Evaluation of new grizzly bear genetic scat results with DNA results from hair collection – a test and comparison of population monitoring for the future of provincial grizzly bear monitoring.

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Final Report

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Note: This research has now been published in the following scientific journal and the reader is referred to this manuscript for complete details and analysis. A PDF version of this publication has been provided to AUPRF. The information contained in this AUPRF report is a general summary of the results of this research along with additional materials regarding application of findings. New findings since the completion of this work are also included for interest.

Comparison of grizzly bear hair-snag and scat sampling along roads to inform wildlife population monitoring Isobel Phoebus, John Boulanger, Hans Geir Eiken, Ida Fløystad, Karen Graham, Snorre B. Hagen, Anja Sorensen and Gordon Stenhouse. Wildlife Biology 2020: wlb.00697 doi: 10.2981/wlb.00697

Abstract

Wildlife managers conduct population inventories to monitor species, particularly those at-risk. Although costly and time consuming, grid-based DNA hair-snag sampling has been the standard protocol for grizzly bear inventories in North America, while opportunistic fecal DNA sampling is more commonly used in Europe. Our aim is to determine if low-cost, low-effort scat sampling along roads can replace the current standard. We compare two genetic non-invasive techniques using concurrent sampling within the same grid system and spatially explicit capture–recapture. We found that given our methodology and the present status of fecal genotyping for grizzly bears, scat sampling along roads cannot replace hair sampling to estimate population size in low-density areas. Hair sampling identified the majority of individual grizzly bears, with a higher success rate of individuals identified from grizzly bear samples (100%) compared to scat sampling (14%). Using scat DNA to supplement hair data did not change population estimates, but it did improve estimate precision. Scat samples had higher success identifying species (98%) compared with hair (80%). Scat sampling detected grizzly bears in grid cells where hair sampling showed non-detection, with almost twice the number of cells indicating grizzly bear presence. Based on our methods and projected expenses for future implementation, we estimated an approximate 30% cost reduction for sampling scat relative to hair. Our research explores the application of genetic non-invasive approaches to monitor bear populations. We recommend wildlife managers continue to use hair-snag sampling as the primary method for DNA inventories, while employing scat sampling as supplemental to increase estimate precision. Scat sampling may better indicate presence of bear species through greater numbers and spatial distribution of detections, if sampling is systematic across the entire area of interest. Our findings speak to the management of other species and regions, and contribute to ongoing advances of monitoring wildlife populations.

Best Practices / Tangible Project Outcomes

While hair-snag sampling retains its position as the standard for grizzly bear population estimates with superior individual identification rates, scat sampling holds promise as a complimentary monitoring technique. The ability to better determine species distribution, increase estimate precision and, with improved field techniques, conduct DNA inventories using a cost-effective scat approach is a major step forward for long-term grizzly bear monitoring efforts in Alberta. On a broader scale, our research has

demonstrated the value of comparative studies where two gNIS approaches were applied and evaluated under similar rules. With this comparison, we were able to identify strengths of stand-alone methods and show that despite differences in field and genetic success, an appropriate approach is purpose-specific and depends on monitoring objectives. Our research provides insights for managers as they balance scientific rigor and cost-effectiveness while striving to collect consistent and comparable data for adaptive, long-term, and sustainable wildlife monitoring and conservation.

Background

In 2018 the fRI Research Grizzly Bear Program was asked to lead and complete two large scale grizzly bear population inventory projects, one in BMA 7 (the first one in provincial history for the Swan Hills) and the second one, a repeat inventory, in BMA 4 (Clearwater). Both these projects utilized the "gold-standard" barb wire hair snagging grid SECR approach which is both labor intensive and costly. Midway through the field work for these projects funding issues required our teams to suspend work, and hence these projects were not completed in 2018. Although the largest percentage of the budgets for these 2 inventories had been spent, when work was stopped, we had completed all the field work and gathered all the needed samples based on our planned sampling design.

The needed funds to allow us to complete these inventory project by have the laboratory genetic analysis completed and the statistical analysis undertaken, was not received until the late winter of 2020 and unfortunately coincided with the Covid 19 global pandemic. Thus sample analysis was further delayed until laboratories could safely resume operations. Our final genetic data sets were therefore not received back from the laboratories until the August 2020.

It is important to understand these events and the timelines associated with these larger inventory efforts in the context of the current (AUPRF) funded project.

Over the past 6 years AUPRF funding, with additional support from companies in the energy and forestry sectors, has allowed the fRI Grizzly Bear Program to move forward with a structured and well planned program to evaluate the use of new approaches to support and monitor the recovery of grizzly bears in Alberta. Specifically our team has focused on using grizzly bear scat collection as a means to monitor a variety of metrics deemed to be important in status evaluation.

In our work on this topic, and research direction, we have approached and successfully completed a variety of linked projects related to this grizzly bear scat sampling methodology in a step wise manner which included:

1. Laboratory validation and improvements of DNA extraction from Alberta samples
2. Scat collection protocols for field workers
3. The development and application of a scat APP for use by field staff and citizen scientists
4. Testing of field collection efficacy by citizens and directed biologists
5. Implementation of industry collection program and evaluation of findings

6. Expansion of genetic markers from grizzly bear scat (from 9 to 23 genotypes)

Throughout this time we have approached this applied research effort in a careful and well thought out manner which has brought us to undertake the final and most important step on this research topic project. Specifically we are interested in simultaneously collecting both hair and scat samples with a well-designed and thorough sampling design that will provide important answers to the application of scat sampling for future population monitoring. We believe this work will allow managers to determine if or how scat sampling may assist in future grizzly bear population monitoring efforts.

