place to study the effectiveness of road mitigation measures.

Recent evidence indicates that healthy populations of apex consumers (e.g. carnivores) are critical to maintaining top-down ecological processes, but many carnivore species are experiencing a rapid global decline (Estes et al. 2011). Wide-ranging, large-bodied carnivores that inhabit BNP such as grizzly (*Ursus arctos*) and black bears (*U. americanus*) are easily susceptible to road-caused fragmentation due to their low densities and reproductive rates combined with large home ranges (Brody & Pelton 1989;

Proctor et al. 2005; Rytwinski & Fahrig 2011). Wildlife crossing structures in BNP and the bears that use them represent the ideal study system and model study species to address huge gaps in our understanding of how, when, and why road mitigation measures provide connectivity for individuals and populations.

Until recently, remote cameras and track pads have been the most frequently used methods to evaluate the effectiveness of wildlife crossing structures (Ford et al. 2009; Long et al. 2008), but these methods cannot reliably distinguish individuals or genders so the research questions they can address are limited (Clevenger & Sawaya 2010). For instance, hundreds of grizzly and black bear passages have been recorded with track pads at the wildlife crossing structures in BNP, but due to methodological limitations the ability of crossing structures to serve as movement corridors for multiple individuals of different genders remains completely unknown. Samples collected from noninvasive genetic sampling can identify male and female individuals and may provide information on abundance, vital rates, and genetic interchange, but wildlife managers have been reluctant to embrace genetic monitoring methods because they are relatively new (Schwartz et al. 2006). A new noninvasive genetic sampling method recently developed in BNP allows the opportunity for evaluating the ability of wildlife crossings to connect grizzly and black bear populations (Clevenger and Sawaya 2010)

In April 2006, we initiated a three-year investigation to evaluate the effectiveness of wildlife crossing structures to provide connectivity for grizzly and black bears in the Bow Valley of BNP. We sampled wildlife crossings structures using the hair collection method developed by Clevenger and Sawaya (2010) and sampled the surrounding grizzly

and black bear populations using a combination of hair traps and rub tree surveys (Sawaya et al. *In Review*). Our main objectives were to: 1) determine the number of individual male and female grizzly and black bears using wildlife crossings to traverse the TCH, 2) examine the spatial and temporal patterns of bear movements at wildlife crossings, and 3) estimate the proportions of the bear populations in the Bow Valley using wildlife crossings.

STUDY AREA

Our 2246 km² Bow Valley study area (BVSA) was located in Banff National Park approximately 120 km west of Calgary, Alberta, east of the Continental Divide in the central Rocky Mountains (Fig. 1). BNP is the flagship of Canada's extensive national park system and was established in 1885, making it the third national park created in the world. BNP along with three other national parks, Yoho, Kootenay, and Jasper, comprise the UNESCO Rocky Mountain World Heritage Site. The lower Bow Valley is a human-dominated landscape with the TCH, the Banff Townsite (=8000 residents), a golf course, 3 ski areas, a railway, and a secondary highway. Between 1982 and 1997, 45 km of the TCH extending west from the eastern park boundary were widened from two to four lanes for safety reasons (McGuire & Morrall 2000). A total of 25 wildlife crossing structures, 2 overpasses and 23 underpasses (e.g. box culverts, creek and open-span bridges) were constructed, along with 2.4 m high fencing to reduce wildlife vehicle collisions and facilitate wildlife movement across the four-lane section of the TCH (Ford

et al. 2010). Detailed ecological descriptions of the study area can be found in Holroyd and Van Tighem (1983) and Holland and Coen (1983).

METHODS

Sampling

We conducted hair sampling concurrently with track pad monitoring at 20 of 25 wildlife crossings structures in the Bow Valley between 15 May – 18 October 2006, 22 April – 29 October 2007, and 22 April–18 October 2008 (Table 1, Fig. 1). Five were not included in this study because they receive high levels of human use and low levels of bear use (Clevenger and Waltho 2005). We set up our hair-sampling system by stretching two lengths of barbed wire perpendicular to the line of movement in order to snag hair from passing bears (Clevenger and Sawaya 2010). We conducted hair sampling in conjunction with track pad monitoring. Track pads consisted of strips of sandy loam 1.5 -2 m wide, running perpendicular to the line of movement and spanning the length of the wildlife crossing (Clevenger & Waltho 2000; Clevenger & Waltho 2005). We checked track pads every two days and recorded species, direction of travel, and number in group (Ford et al. 2009). Species identification was based on diagnostic characteristics of tracks. We calculated total number of passages by summing discrete crossing events over the 3-year sampling period.

For hair sampling at the crossing structures, we secured two metal stakes at each end of a track pad, and attached the two strands of wire at 30 cm and 70 cm, respectively. Page wire and brush were piled at the ends of the wire to funnel animals through the hair

sampling system. Illustrations along with detailed descriptions of the hair sampling system and related pilot study can be found in Clevenger and Sawaya (2010). We checked the barbed wire for hair every two days in conjunction with track pad monitoring. We collected hair samples from each barb separately and also collected discrete clumps of hair below the wire. We placed samples in paper envelopes labelled with a uniquely numbered barcode and sterilized every barb from which we collected hair with an open flame in order to prevent DNA contamination. We extracted and amplified DNA using procedures designed for noninvasively collected samples and identified individuals using seven microsatellite loci (Sawaya et al., *In Review*). After we compiled multilocus genotypes for hair samples, we counted the number of unique individuals detected at a given time or place.

In a previous analysis, we used two noninvasive genetic sampling methods, hair traps and bear rubs, to collect hair from black and grizzly bear populations surrounding the TCH and estimate grizzly and black bear population abundance for the Bow Valley Study Area in 2006 and 2008. Detailed descriptions of hair collection from hair traps and bear rubs and modeling methods and results can be found in Sawaya et al. (*In Review*).

Connectivity

We examined individual patterns of bear passages across wildlife crossings along the TCH. We used multi-locus genotypes to count the number of unique male and female bears using crossing structures each year and over the three year sampling period. We calculated mean and standard error for frequency of individual bear passages by year and across years. We examined spatial and temporal patterns of individual bear use with

graphical plots.

We assessed population-level connectivity by comparing data from crossings with abundance estimates of grizzly and black bears in the Bow Valley Study Area (Sawaya et al. In Review).

We estimated minimum proportions of grizzly and black bear populations using crossings by dividing the number of males and females detected at crossings in a year by the annual abundance estimates for each species and gender.

RESULTS

Sampling

We recorded 91 grizzly bear passages at track pads in 2006, 115 in 2007, and 180 in 2008 (Fig. 2). We recorded 129 black bear passages at track pads in 2006, 98 in 2007, and 88 in 2008 (Fig. 2). We collected 348 hair samples from wildlife crossings in 2006, 416 in 2007, and 553 in 2008 (Table 1). Many samples came from the same individual bears or non-target species such as cougars (*Puma concolor*) and wolves (*Canis lupus*). We collected hair from 100% of wildlife crossings (n = 20) monitored in all years, but not all passages yielded useable DNA (i.e. identify individuals) or DNA from the target species. We collected useable DNA from 37% of grizzly (n = 386) and 33% of black bear passages (n = 315).

Connectivity

We identified 15 grizzlies (8 M, 7 F) and 17 black bears (9 M, 8 F) using the wildlife crossing structures over the course of the study. We identified 11 grizzlies and 11 black

bears using crossings in 2006, 12 grizzlies and 8 black bears in 2007, and 10 grizzlies and 9 black bears in 2008 (Table 2). A high percentage of individual grizzly bears detected at crossings were detected in >one year (71% of females and 75% of males). A lower percentage of black bears were detected in >one year (37% of females and 55% of males). In fact, 57% of female and 37% of male grizzlies were detected all three years of sampling compared to only 25% of female and 11% of male black bears. The average number of passages per individual was higher for grizzly bears than for black bears (Table 3). Variability in individual passage frequency was high for both species. Of the 247 total passages that produced useable DNA samples, grizzly bear males had the highest number of total passages (n=97) and the highest mean number of passages per individual ($\bar{x}$ =12.1, SD=12.8). One grizzly bear male accounted for 34 of the 97 (35.1%) passages by male grizzly bears for which we had useable DNA, including the highest number we recorded for a single year, 17. One grizzly bear female accounted for 18 of 47 (38.3%) female grizzly bear passages. Single individuals accounted for an even larger percentage of black bear passages (24 of the 55 (43.6%) passages by one male and 21 of 48 (43.8%) female black bear passages by a single female).

The number of grizzly and black bear passages appears to be spatially correlated with the number of unique individuals detected at wildlife crossing structures (Pearson's $r=0.89$ for black bears and $r=0.95$ for grizzly bears; Fig. 3). Grizzly bear passages and individuals are highly concentrated at two overpasses and one open-span underpass to the west of Banff Townsite, while black bear passages are evenly distributed and more concentrated to the east (Fig. 3). High proportions of grizzly bear males and females

were detected at the most open wildlife crossing types, overpasses, and open-span underpasses (Fig. 4a). Over 70% of female and 75% of male grizzly bears detected at wildlife crossings over the three year period were detected at one of just two overpasses (Fig. 4a). The proportion of black bears detected at each crossing type was more evenly distributed (Fig. 4b).

The number of grizzly and black bears detected at wildlife crossing structures during each month was highly correlated with the number of passages detected with track pads (Pearson's $r=0.99$ for black bears and $r=0.98$ for grizzly bears; Fig. 5). The number of grizzly bear individuals identified using wildlife crossings peaked in July, but showed a slight uptick in September (Fig. 5a). The number of black bear individuals and passages showed an even stronger peak in July (Fig. 5b).

We obtained precise abundance estimates for grizzly and black bears in the Bow Valley (Sawaya et al. *In Review*) from which we determined that high percentages (>10%) of both species used wildlife crossing structures to traverse the TCH. We estimated the minimum proportion of the grizzly bear population using crossings as 15.0% in 2006 and 19.8% in 2008 (Table 4). We estimated the minimum proportion of the black bear population using crossings as 17.6% in 2006 and 11.0% in 2008 (Table 5).

DISCUSSION

Transportation agencies and wildlife managers need to know whether wildlife crossings provide connectivity for individuals and populations in order to justify the continuation of expensive road mitigation projects. Few if any studies, however, have collected

empirical data on individual use of wildlife crossings and provided population-level context to those movements (Corlatti et al. 2008; Kaplan 2009). We present the first in depth examination of individual movements, spatial and temporal patterns of use, and population-level connectivity afforded by wildlife crossing structures and we conclude that wildlife crossings provide connectivity for grizzly and black bear populations.

We collected hair samples successfully from all of the wildlife crossings that we monitored ($N = 20$) with our hair sampling system. Although we were unable to produce genetic results for many of the samples we collected at crossings, we still managed to obtain individual identities for 37% of grizzly and 33% of black bear passages, thus giving us a robust minimum count of bears using wildlife crossings to traverse the TCH.

An examination of spatial patterns of individual bear use revealed that the type of crossing structure is important in determining where certain species are likely to cross. We found a much higher percentage of male and female grizzly bears using large, open crossing structure types, while black bears were found using smaller, more constricted types of crossings (Fig. 4). These findings support earlier research based on track pad counts (Clevenger & Waltho 2005). Considering the strong preference by many individual grizzly bears for more open, less constricted crossing types, we strongly recommend transportation planners and engineers consider overpasses and open-span underpasses when mitigating highways for grizzly bears. It appears that black bears are more adaptable and use a wider variety of crossing types than grizzly bears (Fig. 4), so mitigation targeted for black bears could involve a broader array of smaller crossing types (Clevenger & Huijser 2011).

Given the high degree of variability in the number of passages per individual (Table 3), it was surprising to find that the number of passages from track pad counts was highly correlated with the number of individuals for both bear species (Fig. 3). This provides some independent validity to studies of crossing structure effectiveness that make assumptions about higher order processes based solely on track count data. Our results demonstrate that wildlife crossings provide spatial connectivity by allowing frequent movements by a number of individuals in a variety of locations and through a number of different crossing structure types.

An examination of movements at the wildlife crossings also reveals some interesting temporal patterns. We detected a strong peak in July for the number of grizzly and black bear passages and individuals (Fig. 5). This is not surprising as we predicted that bears would use crossings most in mid-summer because it corresponds with mating season, dispersal, and the highest foraging activity in the montane valley bottom habitat. We also predicted an abrupt decline in bear use of the crossings as movements decreased due to increased foraging efficiency after buffaloberry patches ripen in late July (Hamer & Herrero 1987). The number of passages we recorded per month at crossings also was highly correlated with the number of individuals detected per month (Fig. 5).

The number of male and female black and grizzly bears detected using the crossings was surprisingly consistent across years (Table 2), especially when considering the upwards trend in grizzly bear passages (Fig. 2) and the recent downward trend in black bear passages. These opposing trends are also complicated by the apparent relationship between the number of track pad detections and number of individuals (Fig.

