

FINAL REPORT

Using scat DNA and citizen science to determine grizzly bear distribution and abundance in West-Central, Alberta

Prepared for

Alberta Environment and Parks | Alberta Conservation Association

Final Report fRI Research Grizzly Bear Program

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Sorensen, A., S. Milligan, T. Larsen, K. Graham, H.G. Eiken, S. Hagen, S.G. Aarne, I.M. Bardalen Fløystad, G. Stenhouse. 2017. Using scat DNA and citizen science to determine grizzly bear distribution and abundance in West-Central, Alberta. Report prepared for Alberta Conservation Association and Alberta Environment and Parks







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January 2017



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Report Summary

Projects that involve citizen scientists are burgeoning, particularly in ecology and the environmental sciences. A significant advantage of citizen science is that it offers a way to collect information across large spatial extents that would otherwise not be economically feasible. In North America, traditional non-invasive hair-based DNA methods that have been used to estimate grizzly bear abundance and distribution remain too costly for long-term monitoring. It has been recognized that a citizen science based approach similar to that used to monitor European brown bear populations may offer a cost-effective solution for Alberta. However, spatial and temporal heterogeneity in detection probabilities are inherent challenges with citizen science, which can lead to lead to imprecise estimates that question the utility of the results for management. Strong sampling designs are very important in these types of projects. New projects involving citizen science should first evaluate potential study designs to determine the amount of volunteer effort that is needed and how data should be collected to ensure acceptable data quality relative to the goal and precision of population estimates. The primary objectives of this project were to (1) develop and test a non-invasive scat-based DNA approach for estimating grizzly bear abundance and distribution that engages citizens — particularly hunters and trappers — in the collection of scientific information. Our second objective was to (2) compare estimates of grizzly bear abundance and distribution from scat-based DNA methods to estimates from hair-based DNA methods, and (3) evaluate the costs and benefits of scat-based and hair-based genetic inventories to further assess the value of engaging citizens in grizzly bear population monitoring in Alberta.

To enable citizen volunteers to collect grizzly bear scat and record other important data (including search effort), we distributed scat collection kits and developed a smartphone application: the Grizzly Scat App. During the same period (August to October), we had fRI staff conduct transect surveys along linear features and quantified search effort. Scat samples were also collected opportunistically by fRI staff involved with the capture program and the hair-based genetic sampling project. Overall, 6 samples were collected by volunteers, 130 samples were collected from 154 transect surveys (with an average transect distance of 4.5 km), and 132 samples were collected opportunistically. A total of 196 samples (73%) were sent to the Norwegian Institute of Bioeconomy Research (NIBIO) in Svanhovd, Norway for genetic analysis. Bear DNA was extracted from 159 samples (81%), of which 105 samples (66%) were determined to be grizzly bear and 54 samples (34%) were determined to be black bear. From samples identified as grizzly bear, 45 samples (43%) successfully provided an individual DNA profile based on at least 2 markers, and 21 samples (20%) successfully provided an individual DNA profile based on at least 5 markers. From these 21 samples, we identified 17 individual bears (9 male, 8 female), including 6 individuals (3 male, 3 female) undetected by hair-based genetic sampling and previously unknown to researchers.

We were unable to directly compare grizzly bear abundance and distribution estimates from scat-based genetic sampling to estimates from hair-based genetic sampling due to the low volunteer participation, which resulted in reduced spatial coverage of sampling effort, fewer than expected scat samples, and thus few individual grizzly bear detections from scat. However, when we compare effort and detectability *post-hoc*, the average number of bears detected per sample unit was nearly the same. This suggested that similar results could be achieved by scat-based DNA inventories as hair-based DNA inventories if the number of sample units (i.e. volunteers) were increased. Significant cost savings could also be achieved with a greater number of citizen volunteers. We conclude that scat-based genetic sampling cannot replace hair-based genetic sampling for the purpose of grizzly bear population inventories at this time; however, the use of scat sampling to provide DNA data to support long-term grizzly bear population monitoring, particularly occupancy showed promise. The results of our study highlight the need to improve the implementation of a large scale large scale citizen science based project specific to grizzly bears in Alberta that focuses significant effort on strategies to improve volunteer participation and engagement.



Acknowledgements

Appreciation is extended to members of the public who participated in data collection for this project. We greatly appreciate the support of Alberta Environment and Parks staff in Hinton, Edson, Drayton Valley, and Rocky Mountain House as well as the staff at High Caliber Sports in Hinton and Trail's End Taxidermy in Drayton Valley for their help in distributing scat collection kits and GPS loggers to the public.

As part of an international research collaboration, scat samples were processed at the Norwegian Institute of Bioeconomy Research (NIBIO) in Svanhovd, Norway. We greatly appreciate the laboratory work of Siv Grethe Aarnes and Ida Floystad.

We thank our field crew members for their efforts in collecting scat samples during the 2014 field season: Cam McClelland, John Saunders, Terry Winkler, Lisa Card, Audrey Lorincz, Leonie Brown, Natasha Mackintosh, Brent Rutley, Sarah Fassina, Ed Healey, Kelly Mulligan, Sean O'Donovan, Theresa Westhaver, Amy Stenhouse, Karen Graham, Tracy McKay, Bryan Macbeth, and Terry Larsen. Administrative support and guidance was provided by Cemil Gamas and Risa Croken. Thanks to Julie Duval, Kevin Myles, and Josh Crough for GIS and data management support.

We greatly appreciate Alberta Environment and Parks providing accommodations for our staff at the Shunda Fire Base.

We thank Sean Kinney, who led the development and provision of the scat application and for support for the media components of this project. Thanks to the team at Punchcard Systems for their hard work and cooperation throughout the process of developing the app.

This project would not have been possible without the financial support and cooperation from our funding partners: Alberta Conservation Association, Alberta Environment and Parks, and partners supporting the grizzly bear program at fRI Research.



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1. Introduction

Grizzly bears are listed as a threatened species in Alberta, based on population inventory work that suggested fewer breeding individuals (359) than what is required for a secure population under IUCN criteria (1000 breeding individuals), high levels of human caused mortality, and habitat pressures related to human activities (Festa-Bianchet 2010). In 2008, a recovery plan was accepted and a second version of the plan is now in preparation (Stenhouse, pers. comm.). Overall, the major focus of management associated with grizzly bear recovery efforts has been to reduce human-caused mortality as a means to increase the provincial population. The plan also highlighted the need to reliably estimate and monitor changes in Alberta's grizzly bear population as part of recovery planning, and recommended that population inventory efforts were to be repeated at 5 year intervals. However, because inventory costs are high and grizzly bears have a number of characteristics (longevity, low reproductive rates, large home range, and cryptic behaviour) that makes them difficult to count (Bellemain et al. 2005, Kindberg et al. 2011), this remains a formidable challenge. As such, there is a need to explore new and alternative inventory methods and evaluate regionally appropriate study designs and sampling protocols.

In North America, researchers have largely relied on non-invasive DNA based methods using barbed wire hair traps (Woods et al. 1999, Boulanger et al. 2004*a*) and rub trees (Kendall et al. 2008) to estimate grizzly bear population size and trend. However, because these techniques are costly, their use as a long-term monitoring technique may be challenging to implement at a bear management area (BMA) scale. In contrast, Scandinavian wildlife managers use a non-invasive DNA based approach that uses grizzly bear scat located by hunter volunteers to estimate population size and trend on a more frequent basis. Recognizing that this may offer a cost-effective solution for Alberta, the fRI Research Grizzly Bear Program, in collaboration with international partners, have conducted extensive research over the past 4 years (2010-2014) to successfully develop common laboratory techniques needed to reliably extract DNA from scat samples collected from grizzly bears in west-central Alberta. These efforts have focused on field collection methods, storage and handling protocols and protocols for comparing genetic results between laboratories and genetic materials (scat vs. hair).

Research to date has focused on improving non-invasive DNA based techniques as follows: 1) understanding the sources of detection heterogeneity and biases associated with barbed wire sampling design and statistical estimators; 2) integrating other data sources (telemetry, rub trees, and habitat models) to improve precision of estimates (Boulanger and McLellan 2001, Boulanger et al. 2004*b*, 2006, 2008, Stetz et al. 2010, Rovang 2013), and 3) improving the analysis of DNA inventory techniques such as spatially explicit capture-recapture methods (Borchers and Efford 2008, Efford et al. 2013). In North America, much of this research stems from expensive mark-recapture studies using barbed wire hair traps, as these methods are well established and successful. In Europe, where wildlife managers successfully use hunter volunteers to collect scat samples for DNA analysis as a means to inventory and monitor bear populations (Kindberg et al. 2011), researchers have cautioned that these methods require careful study design in order to ensure acceptable data quality and precision of estimates. This is due to spatial and temporal heterogeneity in detection probabilities which, when coupled with the generally low detection probabilities of bears in general, can lead to imprecise estimates that question the utility of the results for management (Bellemain et al. 2005). As such, it is important when using volunteers for grizzly bear scat DNA collection that the heterogeneity in detection probabilities is minimized and the overall probability of detection is maximized. This can be achieved through additional sampling in areas where volunteers are unwilling to go or in areas where sampling effort was low (Bellemain et al. 2005).

Bellemain et al. (2005) suggested that hunter participation in Sweden provided the necessary workforce to accurately estimate grizzly bear (brown bear) population size across large spatial extents, and was crucial in local acceptance of the results of the



monitoring surveys. Engaging citizens in scientific enquiry can be a powerful tool for biologists and managers to achieve conservation objectives. Numerous examples of citizen participation in applied research ranging from climate change, invasive species, and population ecology and monitoring (Silvertown 2009) have demonstrated a wide array of benefits. Not only can large volumes of data be gathered across extensive areas and at a reduced cost, but citizen participation in science also contributes to the preventative and educational components of conservation by reconnecting people with nature, and more generally people with science and conservation issues (Devictor et al. 2010). Nonetheless, citizen science is not without challenges and there is often concern that data collected from volunteers is noisy and imprecise and therefore unable to match the question being addressed (Devictor et al. 2010). Others suggest that such assumptions are generally wrong (Schmeller et al. 2009) and that most of the challenges associated with data quality can be overcome if projects are well designed and standardized. For instance, data that has been collected by the public should be validated, assumptions and hypotheses should be stated explicitly and regardless of their simplicity, and as a reward for volunteer participation, researchers should provide volunteers with feedback on their contribution (Silvertown 2009).

With this understanding, the goal of this project was to develop a non-invasive scat based DNA approach for estimating grizzly bear population distribution and abundance that engages citizens, particularly hunters and trappers, in the collection of scientific information. The research team felt that this project would benefit significantly from taking place in the same field season as a concurrent barbed wire hair based DNA population inventory project occurring across Bear Management Area (BMA) 3. The project had three objectives:

- 1. Engage local hunters and trappers to participate in grizzly bear inventory work within the Yellowhead population unit by collecting samples of bear scat for DNA analysis during the fall 2014 hunting season.
- 2. Compare population distribution and abundance estimates using hair and scat datasets alone and in combination (relying on the concurrent 2014 DNA hair inventory project within the Yellowhead population unit).
- 3. Evaluate the costs and benefits associated with genetic inventories using hair and scat relative to these estimates to maximize the efficiency of future sampling designs and assess the value of engaging citizens in data collection.

2. Study Area

The study area (549181E, 5868258N, NAD 83 UTM Zone 11N; 28,529 km²) encompasses the Yellowhead population unit (BMA 3) (Figure 1). The study area is bordered by highway 16 to the north, highway 11 to the south and, for the purposes of this study, the federally protected National Parks to the west. However, the southern portion of Jasper National Park is considered at the provincial level to be part of BMA 3. Portions of the area have been designated as core and secondary grizzly bear conservation zones (Figure 1). Climate, soil, topography, and vegetation are characteristic of the Rocky Mountain and Foothills natural regions (Downing and Pettapiece 2006). Natural resource exploration and extraction, primarily from oil and gas activities, timber harvest, and open pit coal mining, occurs throughout the central and eastern portions of the study area. While recreational activities such as hunting, trapping, fishing, and off highway vehicle use are prominent in portions of the study area, the density of human activity varies greatly, with limited access in western portions of the study area. Indeed, the distribution of access features, primarily roads for the oil and gas and timber harvesting sectors, was an important factor influencing the feasibility of sampling across the study area, as unlike the concurrent 2014 DNA hair inventory project, we were limited to ground access for scat sampling without helicopter support for more remote areas.



3. Methods

3.1 Citizen science sampling

3.1.1 Public engagement and scat sampling

To connect with and recruit citizens for this project, particularly local hunters and trappers, we focused our communication efforts within the communities of Hinton, Edson, Drayton Valley, and Rocky Mountain House. From July to September 2014, we held public information sessions within each community to describe the project, and additional presentations were given at the annual Alberta Trappers Association Rendezvous in Edson, the Parkland Composite High School Outdoor Education Class in Edson, and the Safari Club International banquet in Drayton Valley. We contacted members of the Alberta Fish and Game Association and placed 75 targeted phone calls to encourage the participation of local hunters and trappers. To connect citizens with the project more widely in Alberta, we released information through a media tour on July 3rd, 2014. Additional communications and media attention occurred following the launch of the Grizzly Scat App and website (grizzlyscatapp.ca), which was an extension from the original project proposal. Advertisements were posted to the fRI Research website and through social media (i.e., Facebook ©, Twitter ©) as well as online advertisements. Announcements were printed and posted in local provincial parks and recreation areas. We also advertised the project using local newspapers such as The Mountaineer (Rocky Mountain House, AB), Parklander and Voice (Hinton), and Fitzhugh (Jasper); articles were published or information was posted to their websites or through their social media. The project also reached citizens by posting to online web forums such as the Alberta Outdoorsmen Magazine.

Through our communications, we encouraged citizens to obtain scat collection kits (i.e., rubber gloves, small wooden sticks, and vials containing silica), which we distributed to the Alberta Environment and Parks offices in Edson, Hinton, Rocky Mountain House, and Drayton Valley. Scat collection kits were also provided to High Caliber Sports in Hinton and Trail's End Taxidermy in Drayton Valley for distribution. Staff members at each of the distribution sites were responsible for signing out scat kits and collecting and storing any incoming samples. In addition to scat collection kits, we also provided GPS loggers (i-gotU - Mobile Action Technology Inc.) to distribute to individuals participating in the project who could not use the ScatApp (see below). Loggers would allow users to track their path and obtain GPS locations for scat samples collected within the study area. We require this information to spatially and temporally represent the data and the sampling efforts of citizens, even if no scats were encountered. After sample selection and analysis, we would contact each participant to indicate the status (sent for analysis of not) and results (grizzly bear or other) of scat sample(s) provided.