3 and 5). The number of grizzly and black bear crossers was fairly constant across years (Table 2). We detected a very high percentage of grizzly bears using wildlife crossings from year to year, but we recaptured far fewer black bears across years. The obvious upward trend in grizzly bear passages coupled with the relatively stable numbers of crossers suggests the increase in grizzly bear use can most likely be explained by increasingly frequent use by a few individuals with a disproportionately high number of passages. Previous researchers have proposed that there may be a strong learning curve for some species to find and use wildlife crossings and the strong upward trend (Fig. 2) shows the importance of long-term monitoring of crossing structures (Clevenger & Waltho 2005; Gagnon et al. 2011).

We were able to provide population-level context to our wildlife crossing data by obtaining precise estimates of abundance (Sawaya et al. *In Review*) and directly comparing them to the minimum number of individuals we detected with our hair sampling system. Large percentages of the populations of grizzly (15-20%) and black bears (11-19%) in the Bow Valley used wildlife crossings to traverse the TCH. These minimum percentages are especially high when considering that many bears are unlikely to have home ranges overlapping/ bordering the TCH. Regardless, nearly equal proportions of males and females of both species were detected at crossings in two separate years; a particularly encouraging finding as Proctor et al. (2005) found significant fragmentation effects and male-biased dispersal of grizzly bears across a major east-west highway in Alberta and British Columbia.

It is worth noting that although the minimum number of individuals detected using the crossings were relatively consistent from year to year, the population estimates for grizzly bears decreased while the estimates for black bears increased between 2006 and 2008. Changes in abundance explains much of the variability in minimum estimates of the proportions of crossers between years, but the opposing trend shown in passages from track pads (Fig. 2) reinforces the evidence that the number of passages is driven by a few individuals, irrespective of abundance. It is clear that it takes time for grizzly bears to learn how and where to incorporate wildlife crossings into home range movements. But as grizzly bears become comfortable with crossing structures it appears that they will use crossings with more and more regularity. In situations where adult survival is lowered (i.e. hunted populations), grizzly bears may not survive long enough to learn to adapt to wildlife crossings, therefore crossing structures may not be as effective for an unprotected population as they are for a protected population such as BNP.

Ideally, wildlife crossings should increase the permeability of roads and, therefore, survival of animals that live near them, but direct and indirect impacts of road mitigation are complicated and difficult to evaluate without pre-mitigation data (Roedenbeck et al. 2007). Wildlife crossings can positively affect bear survival through reduced road-kill rates, increased access to food and shelter, and by allowing escape from predation by other bears. Repeated visual observations of bears foraging in the small strip of riparian habitat between the highway and the Bow River suggest that wildlife crossings in BNP play an important role in allowing access to seasonally productive food resources which could be especially important to one of the most fragmented, food-

stressed, and human-influenced populations of grizzly bears on the planet (Gibeau et al. 2002; Chruszcz et al. 2003). Even though just a few individuals accounted for the majority of female grizzly and black bear crossings in our study, their use of crossings could be crucial to maintaining bear population viability because even slight changes in adult female survival can strongly influence population growth rates for grizzly and black bears in BNP (Hebblewhite et al. 2003; Garshelis et al. 2005).

Indirect effects of road mitigation are rarely considered when mitigating roads, but could be just as influential on population viability as direct effects. Research has shown that stress can affect reproductive physiology and demography (Romero 2004). Wildlife using crossings may experience reduced stress thus leading to increased health and reproductive fitness. Survival could be negatively affected if wildlife crossings allow movements into areas with high habitat quality and high mortality rates. For example, the Bow Valley could be an attractive sink for grizzly bears (Nielson et al. 2004) and it is possible that wildlife crossings allow movements into areas with high mortality risk. The railroad is one of the highest causes of mortality for grizzly and black bears in BNP (Bertch & Gibeau 2009, 2010) and it is conceivable that wildlife crossings could inadvertently allow access to grain spills on the railroad, thus increasing risk from railway strikes. Wildlife crossings are no guaranteed solution for landscape and species conservation and the potential merits of building each crossing should be weighed carefully against the costs and potential direct and indirect impacts of road mitigation.

We conclude that wildlife crossings provide connectivity for grizzly and black bear populations in BNP, but we also suggest further study is warranted to understand the

ability of wildlife crossings to prevent genetic isolation and enhance population viability. The one-to-ten-migrant-per-generation rule of thumb proposed a threshold of genetic interchange that should minimize short-term effects of inbreeding while allowing for local adaptive differentiation (Mills & Allendorf 1996; Wang 2004). Although studies have shown that movement does not necessarily translate to the flow of genes (Riley et al. 2006; Strasburg 2006), we are fairly confident that wildlife crossings maintain genetic connectivity across the TCH because the observed crossing rates far exceeded those needed to prevent inbreeding or population differentiation. In short, we have demonstrated the ability of wildlife crossing structures to provide spatial and temporal connectivity for wildlife populations across a major trans-continental transportation corridor.

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TABLES**Table 1.** Number of stations monitored and hair samples collected for three noninvasive genetic sampling methods, wildlife crossings, hair traps, and bears rubs, in the Bow Valley of Banff National Park, Alberta, Canada between April 2006 and October 2008.

	2006	2007	2008	Total
<u>Wildlife crossings</u>				
No. stations	20	20	20	20
No. hair samples	348	416	553	1317
<u>Hair traps</u>				
No. stations	210	0	210	420
No. hair samples	884	N/A	1125	2009
<u>Bear rubs</u>				
No. stations	284	321	313	321
No. hair samples	408	1221	1281	2910

* Some stations monitored more than one year.

Table 2. Number of individual bears detected with three noninvasive genetic sampling methods, wildlife crossings, hair traps, and bears rubs, in Bow Valley of Banff National Park, Alberta, Canada between April 2006 and October 2008.

		2006	2007	2008	Total
<u>Wildlife crossings</u>					
Grizzly bears		11	12	10	15
	Males	6	6	5	8
	Females	5	6	5	7
Black bears		11	8	9	17
	Males	7	4	4	9
	Females	4	4	5	8
<u>Hair traps</u>					
Grizzly bears		31	N/A	39	42
	Males	16	N/A	12	20
	Females	15	N/A	17	22
Black bears		40	N/A	57	76
	Males	16	N/A	19	30
	Females	24	N/A	38	46
<u>Bear rubs</u>					
Grizzly bears		40	46	43	73
	Males	25	30	24	44
	Females	15	16	19	29
Black bears		2	11	6	16
	Males	1	9	2	12
	Females	1	2	4	4

* Some individuals detected with more than one method or in more than one year.

Table 3. Frequency of grizzly and black bear passages^a at wildlife crossing structures per individual^b detected from hair samples^c collected in Banff National Park, Alberta, Canada between April and October of 2006-2008.

Species	Parameter	2006	2007	2008	Total
<u>Grizzly bears</u>	Male Total	23	36	38	97
	Male Mean	3.8	6.0	7.6	12.1
	Male SD	2.7	6.1	6.3	12.8
	Female Total	9	20	18	47
	Female Mean	1.8	3.3	3.6	6.7
	Female SD	0.8	3.3	2.2	5.5
	Male Total	20	16	19	55
	Male Mean	2.9	4.0	4.8	6.1
	Male SD	2.8	2.9	3.9	7.4
<u>Black bears</u>	Female Total	22	14	12	48
	Female Mean	5.5	3.5	2.4	6.0
	Female SD	3.3	5.0	1.7	7.1

^a The number of passages was the sum of unique crossing events for each individual that produced a hair sample with amplifiable DNA.

^b Individual identity was determined from DNA analysis of hair samples collected at wildlife crossings.

^c Many crossing events resulted in the collection of multiple samples from the same individual, but passages were only counted once for each combination of space (i.e. crossing) and time (i.e. collection date).

Table 4. Total minimum counts from noninvasive genetic sampling, model-averaged estimates of grizzly bear abundance and number and proportion of population of grizzly bears detected using wildlife crossings to traverse the Trans-Canada Highway in the Bow Valley Study Area of Banff National Park, Alberta, Canada, in 2006 and 2008.

Grizzly bears	Min. count	Abundance estimate	SE ($\hat{N}$)	No. detected at crossings	% of population detected at crossings
<u>2006</u>					
Males	32	39.9	4.7	6	15.0
Females	25	33.6	5.3	5	14.9
Total	57	73.5	7.2	11	15.0
<u>2008</u>					
Males	26	28.1	2.1	5	17.8
Females	22	22.3	0.6	5	22.4
Total	48	50.4	2.2	10	19.8

* Proportion of population using wildlife crossings was estimated by dividing the number detected at the wildlife crossings by the population estimate.

Table 5. Total minimum counts from noninvasive genetic sampling, model-averaged estimates of black bear abundance and number and proportion of population of black bears detected using wildlife crossings to traverse the Trans-Canada Highway in the Bow Valley Study Area of Banff National Park, Alberta, Canada, in 2006 and 2008.

Black bears	Min. count	Abundance estimate	SE ($\hat{N}$)	No. detected at crossings	% of population detected at crossings
<u>2006</u>					
Males	19	30.2	7.3	7	23.2
Females	24	32.3	4.9	4	12.4
Total	43	62.6	9.0	11	17.6
<u>2008</u>					
Males	22	28.3	4.1	4	14.1
Females	41	53.5	5.7	5	9.3
Total	63	81.8	7.2	9	11.0

* Proportion of population using wildlife crossings was estimated by dividing the number detected at the wildlife crossings by the population estimate.

** 13 black bear samples from 6 hair traps were not analyzed in 2006.

FIGURE LEGENDS

Figure 1. Study area, sampling grid, and noninvasive genetic sampling locations used to investigate conservation benefits of wildlife crossing structures for grizzly and black bear populations in the Bow Valley of Banff National Park (BNP), Alberta, Canada, between 21 April and 31 October of 2006-2008. We monitored 20 wildlife crossing structures with a hair sampling system developed in BNP (Clevenger and Sawaya 2010), 420 hair traps, and 321 bear rubs; we did not monitor five wildlife crossings due to high levels of human use and low levels of bear use (i.e. passages determined from track pads).

Figure 2. Frequency of grizzly and black bear passages detected with track pads at wildlife crossing structures monitored in Banff National Park, Alberta, Canada between April 1997- October 2008. The number of passages was the sum of unique crossing events recorded at track pads over the three year sampling period for each species.

Figure 3. Total number of grizzly and black bear passages (Fig 3a) and individuals (Fig 3b) detected at each wildlife crossing structure between 2006 and 2008. The number of passages was the sum of unique crossing events recorded at track pads over the three year sampling period. The number of individuals was the sum of unique individuals detected at a given crossing structure; individual identity was determined from DNA analysis of hair samples collected at wildlife crossings over the three year sampling period. Many crossing events resulted in the collection of multiple hair samples from the same

individual and some individuals were detected at the same wildlife crossing on multiple occasions, but only counted once per wildlife crossing. Crossing structure types from small to large are abbreviated as follows: M=medium culvert (n=1), B=box culvert (n=4), L=large culvert (n=5), C=creek bridge (n=3), S=open-span (n=5), and O=overpass (n=2). Crossing structures are ordered from west (left) to east (right).

Figure 4. Total number of grizzly (Fig 4a) and black bear (Fig 4b) individuals detected at each of 6 crossing structure types monitored with noninvasive genetic sampling between 2006 and 2008. Proportions were based on the number of unique individuals detected at a crossing type over the 3-year sampling period broken into females (F) and males (M). The number of individuals was the sum of unique individuals detected at a given crossing structure type; individual identity was determined from DNA analysis of hair samples collected at wildlife crossings over the three year sampling period. Many crossing events resulted in the collection of multiple samples from the same individual and some individuals were detected at the same wildlife crossing type, but only counted once per crossing structure type. Crossing structure types are ordered from more constricted (left) to more open (right): medium culvert (n=1), box culvert (n=4), large culvert (n=5), creek bridge (n=3), open-span (n=5), and overpass (n=2).

Figure 5. Total number of individual grizzly bears (Fig 5a) and black bears (Fig 5b) detected each month with noninvasive genetic sampling at wildlife crossings in Banff National Park, Alberta between 2006 and 2008. The number of passages was the sum of

unique crossing events recorded at track pads over the three year sampling period; species identification was determined based on diagnostic characteristics of tracks. The number of passages with DNA was the sum of unique crossing events that produced a hair sample with amplifiable DNA. The number of individuals was the sum of unique individuals detected at a given crossing structure; individual identity was determined from DNA analysis of hair samples collected at wildlife crossings over the three year sampling period. Many crossing events resulted in the collection of multiple samples from the same individual and many individuals were detected more than once in a given month or during more than 1 month in the same year or across years, but only counted once per month.