3.1.2 Smartphone application

In addition to collecting the biological samples (scat) from grizzly bears, it was important that spatiotemporal measures of search effort were recorded in order to develop and evaluate the efficiency and cost-effectiveness of non-invasive scat based DNA methods and future sampling designs. The complexity of using volunteer citizens to collect data across a large study area required a new approach for scientific data collection that could be used remotely and handle multiple types of data. In anticipation of this need, a smartphone application – The Grizzly Scat App – was developed and tested prior to this project. We made the Grizzly Scat App available to iOS (Apple) and Android (primarily Samsung devices) smartphone users at no cost. For this project, users simply downloaded the app and registered their information, after which the app would instruct the user to visit one of six distribution/collection depots in our four targeted communities to pick up a scat collection kit. Once the app was initiated by the user to log their route, it would run in the background collecting time and location information every 15 minutes (a cellular connection was not required). If scats were encountered, we instructed volunteers to collect approximately 1 cm³ of



biological material from the center (inside) of each scat and place the material into a vial containing silica beads. If multiple scats were encountered at a single location but could not all be sampled, we instructed volunteers to sample the freshest scat(s) or scat(s) with the most berries as these samples typically had the greatest success with DNA extraction and genotyping (unpublished data). Once the scat sample was placed inside the vial, the volunteer was instructed to open the smartphone application to (1) scan the unique bar code on the vial, (2) take a photo of the site, and (3) log any relevant notes. Time and location data were captured at the time of bar code scanning. When forays were completed and the app session was terminated, the volunteer's path and/or scat data was automatically transmitted securely to the fRI Research database when a cellular network connection is in place. However, cell service is not required for the app to function; data is simply stored until such a time that cellular service is regained. To keep each citizen volunteer engaged in the project and informed about their individual contribution, the app would send users updates regarding the status and result of their sample(s).

3.2 Supplemental sampling

3.2.1 Transect sampling and design

Due to our concern that citizen participation in this project might be low, we anticipated that public participation alone would not allow us to use scat sampling as a method for estimating grizzly bear population size across such a large study area. Therefore, fRI Research field staff established and sampled transects throughout the study area to conduct targeted searches for scat with a quantifiable measure of search effort. Transects were searched during the months of August and October. Efforts were made to distribute transects evenly across watershed units (Figure 1) deemed as core and secondary grizzly bear habitat, targeting areas where habitat values were estimated to be high based on resource selection function models (Nielsen et al. 2002). However, distribution of these transects were limited by road access, as we did not have helicopter support or the resources to dedicate hours of staff time accessing remote backcountry areas on foot. Transects were established along linear features such as seismic lines or pipelines as well as forestry cutblock edges. These features are presumed to act as low resistance travel corridors and foraging sites for grizzly bears (Mclellan and Hovey 2001, Nielsen et al. 2004, McKay et al. 2014). Transect length varied based on the linear features available, but averaged 4.45 km (min= 830 m, max= 10.95 km), typically in a path that routed field staff back to their starting point.

3.2.2 Opportunistic scat collection

In addition to transect searches, scat samples were also collected opportunistically by fRI Research grizzly bear program staff throughout the field season associated with concurrent projects. This included the previously mentioned DNA census that took place in BMA 3 during May to August 2014, along with capture work that occurred in BMA 3 during May to July. During the DNA census, barbed wire hair snag sites, which were distributed in a grid configuration (7 x 7 km cells) (Figure 5), were checked every two weeks for hair samples, for a total of 4 sessions (Stenhouse et al. 2015). Due to late spring snow conditions at higher elevations, hair snag sampling sessions for sites within the White Goat Wilderness Area (n=25) occurred about one month behind schedule of sites accessed by road (n=172). Scats discovered by staff en route to or near hair snag sites were collected, which included scats observed on roads. During capture operations, scats were also sampled when encountered, typically at trap sites or on roadways. The rationale for opportunistic sampling was to obtain genotypes for as many bears as possible, particularly cases where either bear hair was not snagged or animals were not captured.



3.3 Sub-selection of scat samples and genetic analysis

All samples collected by the public were submitted to the lab for genotyping. However, in an effort to minimize lab costs associated with duplicating unique genotypes, we developed a sub-selection protocol for scat samples collected on transects and for scat samples collected opportunistically.

Transects— Scat samples collected by field staff on transect routes that were greater than 200 m apart were considered unique samples, whereas adjacent samples within 200 m of each other were presumed to be deposited by the same bear, unless comments by staff regarding the size, age or contents of the scat indicated that more than one bear was likely present. If duplicate samples were suspected, the samples were ranked - based on direct sunlight (shadier ranks better), moisture level (drier ranks better), and size (too much or too little) - to indicate the order by which the samples should be analyzed at the lab. If the first ranked sample was genotyped successfully, the remaining samples were not analyzed. For adjacent samples that were not suspected to be duplicates, all samples were selected for genetic analysis.

Opportunistic samples collected by capture crews— During grizzly bear capture and collaring events, a hair sample is collected from each bear and genotyped at the lab for individual identification, therefore scat samples collected in the immediate vicinity of a capture site were not sent to the lab. However, all scat samples collected opportunistically by capture crews (e.g. encountered on roads) were submitted to the lab for genetic analysis.

Opportunistic samples collected by hair DNA crews— For scat collected opportunistically at barbed wire hair snag sites, we assumed that any sample within 200 m of the site was likely from the same individual bear(s) that 'deposited' a hair sample(s). Depending on whether hair samples were collected and sent to the lab for genetic analysis, the following rules applied:

- If hair samples from that site and time period were already sent to the lab for genotyping, any scat samples within 200
 m of the hair snag site were not selected for genetic analysis.
- If the hair samples collected at a site were not sent to the lab because they had a high probability of being black bear (based on visual inspection of hair), any scat samples collected within 200 m of the hair snag site were also removed from lab analysis.
- If hair was collected at a barbed wire hair snag site but there were insufficient quantities for genotyping, any scat samples found within 200 m were sent to the lab for genotyping.

Lastly, we could not assume that a scat sample(s) located greater than 200 m from a barbed wire hair snag site was likely from the same individual bear(s), therefore they were treated as unique samples. If a single scat sample was encountered, it was sent to the lab for genetic analysis; if adjacent scat samples were encountered (at a location >200 m from a barbed wire hair snag site), the same rules used to rank adjacent samples encountered on transects were used to indicate the order by which the samples should be analyzed at the lab.

Laboratory methods— Following sub-selection procedures, we sent a subset of scat samples to the Norwegian Institute of Bioeconomy Research (NIBIO) in Svanhovd, Norway for genotyping. Each DNA extract was first screened for species-diagnostic amplification with one microsatellite marker (G10P) to separate grizzly bear and black bear samples (Paetkau and Strobeck 1994). After this, nuclear DNA profiles to reveal individual identity and gender were established using nine Short Tandem Repeats (STR) microsatellite markers (G10B, GIOH, G10J, G10L, G10M, G1A, G1D, G10P and MU50) developed for bears (Paetkau and Strobeck 1994, Paetkau et al. 1995, Taberlet et al. 1997) and one sex-specific marker (Bellemain and Taberlet 2004). A detailed description of the protocols for molecular analysis of scat samples from European brown bears for individual identification can be found in Kindberg et al. (2011) and Andreassen et al. (2012).



3.4 DNA analysis of hair samples

As we were interested in comparing DNA results from the concurrent hair sampling effort we have included details of laboratory procedures used for hair DNA extraction to allow the reader to understand the different methodologies used for DNA analysis.

Hair samples were sent to Wildlife Genetics International (WGI) in Nelson, British Columbia, Canada for genotyping analysis. DNA was extracted using QiaGEN DNeasy Tissue kits following standard protocols (Paetkau 2003). Samples were examined under a dissecting microscope, and those with the visual appearance characteristics of black bears hair (jet black from root to tip) were removed. Samples that passed the visual examination underwent a prescreen using a species-specific marker (G10J) to distinguish grizzly bear from black bear samples (Paetkau 2003). Individual grizzly bears were genotyped to 7 loci (markers G10J, G1A, G10B, G1D, G10H, G10M, G10P) and sex was assigned either based on size polymorphism in the amelogenin gene or using the ZFX/ZFY gender marker (Paetkau 2003, Waits and Paetkau 2005, Kendall et al. 2009; from Stenhouse et al. 2015).

3.5 Combining genetic datasets from hair and scat samples

It is important to note that there is no international standard for wildlife genetics, therefore genetic laboratories can differ in this regard. Labs have different criteria for what constitutes a successful genotype as well as different standards for matching individuals. These differences can make comparing genotypes from two labs difficult. For example, the NIBIO lab in Norway assigns an annotation to a sample if the sample showed allelic drop out, or the bear identity was assigned based on < 6 loci. Also noted are instances when the allele peak height for a marker was relatively low or the first peak was not higher than the next peak. This means that the client should go through the data and determine which genotypes they deem reliable. In contrast the WGI lab will only provide data for a sample if it has passed all their criteria (every allele met objective peak height thresholds) leaving no ambiguity as to which genotypes to accept. However, the client does not get a chance to examine the incomplete genotypes.

Also, the NIBIO lab used different primers for some markers compared with the WGI lab. In order to compare results, the calibration factor for each marker needed to be determined. Reliable calibration factors for each marker was determined, except for the G1D marker. The G1D and G10H markers have the unusual trait of differing from adjacent alleles by just 1 base pair instead of the usual two (Paetkau pers comm 2005). The use of a different primer between labs could result in variation in the flanking sequence and result in missed alleles, making calibration for the G1D marker between the two labs impossible.

The two labs also differ in their criteria for matching samples to individuals. WGI will only make a match if at least 7 specific markers in the sample match the genotype of a previously identified individual. Seven markers are used because WGI often has hundreds of hair samples to match which results in a higher probability of a matching markers occurring by chance. If two samples mismatch at only one marker, both samples are rerun when possible to investigate the potential for error. If the differing values remain for that marker, the samples are identified as two unique individuals. In contrast, , NIBIO deemed 6 markers as the minimum number required to discern an individual, due to the small number of samples. NIBIO will match a sample to a known bear based on less than the required 6 markers, although these matches are noted with an annotation. In some instances, two samples may have a mismatch in one of the markers but the rest (usually at least 8 markers) match. In this instance, the two samples are assigned to the same individual, as the mismatch is assumed to be a result of allelic dropout.

Once the scat DNA data was calibrated to match genotypes from samples analyzed at WGI, the two datasets were merged and NIBIO staff assigned matched samples to known bears based on their matching criteria. fRI staff reviewed the NIBIO data and matches, taking into consideration the annotations and notes on allele peak height, to determine the final number of individuals detected by scat. We were decidedly conservative in our screening so the final number of individuals is likely a minimum number.



3.6 Estimating grizzly bear abundance and distribution

DNA can be successfully extracted from both hair and scat and then genotyped for individual identification. As a result, both sources of DNA can provide a minimum count of grizzly bears from which population size and trend are estimated using various mark-recapture methods. When this method is the same, the estimates of grizzly bear distribution and abundance from the two different approaches can be directly compared. Advances in mark-recapture methods stemming from mark-recapture studies using barbed wire hair traps has led to the development and widespread use of Spatially Explicit Capture-Recapture (SECR) methods (Borchers and Efford 2008, Efford et al. 2013) in North America (Stenhouse et al. 2015, Morehouse and Boyce 2016). Methods of DNA extraction and genotyping from scat, which is widely used to survey grizzly bear populations in Europe, are detailed by Aarnes et al. (2015). Previous estimates of grizzly bear population size and trend in Sweden have relied on closed-population capture-mark-recapture (CMR) methods described by Kohn et al. (1999) and Kindberg et al. (2011). However, in order to directly compare the estimates of grizzly bear abundance from hair-DNA and scat-DNA approaches in Alberta, only SECR methods were considered for this study.

3.7 Cost-benefit analysis

It was our goal to evaluate the costs and benefits associated with genetic inventories using hair and scat relative in order to maximize the efficiency of future sampling designs and assess the value of engaging citizens in data collection. As a result, we focused on comparing measures of effort, detectability, and laboratory success between the two approaches.

Effort—We recorded the total number of sample units, the total number of sampling days, and the total number of DNA samples that were collected. A sample unit was defined as a hair snag site-session and as a transect for the hair- and scat-based approaches, respectively. Effort was calculated as the average number of units sampled per day.

Detectability—We calculated detectability as the average number of samples detected per sampling day and as the average number of samples detected per sample unit. We also calculated detectability as the average number of individual grizzly bears detected per sampling day and as the average number of individual grizzly bears detected per sample unit.

Genetic results—To compare the success of DNA extraction and genotyping from the samples that were submitted to the lab, we calculated the proportion of samples that were successfully genotyped to species and the proportion of samples that were successfully genotyped to the individual. We also compared the per sample cost for DNA extraction and genotyping.

Project costs—In order to compare the economic costs of the two survey techniques, we calculated expenses incurred throughout the separate projects. As the spatial extent of the DNA hair snag survey far exceeded the extent of the scat transect searches, costs were calculated for only the 4 field crews accessing the 141 hair snag cells accessible by road, and excluded helicopter-access sites and sites within Jasper National Park.

4. Results

4.1 Citizen science participation and supplemental sampling

Citizen Science Participation—In total, 22 scat collection kits and 6 GPS loggers were signed out to participants from the 6 distribution centers within our targeted communities (Table 1). The app was downloaded by 19 and 29 users for iOS and Android, respectively. Unfortunately, this yielded a total of only 6 scat samples collected by citizens, plus an additional three samples collected outside the boundaries of BMA 3. However, none of these samples were accompanied by path data (using either the



app or a GPS logger), thus, we were unable to evaluate search effort for these samples. However, a location for each sample was obtained, which is the minimum amount of information required to assess spatial relationships. Of the 6 GPS loggers retrieved, only 1 user provided about 833km of path information, which was from primarily driving on roads. Finally, of the 3 individuals that used the Grizzly Scat App tracking function, less than 1km of route information was generated, only ~500m of which was within the boundaries of BMA 3.