FIGURES

Figure 1

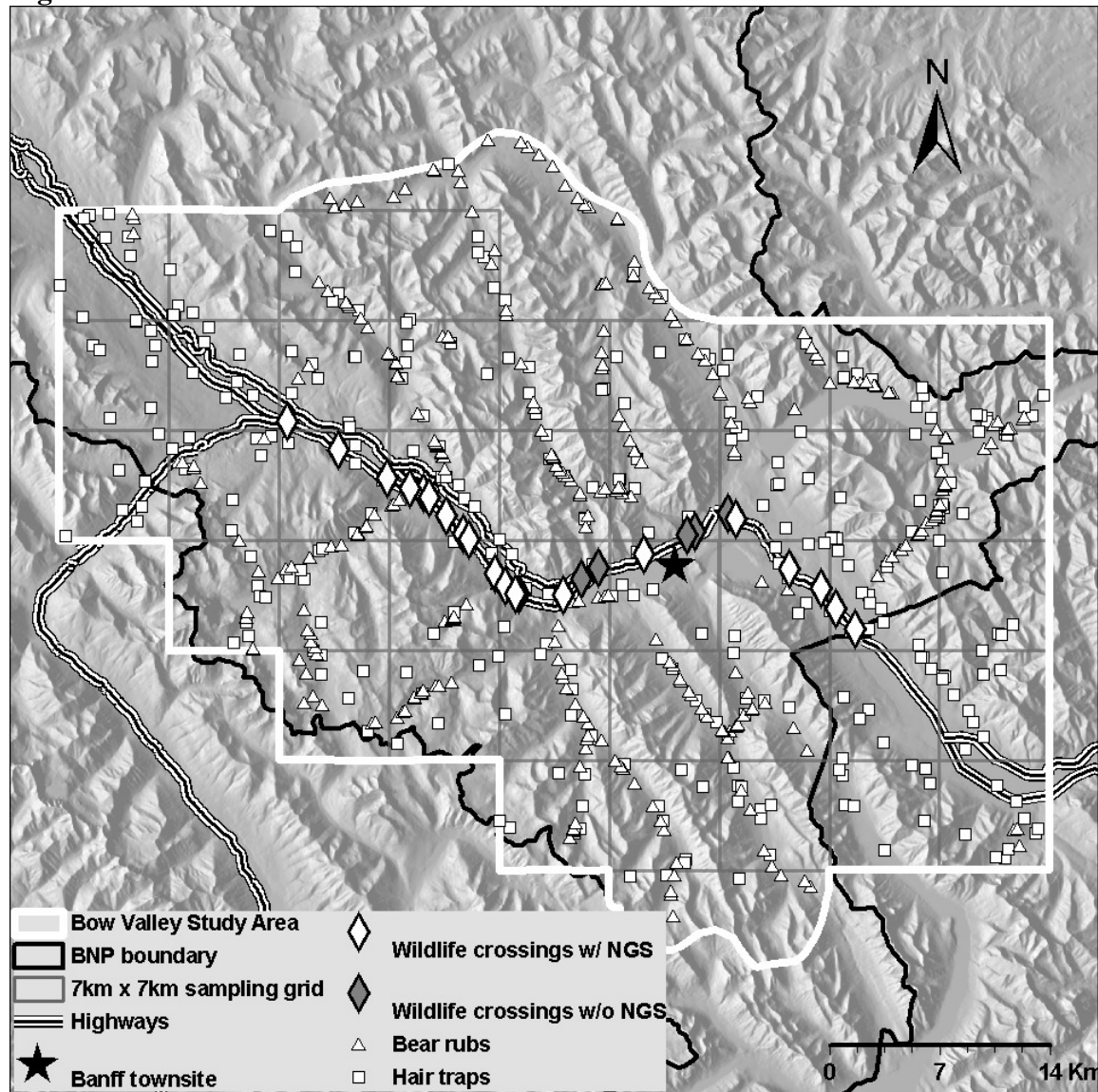


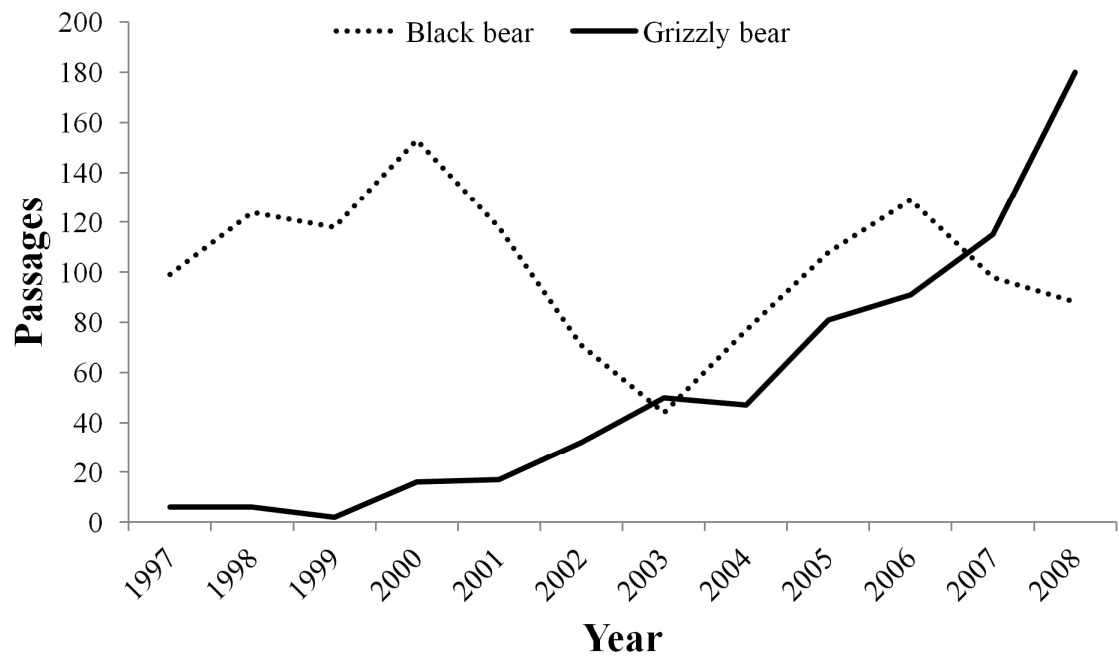
Figure 2

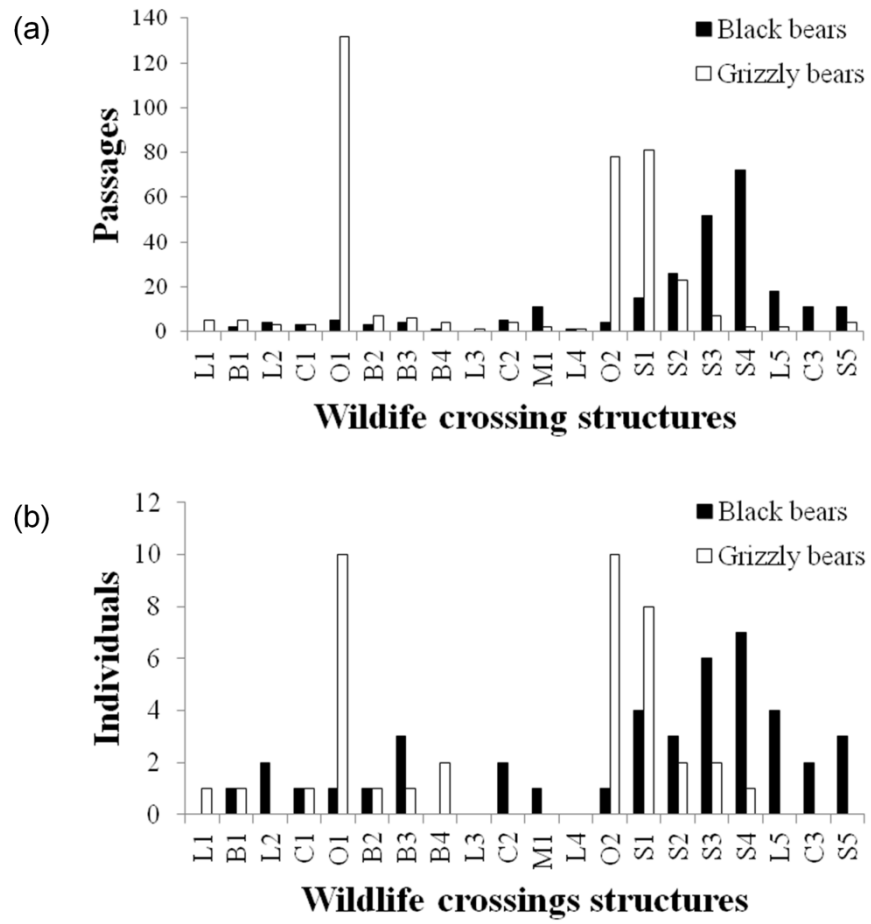
Figure 3

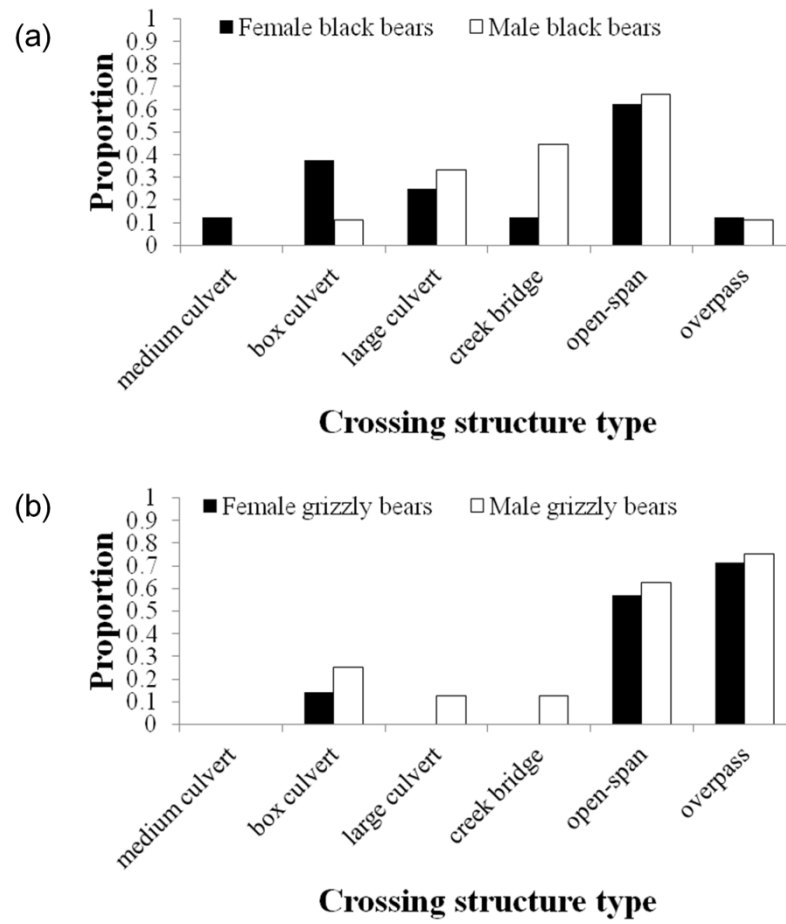
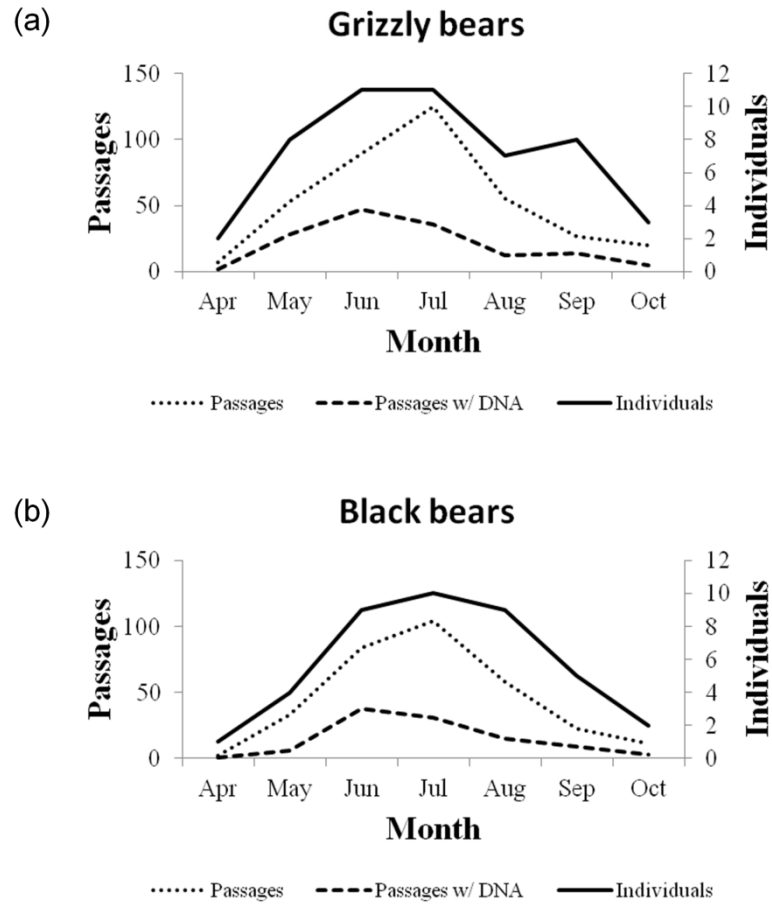
Figure 4

Figure 5

CHAPTER FIVE

GENE FLOW AT WILDLIFE CROSSING STRUCTURES IN
BANFF NATIONAL PARK

Contribution of Author and Co-authors

Manuscript in Chapter 5

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Contributions: conceived the study and figures, designed methods, collected data, and wrote the manuscript.

Co-author: Dr. Steven T. Kalinowski

Contributions: helped conceive the study, discussed the results and implications, and commented on the manuscript at all stages.