Transects—Our field crews completed 154 transects in 19 watersheds over the course of the study (August-October, 2015). The total distance covered was approximately 685 km with transect distance averaging 4.45 km (Table 2). This effort resulted in 130 scat samples, complemented by GPS logger information to quantify search effort over space and time (Figure 2). On average, one scat sample was found for every 5.27 km of transect searched, which varied only slightly by month. In August, one sample was found every 4.94 km of transect search as compared to October where one sample was found every 5.75 km.

Opportunistic Samples—We collected 115 scat samples opportunistically by field crews either checking DNA barbed wire hair snags or on their way to and from scat transect sites (Figure 2). Seventeen samples were also collected during our annual grizzly bear capture and collaring program within this BMA (Figure 2).

4.2 Genetic results from scat

A total of 196 scat samples collected within BMA 3 in 2014 were sent to the NIBIO lab for genotyping following sub-selection procedures (Table 3). From this, 159 samples (81%) were positive for bear DNA in the species test. Of these bear samples, 54 (34%) were found to be black bear and 105 (66%) were grizzly bear (Table 3).

Of the 105 positive grizzly bear samples, 45 samples (43%) provided data for at least 2 markers (Table 3) and 23 samples had data with no annotation associated with the sample. Taking into consideration the annotations and allele peak heights, we determined that 21 samples (20%) provided an individual DNA profile based on at least 6 markers. From these 21 samples, we identified 17 individual bears (9 male, 8 female) (Table 4, Figure 3). Seven of these individuals (6 male, 1 female) were detected in both scat and hair samples from the concurrent barbed wire hair snag census (Figure 4). Four bears (1 male, 3 female) were detected only in scat samples, with no corresponding hair sample in 2014, but were previously known to project researchers. Six individuals (3 male, 3 female) were identified by scat samples with no genetic match to any bears previously identified.

4.3 Comparing estimates of abundance and distribution

Abundance—SECR analyses from the non-invasive hair-based DNA inventory that occurred in BMA 3 (Figure 5) estimated the grizzly bear population at 76.1 grizzly bears (CI = 57.8 - 100.2), with an additional 10.1 (CI=7.5-13.8) bears in the White Goat Wilderness Area (Stenhouse et al. 2015). Given the low volunteer participation — which resulted in fewer scat samples than expected and reduced spatial coverage of sampling effort — and low genotyping success, we did not have an adequate sample size to estimate grizzly bear abundance from scat. Overall, the scat-based survey methods had fewer detections than the barbed wire hair snag methods that were conducted concurrently in BMA 3.

Although we could not directly compare estimates of abundance between the two approaches, we compared the total number of individuals detected, as well as the number of bears detected by scat sampling alone. The barbed wire hair snag inventory method detected a total of 79 unique grizzly bears (46 male, 33 female) in BMA 3 (including the White Goat Wilderness Area), versus a total of 17 individuals (9 male, 8 female) detected from a combination of scat transects, opportunistic scat sampling, and volunteer scat sampling (Table 4). These differences are not surprising given the large difference between the two approaches in the spatial extent of sampling efforts and the number of genetic samples that were collected. However, it is worth noting that 10



of these 17 bears were identified by scat alone and were not detected by any previous hair snag efforts (though four bears were previously known to project researchers as collared bears). Out of a total of 197 grid (sampling) cells used in the hair-based inventory, 99 hair sampling grid cells (50%) intersected with 142 of the 154 scat transects that were surveyed (Figure 5). Within these common sampling cells, 38 unique individuals (19 male, 19 female) were detected using barbed wire hair snags and 15 unique individuals (7 male, 8 female) were detected from scat transect surveys (Table 4).

Distribution—We could not make direct comparisons of grizzly bear distribution in BMA 3 estimated by the two different approaches. Instead, we compared grizzly bear occupancy based on the genetic results from hair- and scat-based survey methods for common sampling cells (n=99) where hair and scat survey efforts overlapped (Figure 5). At the species level, grizzly bears were detected in more cells by scat DNA survey methods (n = 38) than by hair DNA survey methods (n = 25) (Figure 6). Furthermore, 23 of the 38 occupied cells detected by scat DNA were not detected by hair DNA (Table 4). At the individual level, grizzly bears were detected in 23 cells from hair-DNA compared to 13 cells from scat DNA (Figure 7). However, of the 13 occupied cells detected by hair DNA (Table 4).

4.4 Comparing the costs and benefits of two non-invasive inventory methods

In terms of effort, we were able to sample nearly 20 hair snag sites per day using 4 crews and nearly 5 scat transects per day (each transect is approximately 5 km in length) using an average of 3 crews (Table 4). As previously mentioned, the scat-based survey methods had far less detection than the barbed wire hair snag methods; an average of 60 hair samples were collected per day compared to an average of 7 scat samples detected per day (Table 4). However, given the repeated sampling of hair snag sites, this approximates to 3.0 hair samples detected per sample unit (site-session) and 1.6 scat samples detected per sample unit (transect) (Table 3). Despite fewer samples detected overall, a slightly larger proportion of scat samples (0.81) were successfully genotyped to *Ursus spp.* than hair samples (0.71), and approximately half of all the samples sent to the lab were identified as grizzly bear at the species level for both approaches (Table 4). Nonetheless, a lower proportion of scat samples (0.86) (Table 4). This difference is less pronounced if you consider the proportion of samples sent to the lab (Table 4). Approximately 2 bears were detected per day during hair sampling efforts compared to 0.5 bears detected per day during scat sampling efforts (Table 4). However, when you consider the repeated sampling of hair snag sites, the average number of bears detected per sample unit is nearly the same (Table 4), which suggested that similar results could be achieved by scat-based inventories as hairbased inventories if the number of sample units (i.e. volunteers) were increased.

A simple cost comparison between hair-DNA and scat-DNA population inventory projects revealed the economic benefits of using a scat-based sampling approach (Table 5). Currently, the lab cost per sample is approximately 30% greater for scat than hair, but the set up and repeated sampling of barbed wire hair snag sites proved labour intensive, and thus costly. While the greatest cost of the scat sampling program was the salaries of staffed transect searches, the potential remains for scat-based inventories to achieve even lower project costs provided that an adequate number of citizen volunteers can be used. Given that our effort to engage citizen science in grizzly bear research was the first of its kind in Alberta, there is immense opportunity for improvement and versatility with non-invasive scat-based DNA survey techniques as discussed in the next section.



5. Discussion

It is clear that despite a great deal of effort in communicating with potential "citizen scientists" from the local hunting and trapping communities within and bordering the study area, there was very little active participation either in the form of using the Scat App and GPS tracking devices or, most importantly, in providing scat samples for analysis within this project. Without the use of our research team staff there would have been a total of 6 scat samples obtained from the general public. This small number of samples was not accompanied by any track data and thus provided no data on search effort. Active and ongoing volunteer participation in this type of program is essential, not only for strong data, but also to keep this technique affordable and thus attractive to wildlife managers.

The lack of participation was particularly surprising and disappointing. The public seemed genuinely supportive and excited about the project and the prospect of being involved. However, the lack of participation reinforced that fact that active engagement and getting the public onboard with data collection are two distinct but equally important phases of a citizen science project. Both phases require substantial lead time and sustained effort. Although the iOS version of the app was expected to be available in late August, it wasn't available for download until September 18, 2014. This delay combined may have contributed to the lack of participation. Although we suspect that it may take many years, simplifying the recruitment process and/or generating other incentives to reach a critical mass of engaged citizens that can contribute to data collection may be necessary. If one is considering using some type of reward or recognition s to increase participation within the stakeholder groups, caution is warranted to avoid a payment per sample approach that could bias results and be a challenge for project budgeting purposes.

This lack of participation indicates to us that different approaches are needed to work with "volunteer" teams, groups, or individuals if this type of data collection is to be used for grizzly bear population inventory or trend monitoring in other BMA's in Alberta. New approaches might need to consider being more inclusive of other stakeholder groups (e.g., ATV clubs, naturalist groups, other outdoor user groups, etc.) along with local hunters, trappers, and other organizations (Fish and Game Association). In addition, consideration should be given to work with industrial stakeholders who are active on the same landscape from which scat samples are required. This type of approach is now being pursued within our ongoing research program.

This project showed that despite challenges in engaging local hunters with data collection support, individuals can successfully encounter and collect bear scat while out driving, using ATV's, or walking in habitat inhabited by grizzly bears. We have developed an app and other techniques to make data collection simple and provide a platform for self-populating our database at fRI Research (Appendix A). These data can generate information on search area and effort from volunteers and aid in identifying specific geographic areas where additional sampling effort is required or where sampling effort should be redirected. Sample containers using a bar code and scanning system worked well and aided in sample tracking and management both at collection stations and in the laboratory.

Making comparisons between genetic profiles generated from hair and scat samples processed in different laboratories is not a simple nor straightforward task. In this report we have taken a conservative approach in matching and comparing bears between our data sets prepared from different biological samples (hair and scat). Genotyping errors will be an ongoing challenge and requires an establishment and acceptance of error rates when comparing genetic results. These acceptable genotyping error rates should be viewed in the context of management risk for conclusions drawn from the data set and how the results will be utilized for conservation purposes. Perhaps the most important consideration is what the study objective is with the use of scat sampling. This scat collection technique for population estimation would require extensive sampling along predetermined routes, which would likely be difficult to achieve with volunteer field workers within the area of a BMA. However in a parks and



protected area, using established trail networks would allow this approach to be more feasible. Irrespective of the study objectives for using scat DNA techniques, adequate and directed sample collection is vital in acquiring needed samples. We recommend further work with genotyping questions and error rates and the establishment of defined acceptable standards between laboratories working with different biological samples. Additional research is also needed to develop appropriate study designs and sampling protocols that are linked to specific management questions.

The use of scat sampling from grizzly bears in the boreal forests of Alberta to provide DNA data to support population inventory and monitoring efforts shows promise. From a long term perspective we believe that scat sampling will not replace hair sampling techniques for the purpose of BMA wide grizzly bear population inventories. However with increased level of participation in collecting samples this technique does have important benefits for provincial grizzly bear population monitoring to evaluate recovery progress. In addition, through the collection of hair samples we are now able to provide many other pieces of data (health measures) that are not possible from scat samples. However, we also recognize that the scat sampling technique does have tremendous value in grizzly bear population monitoring if it supplements the hair based population estimation approach. For example once a population estimate and distribution have been determined there will be areas (watersheds or grid cells) where occupancy is low or no bears were found. From a management perspective, there would be no need to repeat an expensive hair snag based population inventory over the next five years, but with the scat method low or zero occupancy watersheds could be targets to monitor short term changes. These results would provide an indication of population recovery and expansion. This assumes that as the population does recover that unoccupied habitats would be occupied and have a greater number of individual bears. The scat based DNA data, with adequate sampling, can also identify the gender of bears and provide information on survival over longer time periods. Taken collectively, and with relatively little cost, these data could help inform managers when the next larger scale (hair based) population inventory should take place. This could be viewed as a population sub-monitoring system that could be carried out at a regional or BMA scale. It is also important to highlight the value (and need) of establishing a provincial grizzly bear genetic data base to allow searching and monitoring of populations over time and in a coordinated fashion. This provincial data base should be viewed as a key element for grizzly bear population recovery efforts and for the conservation of this species for future generations.



6. Literature Cited

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7. Tables

Table 1. Number of scat kits distributed and the number of scat samples collected by the public within BMA 3, across the six collection depots.

Distribution Sites		# Kits Distributed	# Scat Samples	
GoA	Hinton	8	3	
	Edson	6	1	
	Drayton Valley	3	2	
	Rocky Mountain House	5	0	
Private	High Caliber Sports (Hinton)	0	0	
	Trails End Taxidermy (Drayton Valley)	0	0	
	Total	22	6	



Watershed	Number of transects searched	Total transect length (km)	Number of scat samples found	Samples found per km searched
16	9	36.27	8	0.22
46	9	37.06	10	0.27
48	6	24.95	1	0.04
49	3	15.99	0	0.00
52	7	28.09	2	0.07
50	7	31.99	3	0.09
65	4	15.79	8	0.51
66	4	16.78	0	0.00
71	2	18.86	3	0.16
73	4	18.57	2	0.11
80	14	63.01	15	0.24
81	26	112.28	30	0.27
82	24	110.45	26	0.24
83	11	44.93	8	0.18
103	5	22.89	0	0.00
104	7	32.93	1	0.03
105	4	19.89	2	0.10
107	4	16.92	7	0.41
111	4	18.14	4	0.22
Total	154	685.80	130	0.19

Table 2. The number of transects by watershed completed, total transect distance, number of scat samples collected, and the number of samples per km searched by field staff.



			Sampling Type		
	Citizen /		Opportunistic	Opportunistic	
	Volunteer	Transects	(DNA crew)	(Capture crew)	Total
Total number of samples collected	6	130	115	17	268
Number of samples sent for genetic analysis	6	128	45	17	196
Number of samples genotyped to species	6	96	40	17	159
Number of samples identified as Ursus arctos	3	63	27	12	105
Number of samples with individual DNA profile					
based on at least 2 markers	0	23	13	9	45
Number of samples with individual DNA profile					
based on at least 6 markers	0	16	3	2	21

 Table 3. The number of samples collected and the number of samples sub-selected for genetic analysis by sampling type.

 The number of samples with successful DNA extraction and genotyping are also reported.



Table 4. Cost-benefit evaluation of non-invasive scat-based DNA grizzly bear survey method as compared to traditional hair-based survey method.

tegory for comparison	Description	Hair-DNA Method	Scat-DNA Metho
ABUNDANCE	Total number of individuals detected §	79	17
	Number of new individuals detected	46	13
	Number of individuals detected only by scat		10 ‡
DISTRIBUTION	Common sampling cells	99	99
	Number of individuals detected within common sampling cells	38	15
	Number of cells occupied at the species level	25	38
	Number of occupied cells detected only by scat		23
	Number of cells occupied at the individual level	23	13
	Number of occupied cells detected only by scat		9
EFFORT	Total number of sampling units	788 †	154
	Total days of sampling	40	33
	Average number of crews	4	3 ¥
	Average number of units sampled per day	19.70	4.67
	Average number of units sampled per day per crew	4.93	1.56
DETECTABILITY	Total number of samples collected §	2,418	245
	Average number of samples collected per sample unit	3.07	1.59
	Average number of samples collected per day	60.5	7.42
	Average number of individuals detected per sample unit	0.10	0.11
	Average number of individuals detected per day	1.98	0.52
LAB SUCCESS	Number of samples sent for genetic analysis	698	196
	Proportion of all samples genotyped to Ursus spp.	0.71	0.81
	Proportion of all samples identified as Ursus arctos	0.51	0.54
	Proportion of *Ursus spp. samples identified as Ursus arctos	0.72	0.66
	Proportion of all samples genotyped to individual	0.44	0.10
	Proportion of *Ursus arctos samples genotyped to individual	0.86	0.20





‡ 6 bears identified only by scat with no genetic match to any bears previously identified; 4 bears detected only by scat but previously known to project researchers.