Co-author: Dr. Anthony P. Clevenger

Contributions: helped conceive the study, designed methods, collected data, and wrote the manuscript.

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RH: Wildlife crossing structures

Gene Flow at Wildlife Crossing Structures in Banff National Park.

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ABSTRACT

Roads can fragment and isolate wildlife populations which will eventually decrease the amount of genetic diversity within populations. Wildlife crossing structures may counteract these impacts by maintaining or restoring gene flow, but most crossings are relatively new, and there is little evidence that they facilitate gene flow. We conducted a three-year research project in Banff National Park, Alberta to evaluate the effectiveness of wildlife crossings to provide genetic connectivity. Our main objective was to determine how the Trans-Canada Highway and the wildlife crossing structures along it affect gene flow and population structure in grizzly and black bears. To address these questions we compared genetic data generated from individuals detected at wildlife crossings with data collected from greater populations using noninvasive genetic sampling. We detected a genetic discontinuity in grizzly bears at the highway but not in black bears using population-based and individual-based analyses. We used three genetic methods for identifying bears that crossed the highway, and all gave comparable estimates of migration rates. Grizzly bears detected at crossings assigned to populations north and south of the highway, providing evidence of bidirectional gene flow and genetic admixture at wildlife crossings. Genetic parentage tests showed that 47% of black bears and 27% of grizzly bears that used crossings successfully bred, including multiple males and females of both species. Differentiating between dispersal and gene flow is difficult, but we documented gene flow at wildlife crossings by showing a migration event followed by successful reproduction and genetic admixture. We conclude wildlife crossings allow sufficient gene flow to prevent genetic isolation of

ursid populations in Banff National Park.

INTRODUCTION

Roads connect human populations, but often fragment wildlife populations (Forman et al. 2003). Busy roads such as highways can act as barriers to movement through direct mortality of dispersers or through behavioral avoidance (Trombulak & Frissell 2000, Shepard et al. 2008). This can reduce access to habitat and mates (Forman and Alexander 1998), and thereby reduce survival and breeding opportunities. In addition, when roads prevent migration between populations, genetic drift and inbreeding will gradually reduce the amount of genetic diversity in populations and this will happen fastest in smaller, more isolated populations (Epps et al 2005, Holderegger and Di Giulio 2010). Barrier effects may be particularly harmful to fragmented wildlife populations in the face of climate change because animals will be less able to move in response to changing environmental conditions. Landscape corridors, however, may allow animals to change movement patterns (i.e. latitude, elevation) in response to rising temperatures and allow the flow of genes so that populations can adapt and evolve to rapidly changing environmental surroundings (Krosby et al. 2010). Wildlife crossing structures are one solution to mitigating the barrier effects of roads on wildlife and maintaining or restoring functional connectivity (Clevenger and Wierchowski 2006).

Wildlife crossing structures such as overpasses and underpasses are designed to serve as small-scale corridors to increase permeability across highways, but few studies have examined their effectiveness beyond simply documenting animal movements.

Often, highways have been fenced for the purpose of decreasing wildlife-vehicle collisions and research has shown that exclusion fencing helps to reduce highway accidents leading to less wildlife mortality and greater public safety (Clevenger et al. 2001, Jaeger and Fahrig 2004). Numerous species will use a variety of crossing structure types (i.e. overpasses, culverts, open-span underpasses, etc) to traverse busy highways (Clevenger et al. 2000, 2005, Ng et al. 2004, Gagnon et al. 2011). Most studies that have assessed the effectiveness of wildlife crossing structures simply document the species and direction of movement with track pads and remote cameras, but these methods cannot accurately identify male and female individuals or address questions at the population-level (Clevenger and Sawaya 2010). Recent studies have demonstrated that wildlife crossing structures allow considerable movement for populations (Sawaya et al. *In Review*); however, the ability of wildlife crossing structures to provide genetic connectivity and counteract the effects of genetic isolation remains relatively unknown (Corlatti et al. 2009, Kaplan 2009).

Fragmentation divides large populations into smaller populations that are more prone to extinction due to demographic and environmental stochasticity (Berger 1990). Small, isolated populations also have less genetic diversity and are more prone to extinction due to inbreeding depression than larger, connected populations (Frankham 1996, 1997, 1998). Genetic variation within a population can be lost due to genetic drift following population fragmentation (Mills 2007). Small populations that are isolated from other populations are more likely to experience inbreeding, the mating of related individuals, because the smaller the population the more change there will be between

parental and offspring gene pools. Small populations are also more susceptible to disease epidemics and less able to cope with extreme environmental conditions due to a lack of genetic variation. Migration plays an important role in maintaining genetic diversity along with mutation, selection, and genetic drift (Wright 1931), particularly within fragmented populations (Couvét et al. 2002).

Landscape genetics studies have revealed that roads can impact the genetic structure of wildlife populations in a number of ways (Balkenhol and Waits 2009, Simmons et al. 2010). Several studies have looked at geneflow across major roadways and found that reduction in migration rates led to population differentiation (Proctor et al. 2005, Kuehn et al 2006, Riley et al 2006). Other research has shown that road caused fragmentation can lead to loss of genetic variability (Keller et al. 2003). Roads can even act as partial filters to migration resulting in sex-biased dispersal or reduced gene flow. For example, Proctor et al. (2005) found substantial male-biased dispersal of grizzly bears (*Ursus arctos*) across a major highway in Southern Canada using population assignment tests. Riley et al. (2006) examined dispersal and genetic differentiation in coyote (*Canis latrans*) and bobcat (*Lynx rufus*) populations separated by a major California freeway. They found using radio-telemetry that both species frequently crossed the highway, but genetic assignment tests revealed that the level of gene flow was much less than movement rates alone would suggest leading the authors to conclude that the discrepancy was due to a lack of breeding opportunities for typically young dispersers encountering occupied adult home ranges piled up on the other side. As stated in a related article in *Nature*, “if the discrepancy between dispersal and geneflow that

Riley et al. found is widespread, as seems likely, the effectiveness of highway dispersal corridors needs to be tested to see how well they meet their goal of ensuring genetic connections among populations” (Strasburg 2006).

Wildlife crossing structures in Banff National Park (BNP) are an ideal study system to study how roads and crossing structures affect gene flow and population structure. One of the worlds’s most developed and a well-known system of wildlife crossings is located within BNP in the Central Rocky Mountains of Alberta, Canada. With over 4 million visitors per year, BNP is one of the most heavily visited national parks in North America and this high level of human visitation and accompanying traffic volume acts as a major stressor on the ecosystem (Banff Bow Valley Study 1996). The Trans-Canada Highway (TCH) is Canada’s busiest highway (17,970 vehicles per day on average through BNP) and serves as the nation’s primary East-West transportation route (Clevenger et al. 2006). Two overpasses and 23 underpasses were built in the 1980s and 1990s to reduce wildlife-vehicle collisions and restore or maintain wildlife movement across the four-lane section of the TCH, (Clevenger & Waltho 2000; Clevenger et al. 2001; Clevenger & Waltho 2005).

Bears are good model study species to investigate the ability of wildlife crossings to move genes across busy highways because they are ecologically important and are sensitive to fragmentation. Recent evidence indicates that the loss of apex consumers (e.g. carnivores) can trigger far-reaching trophic cascades, but many of these species are experiencing rapid global declines due to human causes (Estes et al. 2011). Wide-ranging, large bodied carnivores that inhabit BNP such as grizzly and black bears (*U.*

americanus) are easily susceptible to road-caused fragmentation due to their low densities and reproductive rates combined with large home range requirements (Rytwinski & Fahrig 2011). Numerous studies have shown that roads can significantly reduce bear movements (Brody & Pelton 1989; Proctor et al. 2005) or influence where bears cross busy highways (Lewis et al. 2011). Research on grizzlies using radio and global positioning system (GPS) collars has demonstrated that higher traffic volumes are correlated with stronger barrier effects (Chruszcz et al. 2003, Waller et al. 2005). Not surprisingly, significant genetic discontinuities have been detected in a number of grizzly bear populations fragmented by major highways throughout the western United States and Canada (Proctor et al 2005, Kendall et al 2009, Proctor et al. 2012).

In April 2006, we initiated a three-year investigation to evaluate the effectiveness of wildlife crossing structures to allow geneflow for grizzly and black bears in BNP. We sampled wildlife crossings structures using a hair sampling method developed by Clevenger and Sawaya (2010) and sampled the surrounding grizzly and black bear populations using a combination of hair traps and bear rub surveys (Sawaya et al. *In Review [b]*). Our main objective was to determine how the TCH and the wildlife crossing structures along it affect gene flow and population structure in grizzly and black bears in BNP.

STUDY AREA

Our 2246 km² Bow Valley study area (BVSA) was located in Banff National Park (BNP) 120 km west of Calgary, Alberta, east of the Continental Divide in the central Rocky

Mountains (Fig. 1). BNP was established in 1885 and is the flagship of Canada's extensive national park system. BNP along with three other contiguous national parks, Yoho, Kootenay, and Jasper, comprise the UNESCO Rocky Mountain World Heritage Site. The lower Bow Valley is a human-dominated landscape with the TCH, the Banff Townsite (=8000 residents), a golf course, 3 ski areas, a railway, and a secondary highway. Between 1982 and 1997, 45 km of the TCH extending west from the eastern park boundary were widened from two to four lanes for safety reasons (McGuire & Morrall 2000). In the late 1980s and early 1990s, a total of 25 wildlife crossing structures, 2 overpasses and 23 underpasses (e.g. box culverts, creek and open-span bridges) were constructed, along with 2.4 m high fencing to reduce wildlife vehicle collisions and facilitate wildlife movement across the four-lane section of the TCH (Clevenger and Wierzchowski 2006). Detailed ecological descriptions of the study area can be found in Holroyd & Van Tighem (1983) and Holland & Coen (1983).

METHODS

Sampling

We studied bear crossings at 20 of 25 wildlife crossing structures along the TCH (Fig. 1). Five were not included in this study because they are near Banff Townsite receive relatively high human use, and are rarely used by bears (Clevenger and Waltho 2005). We set up our hair-sampling system by stretching two lengths of barbed wire perpendicular to the line of movement in order to snag hair from passing bears. We secured two metal stakes at each end of a track monitoring pad, and attached the two

strands of wire at a height of 30 cm and 70 cm, respectively. Woven page-wire fence material and tree branches were piled at the ends of the barbed wire in order to funnel animals through the hair sampling system (see Clevenger and Sawaya 2010 for a detailed description). We checked the barbed wire for hair every two days in conjunction with routine track pad monitoring at the wildlife crossing structures. We collected hair samples from each barb separately and also collected discrete clumps of hair below the wire. We placed samples in paper envelopes labelled with a uniquely numbered barcode and sterilized every barb from which we collected hair with an open flame in order to prevent DNA contamination of subsequent samples.

We collected hair from black and grizzly bear populations in the Bow Valley of BNP using hair traps and bear rubs. Detailed descriptions of hair collection from hair traps and bear rubs can be found in Sawaya et al. (*In Review [b]*).

Genetic Analysis

We stored hair samples at room temperature on silica desiccant. Samples were analyzed at Wildlife Genetics International (Nelson, British Columbia), a lab that specializes in analysis of noninvasive genetic samples. We extracted DNA from all samples with ≥ 1 guard hair follicle or 5 underfur hairs, using up to 10 guard hairs with underfur when available. The lab used protocols for DNA extraction and microsatellite analysis of samples described by Paetkau (2003) and validated by Kendall et al. (2009). All DNA extraction was performed using QIAGEN's DNeasy Tissue Kit (Qiagen, Valencia, CA). We used seven microsatellite loci developed by Paetkau et al. (1995) that have become

standard for individual identification of grizzly bears in the Rocky Mountains: G10J, G1A, G10B, G1D, G10H, G10M, and G10P.

Results from noninvasive genetic sampling studies can be biased by low power to distinguish individuals (i.e. shadow effect) and genotyping errors such as allelic dropout and false alleles that result in the creation of false individuals (Waits & Leberg 2000, Mills et al. 2000, Creel et al. 2003). We therefore used variable markers and genotyping error and removal procedures developed by Paetkau (2003). We attempted all seven microsatellite loci for all hair samples for which we were able to extract DNA. We excluded samples from further analysis that produced high confidence genotypes for <3 markers. For the samples that produced 3-5 locus genotypes on the first pass, we re-analyzed the markers, but excluded samples from further analysis if they failed on the second pass.