† 197 hair-snag sites sampled 4 times for a total of 788 sample units.

§ Collected across the respective study areas and not limited to common sampling cells.

¥ 5 crews for 2 weeks and 2 crews for 4 weeks for an average of 3 crews throughout the sampling period.



Table 5. Expenses incurred in a non-invasive scat-based DNA grizzly bear survey method as compared to traditional hairbased survey method.

	Scat tran	sect surveys. 6 weeks	Hair snag surveys. 16 weeks		
Salaries:	\$24,960	11 field staff for 2 weeks, 2 field staff for remaining 4 weeks	\$180,800	8 field staff for 16 weeks, 2 coordinators for 24 weeks.	
Trucks: Rental, fuel, insurance, repair and maintenance	\$10,890	5 trucks for 2 weeks. 2 trucks for remaining 4 weeks	\$38,715	4 trucks for 16 weeks.	
Lab costs:	\$12,320	NIBIO. 196 scat samples	\$10,620	Wildlife Genetics International. 183 hair samples from road- accessible hair snag sites	
Outside contractors:	\$18,000	App development	\$10,000	Study design, spatially explicit capture recapture (SECR) analysis.	
Public engagement	\$2,000	Travel and staff salaries to promote citizen science scat collection program			
Field equipment purchases:	\$1,000		\$9,300		
Total:	\$69,170	\$11,528 per week	\$249,435	\$15,590 per week	



8. Figures

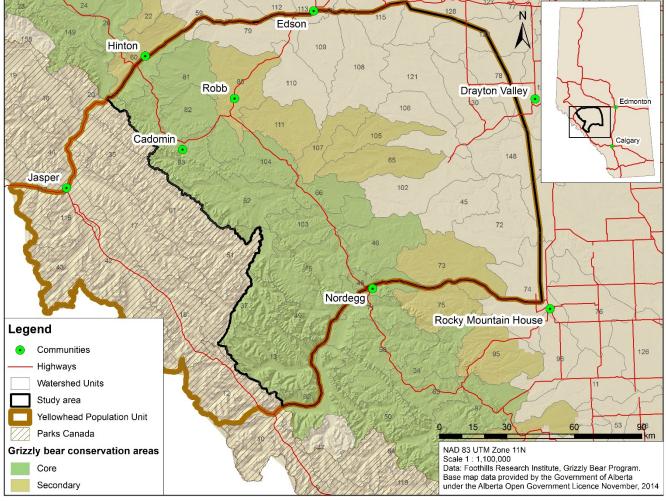


Figure 1. The sampling area by watershed unit within Bear Management Area (BMA) 3.



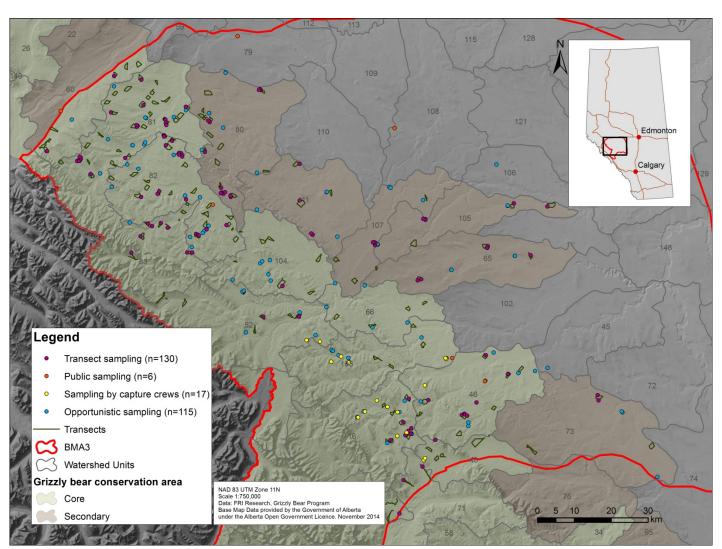


Figure 2. Location of sampling transects and scat samples (n=268) found by transect sampling, by the public, by spring capture crews, and by staff opportunistically throughout the summer field season.



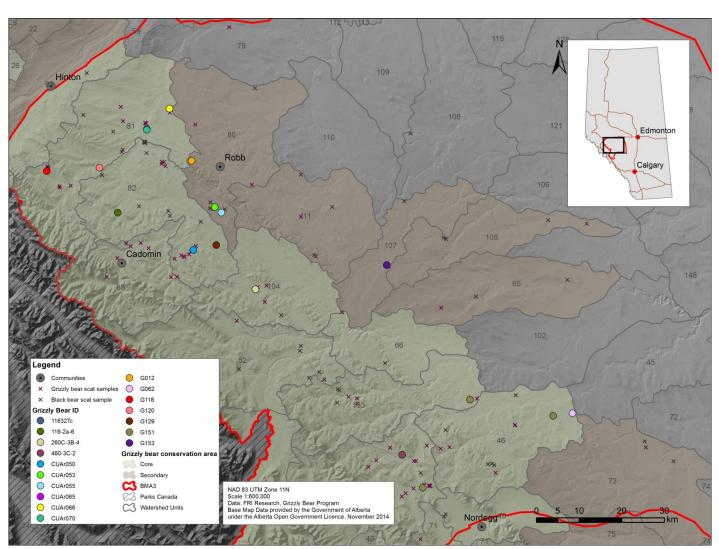


Figure 3. Distribution of grizzly bear scat samples genotyped to unique individuals (>= 6 typed loci), samples as identified as grizzly bear but with incomplete genotyping for individual (<=5 types loci), and scat samples identified only as black bear collected within BMA 3 in 2014.



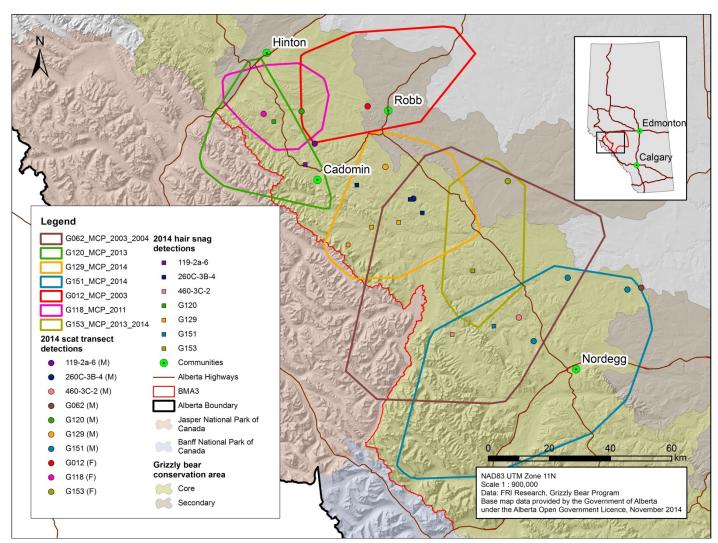


Figure 4. Location of scat samples detecting grizzly bears previously known to fRI Research, and any corresponding hair samples from the 2014 DNA hair snag census. Minimum convex polygon (MCP) home ranges depict the most recent year of available data transmitted from radio collared individuals detected with scat in 2014.



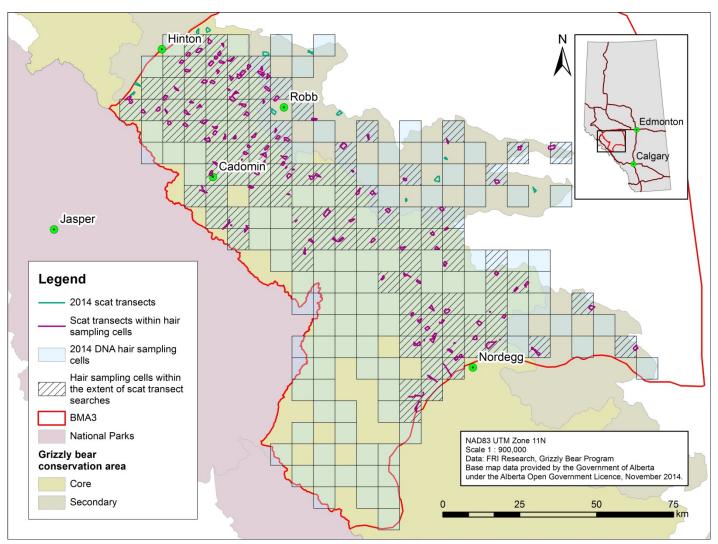


Figure 5. Common areas surveyed between the 2014 barbed wire hair snag population census of the Yellowhead population unit and the 2014 scat sampling transect surveys in BMA3.



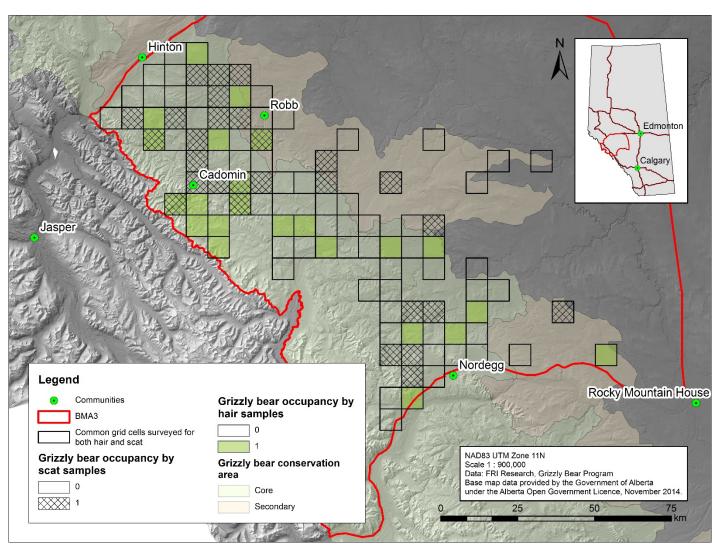


Figure 6. Grizzly bear occupancy of common sampling cells at the species level detected by hair-DNA and scat-DNA survey methods.



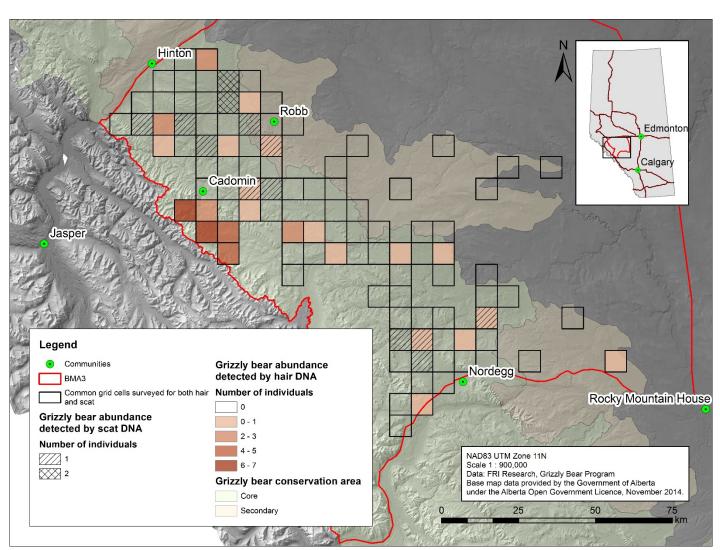


Figure 7. Grizzly bear occupancy of common sampling cells at the individual level detected by hair-DNA and scat-DNA survey methods.



9. Appendix A

■0000 VIRGIN 🗢 2:25 PM 🛞 🖅 79% 📖)		5 PM 🕑 🗡 79% 🔲 🤆	• • • • • VIRGIN		
\equiv I Grizzly Scat App		A Bear! Save	≡ iii.	Collect Sample	Save
Find it. Scoop it. Submit it	Spot a Grizzly Bear o	r a rub tree? Take a	STEP 1: TA	AKE A PHOTO	
Logging your route on every trip helps us identify which areas are being searched.	photo		Take a picture of the site prior to collecting your sample.		
This data is very important, even if you don't collect any scat samples. Please	TAKE PICTURE	SELECT PICTURE	jour oumpre	TAKE PICTURE	
open the app and initiate a trip log for every trip into the study area.					
LOG MY ROUTE					
	WHAT DID YOU SEE?)		OLLECT SAMPLE	
	Rub Tree	Grizzly Bear		k inside the collection kit to ple of the scat.	collect
NEED A COLLECTION KIT?			STEP 3: SO	CAN QR CODE	
	NOTES			uare QR code located on th ction vial. Hold the vial roug	
COLLECT A SCAT SAMPLE				from your phone.	111y 12
REPORT A SIGHTING				SCAN QR CODE	
	WHAT DID YOU SEE	?	NOTES		

Figure A1. Captures of the GrizzlyScat App i.) homescreen, ii) Sight a Bear function, and iii) Sample Collection function within the iOS platform.



Scat Sample - 636105260	399321366.jpg 🗆 ×
Edit	
Save Cancel	Delete Item
Commit Clipboard	Actions
Title	gsa1950
Long	-117.0974119
Lat	53.31189405
Data	{"Id":0,"AppId":0,"UserId":0,"UserName":null,"DeviceId":null,"GPS":n ull,"ServerDate":"2016-09- 26T22:33:59.9321366+00:00","ClientDate":null,"BarCode":null,"FileU RL":null,"files":null,"Status":"Received","DocumentType":"ScatSample" ,"MiniLog":null,"Data
Barcode	gsa1950
Notes	
User	Cam McClelland
Test	False
Email	cammcclell@gmail.com
Status	(None) ▼ This triggers a push notification.
ServerId	11299
UserId	
DeviceId	b36af32800301142
Result	(None)
ClientDate	9/27/2016 10:48:00 AM
ServerDate	

Figure A2. Example of incoming data from the GrizzlyScat App scat collection function, delivered to the fRI Research SharePoint server.