Once we had complete 7-locus genotypes, we began the error-checking and removal process by identifying pairs of genotypes that mismatched by one or two loci, a potential warning sign that a genotyping error occurred (Paetkau 2003; McKelvey & Schwartz 2004). We scrutinized the results from any pairs of samples that differed by one or two loci and re-analyzed loci until we were confident in the results. Once we had complete multi-locus genotypes, we analyzed one sample from each individual for gender using amelogenin (Ennis & Gallagher 1994; Pilgrim et al. 2005). We extended genotypes to 20 loci using the following additional loci: G10C, G10L, CXX110, CXX20, Mu50, Mu51, Mu59, G10U, Mu23, G10X, MSUT-2, REN145PO7, and CPH9 (Taberlet et al 1997, Paetkau et al. 1995, Kitahara et al 2000, and Breen et al. 2001). We calculated

marker power using data from 135 grizzly bears and 103 black bears identified from hair and tissue samples collected in BNP. We used GENALEX (Peakall & Smouse 2006) to calculate expected allele frequencies, expected heterozygosity (H_e), observed heterozygosity (H_o), the probability of identity (P_{ID}) that two randomly drawn individuals would share the same multi-locus genotype and the probability that full siblings would have identical multi-locus genotypes (P_{SIB}). We used GENALEX to evaluate all 20 loci for conformance to Hardy-Weinberg proportions by testing for heterozygote deficiency and linkage disequilibrium.

Genetic structure and gene flow

We used three different methods for identifying bears that crossed the TCH. We defined a migrant as any bear that we detected moving across the highway (Proctor et al. 2012) using any one of three criteria: 1) bear detected with hair collection system at wildlife crossings, 2) bear detected on both sides of the highway using NGS methods (i.e. bear rubs and/ or hair traps), and 3) bear cross-assigned to population of origin using assignment tests. For bears that were detected on both sides of the TCH with NGS, we assigned bears to the north or south populations based on the majority of their locations or their last known location in order to maximize our ability to detect migration events.

We examined isolation by distance patterns in black and grizzly bears using Mantel tests (Mantel 1967, Smouse et al. 1986). Geographic distance was calculated as the Euclidean distance (in meters) between pairs of individual locations. We determined bear locations from sampling points using GPS and recorded geographic locations in the Universal Transverse Mercator coordinate system. Many of the bears in the study were

located more than once. For these bears, we determined geographic coordinates for individual bears by calculating centroids for Minimum Convex Polygons for individuals with ≥ 3 locations. Otherwise, we used 1 point or averaged 2 locations to represent an individual's detection center on the landscape.

We used Alleles In Space (Miller 2005) to calculate Nei's (1983) genetic distances and to perform Mantel tests. For partial Mantel tests, we used GENEPOP (Raymond and Rousset 1995, Rousset 2008) to calculate the individual pair-wise genetic distance of, Rousset's $\hat{a}$ (2000), similar to $F_{ST}/(1-F_{ST})$, which is useful for examining isolation by distance in individuals (Rousset et al. 1997). We employed a partial Mantel test in the R package ECODIST v 1.2.3 (Goslee and Urban 2010) to determine whether the TCH has a significant effect on genetic distance by treating distances from the same or opposite sides of the highway as a variable. We used Alleles In Space to examine our data for spatial autocorrelation and allelic aggregation and to test for the presence of a barrier effect on genetic distance using Monmonie's algorithm (Monmonie 1973).

We calculated population differentiation (F_{ST}) between north and south populations using GENALEX. We used individual clustering methods to examine current population genetic structure and identify recent migrants. We first used multidimensional Factorial Correspondence Analysis (FCA) in program GENETIX (Belkhir 1999) to examine genetic structure in black and grizzly bear populations. FCA uses allele sharing to cluster individuals in a multidimensional hyperspace based on allele sharing similarities using no a priori assumptions about population origin (Proctor et al. 2012). We used program STRUCTURE to determine the number of populations present

in the study area, and to identify admixed individuals or inter-population migrants (Pritchard et al. 2000). We determined the number of populations by testing different values of k from 1 to 4 and using the ad hoc approach described in Evanno et al. (2005). We ran 100,000 burn-in iterations and collected data on the following 100,000 runs. We identified migrants based on cross-population assignment probabilities (Proctor et al. 2012).

We used programs PARENTE (Cercueil et al. 2002) and CERVUS (Marsall et al. 1998, Kalinowski et al. 2007) to conduct parentage analysis using multilocus genotypes. We identified maternal and paternal relationships simultaneously using probabilities of all potential parent pairs. Identifying both parents simultaneously provides more accurate parentage assignments and allows directionality of relationships to be inferred which would not be possible with hair samples alone. We used additional data (e.g., captures, mortalities, remote photographs, etc) to confirm age classes and relationships whenever possible. We examined relative reproductive success of bears using crossings and parental-offspring relationships of crossers.

RESULTS

Sampling

We conducted hair sampling at 20 wildlife crossings structures in the Bow Valley between 15 May – 18 October 2006, 22 April – 29 October 2007, and 22 April–18 October 2008 (Fig. 1). We collected 1317 hair samples from wildlife crossings. We established hair traps from 25 May - 9 August 2006 and 28 May - August 2008. During

five 14-day sessions, we collected 2009 hair samples from 420 traps ($n=210$ in 2006 and 2008). We surveyed bear rubs from 10 September - 21 October 2006, 3 June - 27 October 2007, and 28 May – 27 October 2008. We monitored 330 bear rubs in 2006 and 497 bear rubs in 2007 and 2008. We collected 6543 hair samples from bear rubs. We obtained additional DNA samples (tissue or hair) from bear management actions and wildlife crossings in the BVSA between 15 May – 18 October 2006, 22 April – 29 October 2007, and 22 April–18 October 2008.

Genetic analysis

Marker power was high for grizzly and black bears using twenty loci (Table S1). $P_{(ID)}$ was $5.0E-18$ and for $7.0E-24$ black bears. $P_{(SIB)}$ was $1.2E-07$ for grizzly bears and $3.5E-09$ for black bears. Mean H_o across all 20 loci was higher for black bears (0.79) than for grizzly bears (0.66). The mean number of alleles per locus was also higher for black bears (9.4) than for grizzlies (6.9).

We identified a total of 113 unique grizzly bears and 101 black bears from multi-locus genotypes across all NGS methods and years. Genetic analysis of samples collected at wildlife crossings identified 15 grizzlies (8 M, 7 F) and 17 black bears (9M, 8F) using underpasses and overpasses to traverse the TCH (Table 1). We identified 42 grizzly bears and 76 black bears from hair trap samples and 107 grizzlies and 38 black bears from bear rub samples.

Genetic structure and gene flow

We observed isolation by distance patterns for black and grizzly bears in the Bow Valley (Fig. 2). Mantel test results confirmed a positive correlation for black bears ($r=0.202$, p -

value=0.001) and grizzlies ($r=0.104$, $p\text{-value}=0.002$). The correlation between genetic distance and geographic distance was stronger for female ($r=0.298$, $p\text{-value}=0.001$) than for male black bears ($r=0.137$, $p\text{-value}=0.007$). There was significant correlation for female grizzly bears ($r=0.246$, $p\text{-value}=0.001$), but no statistically significant correlation for male grizzly bears ($r=0.004$, $p\text{-value}=0.444$). The partial Mantel test showed an effect of the highway on genetic distance for grizzly bears ($r=0.098$, $p\text{-value}<0.0001$) but not for black bears ($r= -0.008$, $p\text{-value}=0.725$). The effect of the highway on genetic distance was stronger for female ($r=0.127$, $p\text{-value}<0.0001$) than for male grizzly bears ($r=0.068$, $p\text{-value}=0.008$). Using Monmonie's algorithm, we did not detect significant genetic barriers in black or grizzly bear populations in the Bow Valley.

We found more genetic differentiation for grizzly bears than for black bears across the TCH. F_{ST} was low for both species, but higher for grizzly bears ($F_{ST}=0.02$) than for black bears ($F_{ST}=0.007$). Visual inspection of FCA plots also indicated little genetic structuring in black bears (Fig. 3), but revealed a genetic discontinuity for grizzly bears across the TCH with clustering of individuals from the north and south mostly separated across the primary multidimensional axis (Fig. 4). Program STRUCTURE identified 1 population and indicated no genetic structure in black bears due to the TCH. Conversely, STRUCTURE identified 2 populations of grizzly bears that clustered across the highway (Fig. 5). STRUCTURE identified 2 male grizzly migrants in the north and 6 migrants in the south, including 1 female and 5 males. Many of the migrants identified directly with NGS were also detected at crossing structures (Table 1). The number of male grizzly migrants STRUCTURE identified was comparable to the number identified at crossings

and via NGS (Table 1).

Parentage analysis revealed 27 parent-offspring pairs for black bears and 48 for grizzly bears. We used 136 grizzly bears and 105 black bears in our parentage analysis, including 23 grizzly bears and 4 black bears that were identified from mortalities, bear management actions or during the pilot study at the wildlife crossing structures (Clevenger and Sawaya 2010). Many of the male and female bears using wildlife crossings are successful breeders, including 8 out of 17 black bears (Table 2) and 4 out of 15 grizzlies (Table 3). We identified 27 black bear offspring-parent pair triads and many of the offspring were assigned to parents (18 paternal and 7 maternal relationships) that used crossing structures to traverse the TCH. Many offspring were also assigned to parents (6 paternal and 6 maternal relationships) that used crossing structures to traverse the TCH. More grizzly bear crossers than black bear crossers were assigned to mothers and fathers who also used the crossings (Table 3).

Bears using crossings were assigned to a high percentage of offspring-parent pairs. The male black bear that was detected most frequently at crossings had the highest relative reproductive success of any bear, being assigned paternity in 11 out of 27 triads. This bear mated with 5 different females and fathered numerous offspring which were detected on both sides of the TCH (Table 2). One male grizzly was assigned paternity in 5 out of 48 triads, mated with a female who frequently used crossings and fathered 3 offspring who were all subsequently detected using crossings (Table 3). Another male grizzly assigned to the north of the TCH ($p=0.95$) mated with a female from the south ($p=0.99$) and produced a female offspring who had almost equal assignment probabilities

to both populations ($p=0.42$ to north and $p=0.58$ to south). All 3 bears were recovered as mortalities that provided age information verifying the two putative parents were adults and the offspring was conceived in 2005, born in 2006 and died as a yearling in 2007. The mother was detected using crossings during our pilot study (Clevenger and Sawaya 2010) in 2005, the year of conception.

DISCUSSION

Population fragmentation from roads can reduce migration rates and genetic diversity, yet there is little evidence that highway mitigation measures such as wildlife crossings to allow genetic interchange remains almost entirely unknown (Corlatti et al. 2009, Kaplan 2009). We provide the first empirical evidence of gene flow occurring at wildlife crossings by documenting migration, reproduction and genetic admixture in two wide-ranging fragmentation-sensitive species, black and grizzly bears. We determined there are significant differences between the effects of the TCH on black bears versus grizzly bears, but the highway did not isolate populations of either species. We conclude wildlife crossings provide genetic connectivity for two large carnivore species across a major national transportation corridor that bisects one of the world's preeminent protected areas.

Although distinguishing gene flow from migration between fragmented populations is difficult, our study was able to examine three components of geneflow separately: migration, successful reproduction and genetic admixture. New individual-based genetic analyses are more sensitive to detecting recent gene flow than population

based methods (e.g., F_{ST}) which offer better historical perspectives (Manel et al 2003, Landguth et al. 2010). Significant time lags can exist for genetic discontinuities to develop after barrier formation (Landguth et al. 2010b) and these time lags make it especially difficult to detect changes in gene flow for species with long-generation times such as grizzly and black bears (Proctor et al. 2012). Individual-based methods can detect recent migration events, but many studies that use them infer functional connectivity from detection of inter-population migrants, even though there may be huge discrepancies between migration and gene flow (Strasburg 2006). Our study was able to demonstrate grizzly and black bear gene flow at wildlife crossing structures by combining individual based genetic analyses with parentage analysis to document successful reproduction and genetic admixture in addition to migration.

We observed isolation by distance patterns in black and grizzly bears consistent with other studies on bears in the region (Proctor et al 2012). Isolation by distance was stronger for females than for males (Fig. 2), which we predicted given average male dispersal distances are typically greater than female dispersal distances for black and grizzly bears. Proctor et al. (2004) estimated sex-specific dispersal distances using genetic analysis and found females disperse 14.3 km whereas males disperse 41.9 km on average in the Canadian Rockies. The long dispersal distances for male grizzly bears along with our relatively small study area probably explain the lack of statistically significant correlation we found between genetic and geographic distances in male grizzly bears. The results from our Partial Mantel test indicate the TCH does not affect genetic structure of black bears, but does affect structure of male and female grizzlies.

Results of F_{ST} , Factorial Correspondence Analysis and model-based clustering in STRUCTURE concurred there is only minor structuring in the grizzly bear population and no detectable genetic structure in black bears in the Bow Valley. Detecting genetic structure in the presence of isolation by distance can be quite difficult, but our use of multiple analyses to examine genetic structure allows more powerful inference for linking landscape features and genetic differentiation (Balkenhol et al. 2009b). The FCA plot for black bears shows no visible genetic structure associated with the TCH and indicates there is only one population with almost complete admixture (Fig. 3). The FCA plot for grizzly bears shows genetic differentiation along the primary axis and shows clustering of individuals detected to the north and south of the TCH (Fig. 4). Output from program STRUCTURE agreed there were two populations that clustered north and south of the highway (Fig. 5) providing multiple lines of evidence of genetic structure in the grizzly population.

We documented extensive inter-population movements of black and grizzly bears across the TCH using our 3 different methods for migrant detection. The number of male and female black bear migrants we detected with all 3 methods (Table 1) most likely explains the lack of genetic differentiation associated with the TCH (Fig. 3). The number of male and female grizzly bear migrants detected at the wildlife crossings and with NGS suggest high levels of inter-population movements (Table 1). Of the bears that we detected on both sides of the TCH with NGS methods, very high percentages were using wildlife crossings indicating grizzly and black bears making inter-population movements are highly likely to traverse the TCH at wildlife crossings as opposed to climbing over

the exclusion fence or moving around the ends of the fence (Table 1). All of the female black and grizzly migrants detected with NGS were also detected using wildlife crossings, suggesting wildlife crossing structures likely play a particularly important role in increasing highway permeability for female bears.

Sex-biased dispersal across major highways without mitigation measures has been well-documented in grizzly bears (Gibeau et al. 2002, Proctor et al 2004, Proctor et al 2012). We obtained comparable estimates of grizzly male migration rates with all 3 migrant detection methods (Table 1). The number of female migrants we detected using direct methods (i.e. NGS, wildlife crossings), however, was considerably higher than using indirect, statistical methods (i.e. STRUCTURE). This discrepancy reveals differences between direct and indirect methods for identifying migrants and shows the importance of using multiple, independent methods to examine inter-population movements. One to ten migrants-per generation has been proposed as the amount necessary to overcome genetic isolation while allowing for local adaptation (Mills and Allendorf 1996, Wang 2004). The amount of migration across the TCH we've documented far exceeds the levels of movement that would be necessary to prevent genetic isolation, but only if migrants also successfully reproduce on the other side.

Research suggests that many individuals that use crossings such as culverts to traverse busy highways are juveniles and thus less likely to successfully breed due to their social status (Riley et al. 2006, Kaplan 2006). Contrary to these findings, our results show that relatively high percentages of black (47%) and grizzly bears (27%) using crossings are in fact successful breeders (Tables 2 and 3). Interestingly, males detected

using crossings most frequently also have the highest relative reproductive success of male crossers. This suggests a positive correlation between use of crossings and number of breeding opportunities for males, although further study is warranted to determine if wildlife crossing use actually increases reproductive success. Remote photographs have shown the highly reproductive male black bear (M1, Table 2) aggressively pursuing female black bears through wildlife underpasses, so we speculate it's possible that wildlife crossings act as meeting and courtship sites or provide escape passages for uninterested females. Because we only considered triads in our paternity and maternity assignments in order to infer directionality, we believe that our estimates of relative reproductive success are quite conservative; therefore breeding success of bears using crossings is probably much higher than we've been able to document in our study.

A higher percentage of grizzly bears than black bears detected at wildlife crossings are related (Table 2 and 3). This is not surprising as Sawaya et al (*In Review [b]*) documented a steady increase in grizzly bear use between 1996 and 2008, and we speculate that some of the increase in use is due to offspring learning to use crossings with their mothers. Remote photographs revealed long ago that bear cubs and yearlings of both bear species use crossings with their mothers to traverse the TCH, but now we have independent proof from maternity assignments. We also know now that a single family group accounted for a high percentage of female grizzly bear crossings (Table 3), therefore many of the most important crossings that we documented (i.e. female grizzly bears) were from non-reproductively active bears unable to contribute to the gene pool. The benefit to female offspring using crossings would be that they in turn use crossings

as independent adults after learning the behavior from their mothers. Unfortunately, none of the three daughters in this family group survived long enough to reach adulthood in the Bow Valley, a place where human caused mortality remains high for grizzly and black bears (Benn and Herrero 2002, Bertch and Gibeau 2009, 2010).

Genetic admixture was detected in grizzly bears using FCA and population assignment in STRUCTURE. Research has shown that gene flow can be unidirectional (Dixon et al. 2006, Howes et al. 2009), but our results suggest that geneflow at crossings in Banff National Park occurs in both directions across the TCH. Visual inspection of FCA plots indicates that black bear and grizzly bear individuals using crossings are freely mixing with individuals from the north and south supporting our contention that crossing structures allow gene flow and genetic admixture between populations (Figs 3 and 4). Although we were unable to reliably assign black bear individuals to populations of origin due to a lack of genetic differentiation, we found evidence of genetic admixture in black bears by detecting offspring of black bear parents that use crossings on both sides of the TCH (Table 2). Assignment tests of grizzly bears indicate that male and female grizzlies from north and south populations use crossings, providing evidence of bidirectional gene flow and genetic admixture at crossing structures (Table 3). Our documentation of a female grizzly bear using wildlife crossings to traverse the TCH and successfully mate with a male grizzly from the north provides compelling, though anecdotal, evidence of gene flow at crossing structures.

Genetic methods can be useful to infer demographic as well as genetic processes (Lowe and Allendorf 2010). The majority of grizzly bears we detected at crossings were

assigned to the south (Table 3). In most cases, population assignments were congruent with the frequency of detections to the north or south of the TCH. Given the habitat differences between the north and south of the TCH, we speculate that bears from the south move north of the highway to access seasonally available food resources on south-facing slopes. Black and grizzly bear detection centers were distributed unevenly across the landscape, with black bear locations closer to roadways and developed areas than grizzly bears (Fig. 1). This pattern along with the disparity in the effect of the TCH on genetic structure between black and grizzly populations may reflect differences in tolerance for human disturbance and could be associated with inter-specific resource partitioning.

Although the TCH appears to affect black and grizzly bear gene flow differently, it is clear that male and female individuals using crossings structures are successfully migrating, breeding, and moving genes across a major transcontinental highway. These results are encouraging given that Proctor et al. (2012) found genetic discontinuities in grizzly bears in the Western United States and Canada were most often associated with high traffic volume highways and human settlement. The authors suggested that the relatively weak genetic structure in grizzly bears across the TCH in Banff compared to a section of TCH ~100 km to the west could be attributed to the presence of wildlife crossing structures. We concur that wildlife crossing structures may be helping to counteract the effects of fragmentation on grizzly bears across the TCH.

Before-After-Control-Impact (BACI) study designs provide high inferential strength, however few studies have used them for assessing crossing structure

effectiveness due to difficulties collecting data prior to road mitigation (Roedenbeck et al. 2007, McCollister and van Manen 2010). In the absence of BACI, the use of direct and indirect methods to detect migration, successful reproduction, and genetic admixture provides sound evidence based data to assess the genetic benefits of crossing structures for wildlife populations. In conclusion, we have demonstrated the ability of wildlife crossing structures to provide genetic connectivity for two fragmentation-sensitive carnivore species across a major transcontinental highway.

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TABLES

Table 1: Number of black and grizzly bear migrants detected across the Trans-Canada Highway in Banff National Park, Alberta, Canada using noninvasive genetic sampling (NGS) methods between May 2006 and October 2008.

	Black bears			Grizzly bears		
	Total	M	F	Total	M	F
<u>Total</u>						
Detected with NGS	101	46	55	113	66	47
<u>North population</u>						
Detected to north of highway with NGS	57	24	33	73	41	32
Assignments to north from STRUCTURE	N/A	N/A	N/A	69	40	29
<u>South population</u>						
Detected to south of highway with NGS	52	28	24	54	33	21
Assignments to south from STRUCTURE	N/A	N/A	N/A	44	26	18
<u>Migrants</u>						
Detected at wildlife crossings	17	9	8	15	8	7
Detected on both sides of highway with NGS	6	4	2	11	6	5
Cross-population assignments from STRUCTURE	N/A	N/A	N/A	8	7	1
% NGS migrants detected at wildlife crossings	83%	75%	100%	91%	83%	100%
% STRUCTURE migrants detected at wildlife crossings	N/A	N/A	N/A	13%	29%	0%

* Three different criteria were used to identify migrants: detections at wildlife crossings, NGS detections spanning the highway, and repeated cross-population assignments in program STRUCTURE (Pritchard et al. 2000); black bear assignment probabilities were too low for reliable population assignment.

Table 2: Summary information for black bears detected at wildlife crossing structures in Banff National Park, Alberta, Canada between May 2006 and October 2008.

Black bear	# Crossings	# Offspring	# Mates	Mother	Father	# Detections		Population assignment
						North	South	
F1	21	0	0			1	2	N/A
F2	11	3	1	F7	M1	3	1	N/A
F3	8	1	1			3	0	N/A
F4	3	0	0			0	6	N/A
F5	2	0	0			1	0	N/A
F6	1	0	0			3	0	N/A
F7	1	3	1			3	0	N/A
F8	1	0	0			0	0	N/A
M1	24	11	5			3	2	N/A
M2	11	2	1			1	1	N/A
M3	5	2	1			1	0	N/A
M4	5	0	0			0	0	N/A
M5	4	2	2			0	0	N/A
M6	2	0	0			0	2	N/A
M7	2	0	0			1	1	N/A
M8	1	1	1			3	0	N/A
M9	1	0	0			1	0	N/A

* Bear ID (F=female, M=male), number of crossings, number of offspring, number of mates, ID of mother if she was detected at the crossings, ID of father if he was detected at crossings, number of detections to north or south of Trans-Canada Highway, and population assignment determined using program STRUCTURE (Pritchard et al. 2000); bears were ranked according to their number of crossings. Assignment probabilities were too low for reliable population assignment of black bears.

Table 3: Summary information for grizzly bears detected at wildlife crossing structures in Banff National Park, Alberta, Canada between May 2006 and October 2008.

Grizzly bear	# Crossings	# Offspring	# Mates	Mother	Father	# Detections		Population assignment
						North	South	
F1	18	3	1			1	2	South
F2	9	0	0			22	1	North
F3	7	0	0	F1	M1	1	17	South
F4	4	0	0	F1	M1	1	8	South
F5	4	0	0			4	1	North
F6	3	0	0	F1	M1	0	1	South
F7	2	3	2			0	6	South
M1	34	5	2			16	58	South
M2	29	0	0			3	36	South
M3	15	0	0			7	26	South
M4	7	0	0	F7	M6	12	8	South
M5	6	0	0			3	2	North
M6	4	1	1			0	34	South
M7	1	0	0			0	25	North
M8	1	0	0	F7		0	0	South

* Bear ID (F=female, M=male), number of crossings, number of offspring, number of mates, ID of mother if she was detected at the crossings, ID of father if he was detected at crossings, number of detections to north or south of Trans- Canada Highway, and population assignment determined using program STRUCTURE (Pritchard et al. 2000); bears were ranked according to their number of crossings.

FIGURE LEGENDS

Figure 1. Detection centers for individual black and grizzly bears detected using noninvasive genetic sampling methods in the Bow Valley of Banff National Park (BNP), Alberta, Canada between 21 April and 31 October of 2006-2008.

Figure 2. Plots of geographic distance in meters vs genetic distance (Nei 1983) to examine Isolation By Distance patterns in black bear males (A), females (B), and grizzly bear males (C) and females (D) detected in the Bow Valley of Banff National Park, Alberta, Canada between 21 April and 31 October 2006-2008.

Figure 3. Factorial Correspondence Analysis plot from program GENETIX (Belkhir 1999) showing multidimensional clustering of individual black bears detected north and south of the Trans-Canada Highway in Banff National Park, Alberta, Canada using noninvasive genetic sampling methods. Individuals detected at wildlife crossing structures are displayed as “crossers”.

Figure 4. Factorial Correspondence Analysis plot from program GENETIX (Belkhir 1999) showing multidimensional clustering of individual grizzly bears detected north and south of the Trans-Canada Highway in Banff National Park, Alberta, Canada using noninvasive genetic sampling methods. Individuals detected at wildlife crossing structures are displayed as “crossers”.

Figure 5. Output from program STRUCTURE (Pritchard et al. 2000) showing clustering of grizzly bears detected north (1) and south (2) of the Trans-Canada Highway in Banff National Park, Alberta, Canada using noninvasive genetic sampling methods.

Figure S1. Noninvasive genetic sampling locations used to investigate the ability of wildlife crossing structures to provide genetic connectivity for grizzly and black bear populations in the Bow Valley of Banff National Park (BNP), Alberta, Canada between 21 April and 31 October of 2006-2008. We monitored 20 wildlife crossing structures with a hair sampling system developed by Clevenger and Sawaya (2010), 420 hair traps, and 497 bear rubs; we did not monitor 5 wildlife crossings due to high levels of human use and low levels of bear use (i.e. passages determined from track pads).

FIGURES

Figure 1

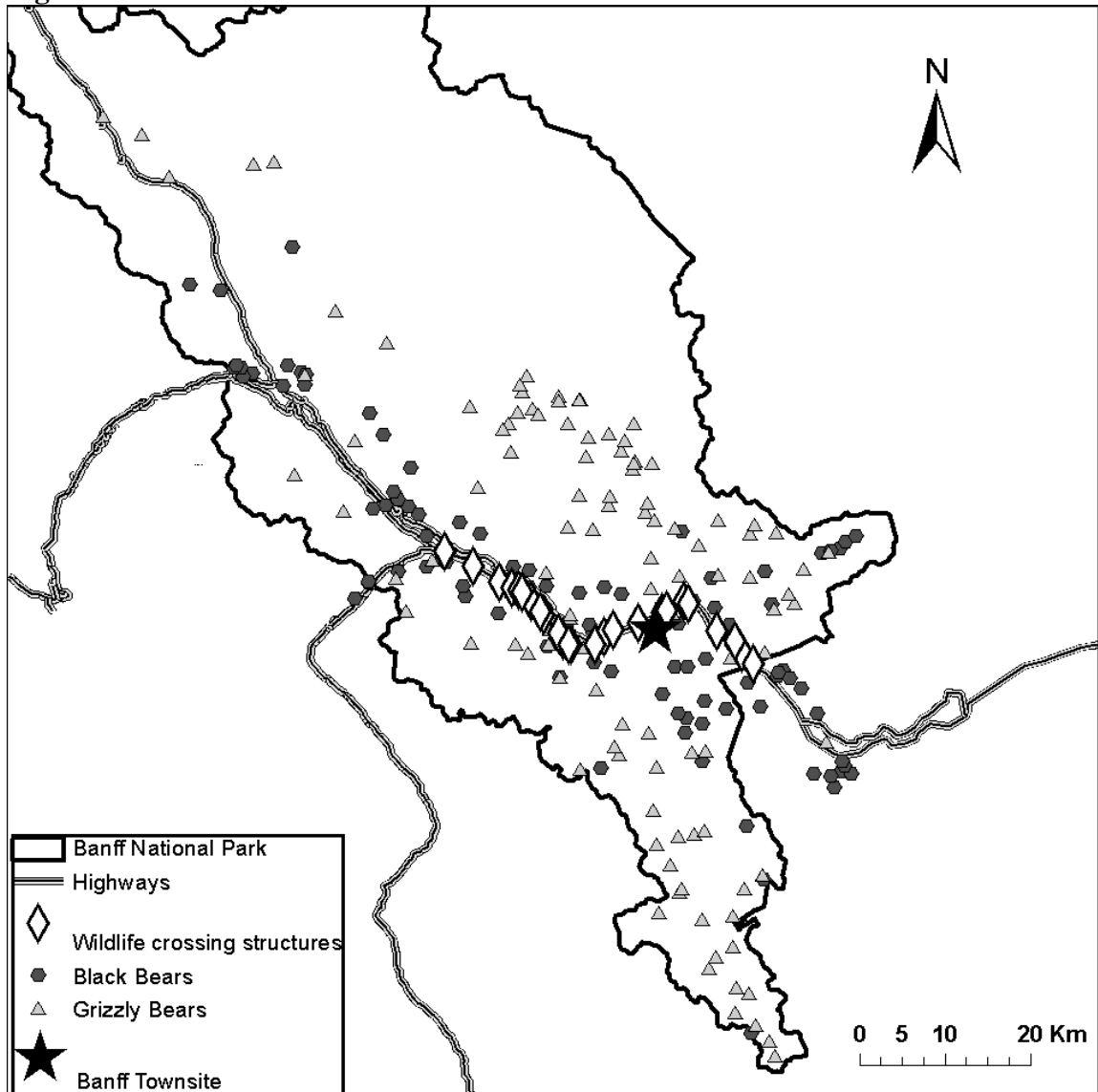


Figure 2

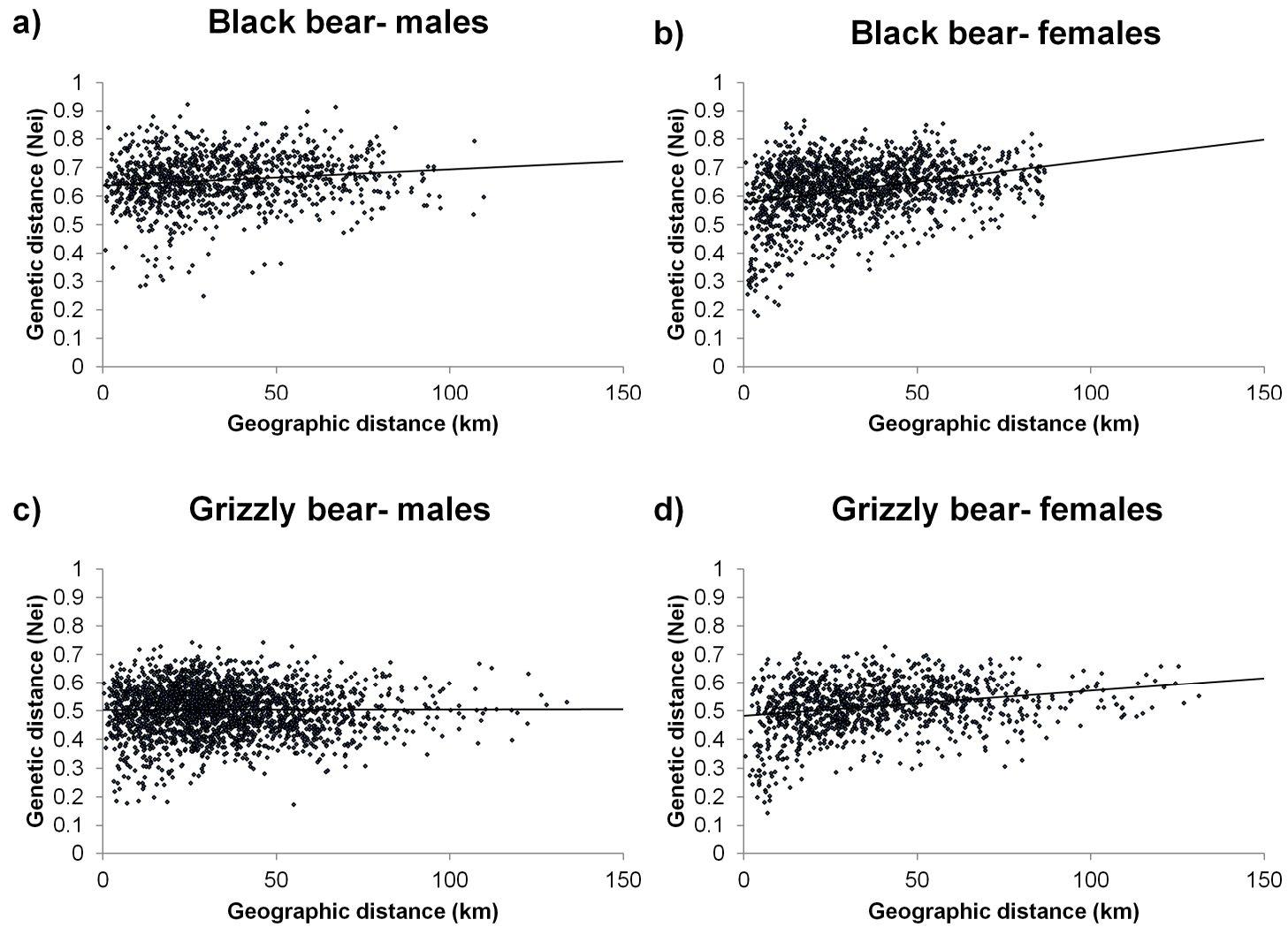


Figure 3

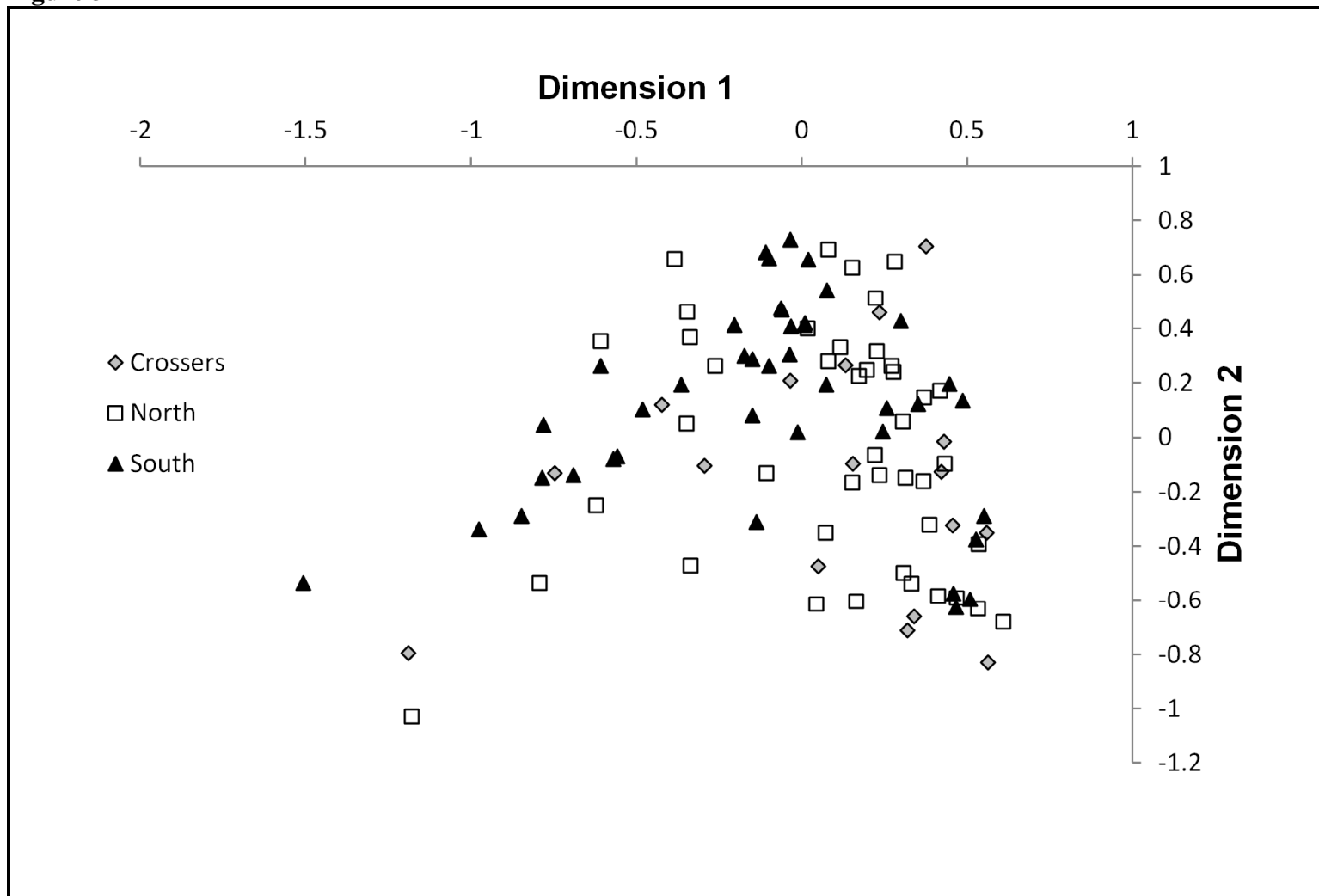


Figure 4

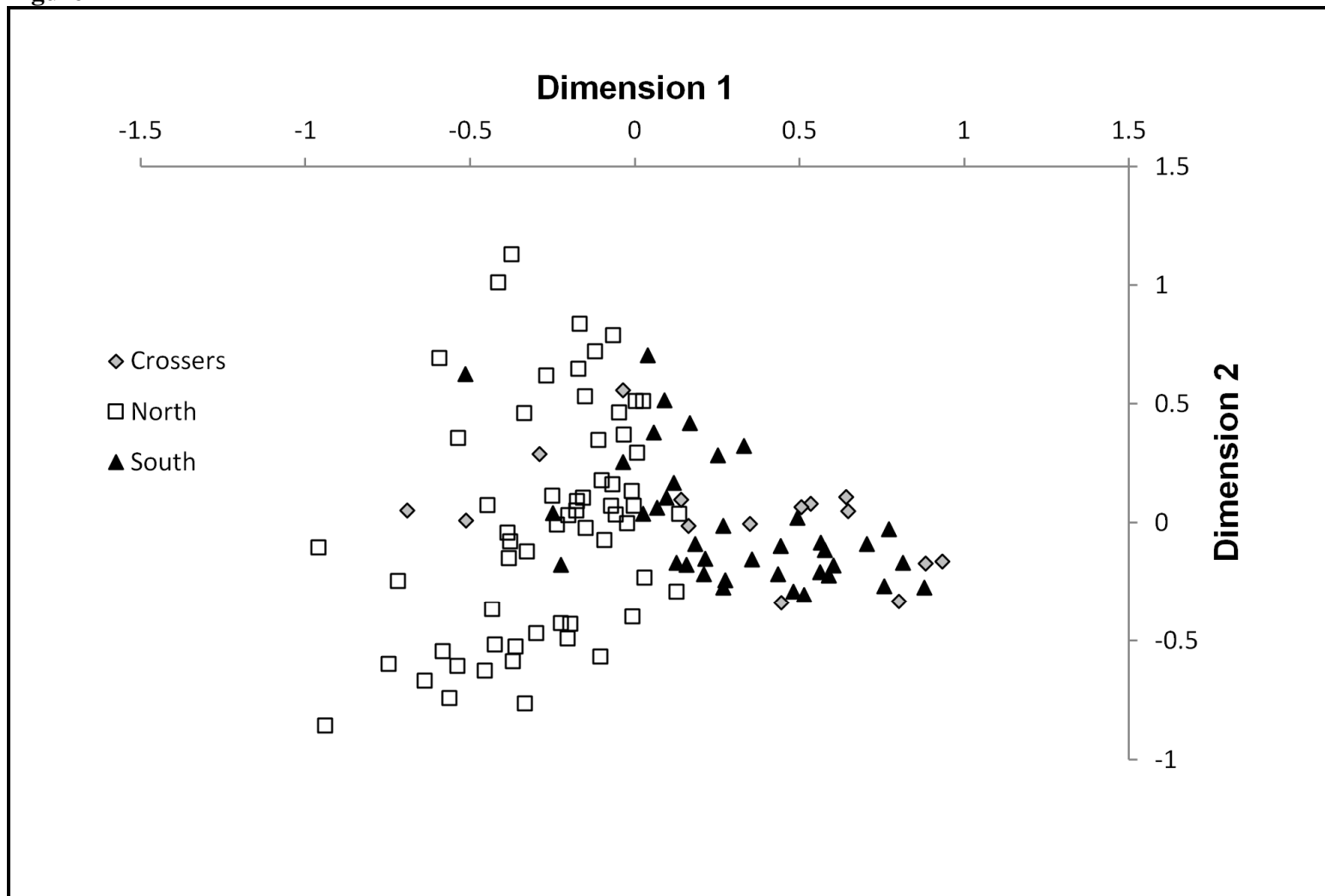
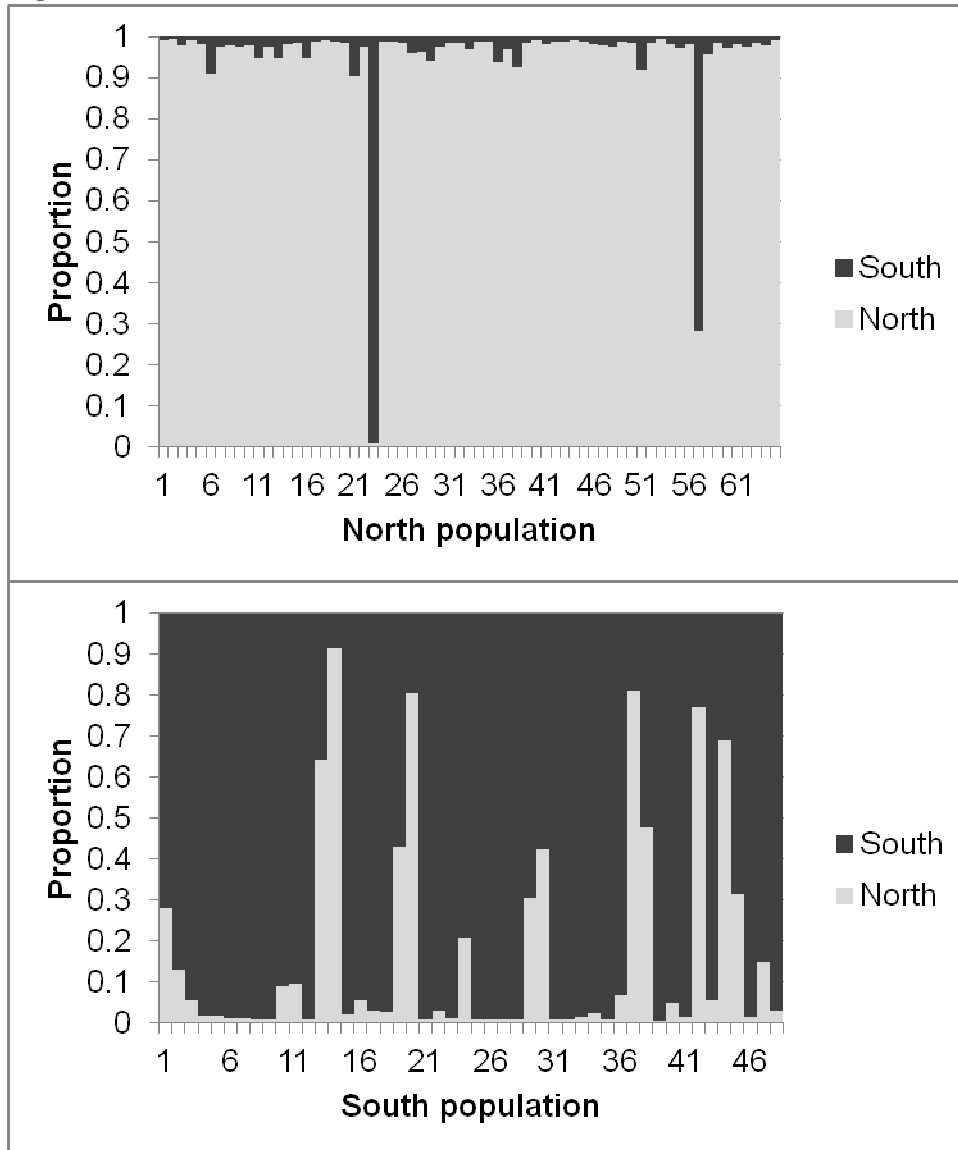
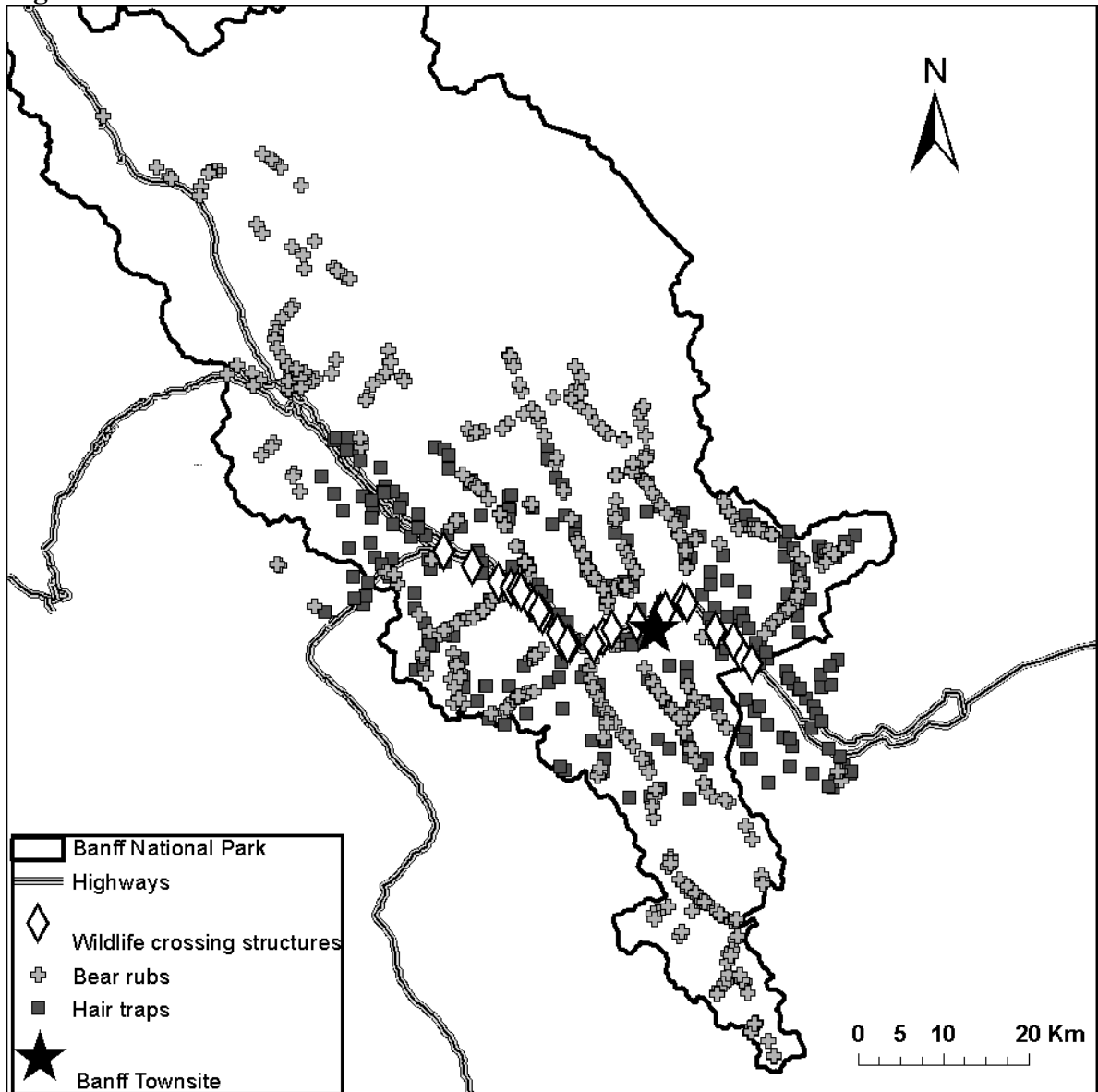


Figure 5

Supplemental Information**Table S1:** Microsatellite marker variability for 113 grizzly bears and 101 black bears detected with noninvasive genetic sampling in Banff National Park, Alberta, Canada.

Locus	Grizzly bears				Black bears			
	N	Na	Ho	He	N	Na	Ho	He
G10J	113	6	0.78	0.76	101	12	0.89	0.86
G1A	113	6	0.73	0.74	101	9	0.72	0.76
G10B	113	9	0.77	0.81	101	7	0.78	0.81
G1D	113	12	0.86	0.84	101	10	0.83	0.81
G10H	113	9	0.80	0.79	101	14	0.92	0.89
G10M	113	6	0.75	0.73	101	11	0.94	0.82
G10P	113	6	0.61	0.66	101	9	0.85	0.84
G10C	113	6	0.53	0.54	100	9	0.77	0.78
G10L	113	4	0.58	0.56	101	13	0.86	0.84
G10U	113	8	0.42	0.42	101	11	0.83	0.80
G10X	113	6	0.27	0.27	101	11	0.89	0.87
CXX20	113	6	0.52	0.55	101	8	0.72	0.77
CXX110	113	9	0.85	0.83	101	10	0.83	0.80
MU50	113	8	0.64	0.62	101	8	0.58	0.60
MU59	113	8	0.70	0.72	101	8	0.88	0.82
MU23	113	6	0.83	0.76	99	8	0.67	0.68
145P07	113	5	0.59	0.66	99	11	0.88	0.84
Msut2	113	6	0.66	0.70	99	9	0.87	0.83
Mu51	113	6	0.78	0.73	99	2	0.43	0.43
CPH9	113	5	0.50	0.54	99	8	0.64	0.73
Mean	113.00	6.85	0.66	0.66	100.45	9.40	0.79	0.78
SE	0.00	0.42	0.03	0.03	0.20	0.57	0.03	0.02

Figure S1



CONCLUSION

Successful conservation of carnivores is dependent on reliable estimates of population parameters which wildlife managers need to respond timely to threats and adapt management strategies before populations go extinct. Human-caused carnivore extirpation has been rapid and widespread due to overexploitation and population fragmentation, but new research opportunities now exist to understand these impacts that were unthinkable not long ago. Over the course of my doctoral research, I found that a wide array of noninvasive genetic sampling (NGS) methods can be used, alone or in combination, to estimate demographic and genetic parameters for large carnivore populations in the Rocky Mountains.

In Chapter 2, I studied cougars in Yellowstone National Park and was able to demonstrate that issues with NGS such as genotyping errors can be overcome with careful analysis procedures. I showed that reliable genetic data can be generated from carnivore hair samples collected in the field. I was also able to develop a new method for DNA collection from bed sites and natural hair snags (e.g. broken branch tips, thorn bushes) while following cougar tracks in snow. This method could be applied at larger scales over longer time periods to estimate abundance and population growth rates for cougars and other carnivore species in similar climates.

In Chapter 3, I showed that one of the few established NGS methods for bear population enumeration, hair traps, may not perform as well as bear rub surveys for estimating grizzly bear abundance in Banff National Park. I also found that hair traps performed better than bear rub surveys for detecting individuals and estimating

abundance of black bears. I estimated realized population growth rates for grizzly bears using data from bear rubs and found that the population may have declined in the Bow Valley during my study. In light of my results and the current status of grizzly bears in Alberta, I strongly recommend that wildlife managers immediately begin a population trend monitoring program in the Canadian Rocky Mountains based on bear rub surveys.

In Chapters 4 and 5, I used the genetic data collected from black and grizzly bear populations in Banff National Park to add context to data from wildlife crossing structures collected using a novel hair sampling system. This comparison allowed me to address pressing questions in road ecology related to the population-level effectiveness of wildlife crossing structures. I conclude that wildlife crossing structures provide demographic and genetic connectivity for ursid populations in Banff National Park; however, further studies using Before-After-Control-Impact study design or Population Viability Analysis are necessary to understand the ability of crossing structures to facilitate gene flow, increase survival, or enhance population viability.

With growing human pressures must come new methods for understanding and mitigating ever-expanding threats to biodiversity. My dissertation reflects a natural progression for me as a doctoral student and scientist as I addressed increasingly complex ecological questions all while using data generated from hair samples. I've shown that NGS can be effectively used to estimate abundance, realized population growth rates, apparent survival, migration rates, genetic structure and gene flow. In summary, I've demonstrated that NGS can provide efficient and reliable alternatives to conventional methods for monitoring large carnivore populations in the Rocky Mountains.

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