Project Overview

Although significant progress has been made on the use of grizzly bear scat collection for monitoring purposes it is still unclear what bear scat DNA can tell us related to bear habitat occupancy and density which are two key metrics in measuring grizzly bear population status and recovery. In the case of most wildlife populations, we typically see an increase in density, and expansion of range or occupied habitat, or both when populations are increasing and the opposite occurs in declining populations.

To answer these questions an experimental validation approach is needed where scat DNA samples are compared against the "gold standard" barbed wire grid cell base hair sampling approach. This has not yet been undertaken due to the costs associated with carrying out sampling of both hair and scat at the same time, within a large study area.

As mentioned we had a unique opportunity in 2018 to directly link and collaborate with an existing Forest Resource Improvement Association of Alberta (FRIAA) supported project in the Pembina River area within BMA 3 north of Highway 11 in west central Alberta. That population inventory project collected grizzly bear hair using the "gold standard" grid sampling approach in a defined study area. The current project allowed us to simultaneously collect both hair and scat samples with a well-crafted sampling design. This study area was selected as we have a current population and density estimate for this area (as well as for the entire BMA) , long term genetic data (hair and scat) from inventory work in 2004, 2013, 2014 and with this project 2018, along with detailed movement data from GPS collared bears spanning the past 20 years.

Methods
Material and Methods

Study Area
The study area covers approximately 2,450 km^2 within the foothills of the Rocky Mountains in Alberta, Canada (Fig. 2.1). The area falls within the southern portion of the Yellowhead Bear Management Area (BMA 3), where forestry, oil and gas exploration, and recreation occur. Elevation ranges from 3,360 to 800 m in a west to east gradient. Vegetation consists of mixed forests with important bear foods including moose (*Alces alces*), deer (*Odocoileus spp.*), alpine sweet-vetch (*Hedysarum alpinum*),

buffaloberry (*Shepherdia canadensis*), cow parsnip (*Heracleum lanatum*) and various blueberry species (*Vaccinium spp.*; Munro et al. 2006).

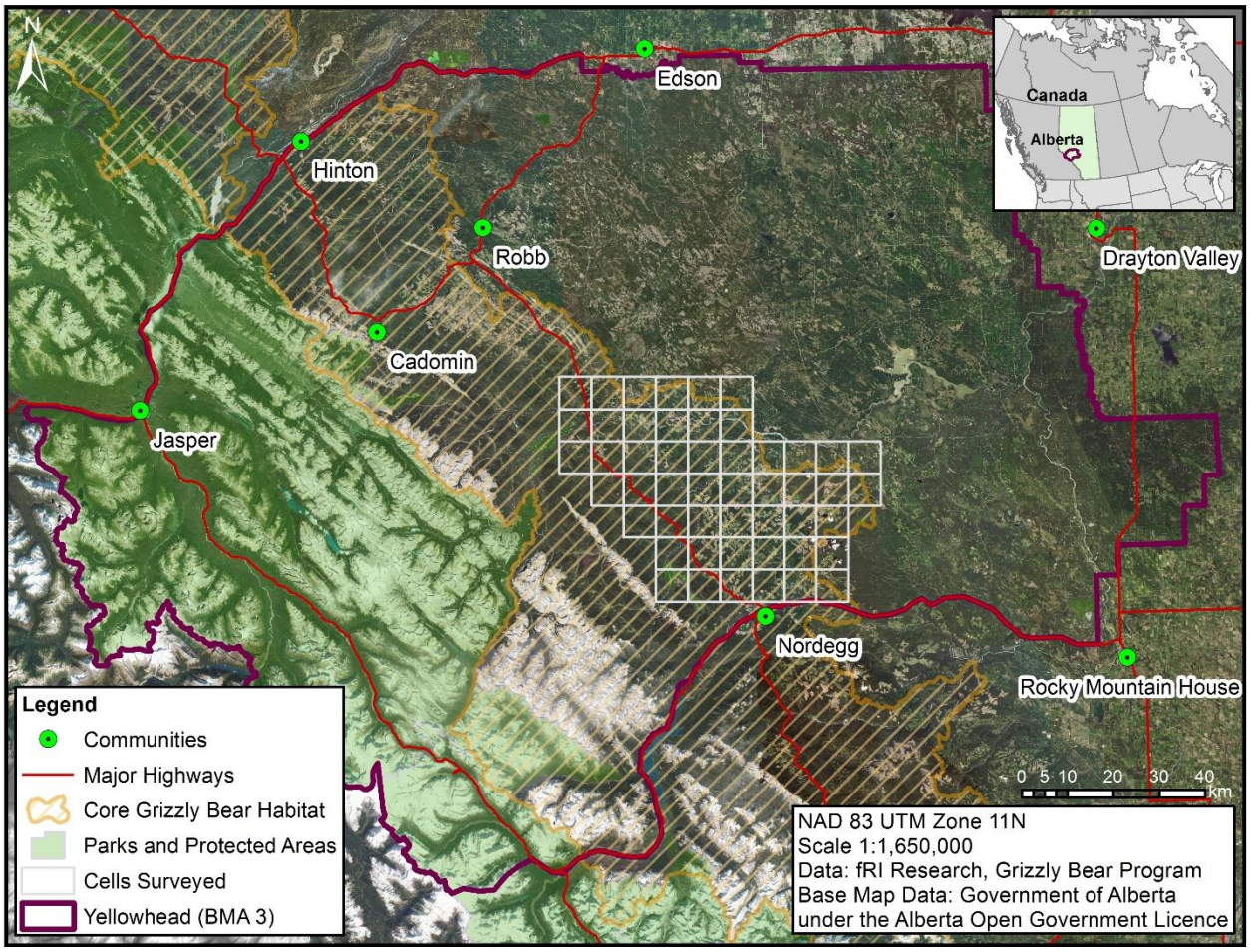
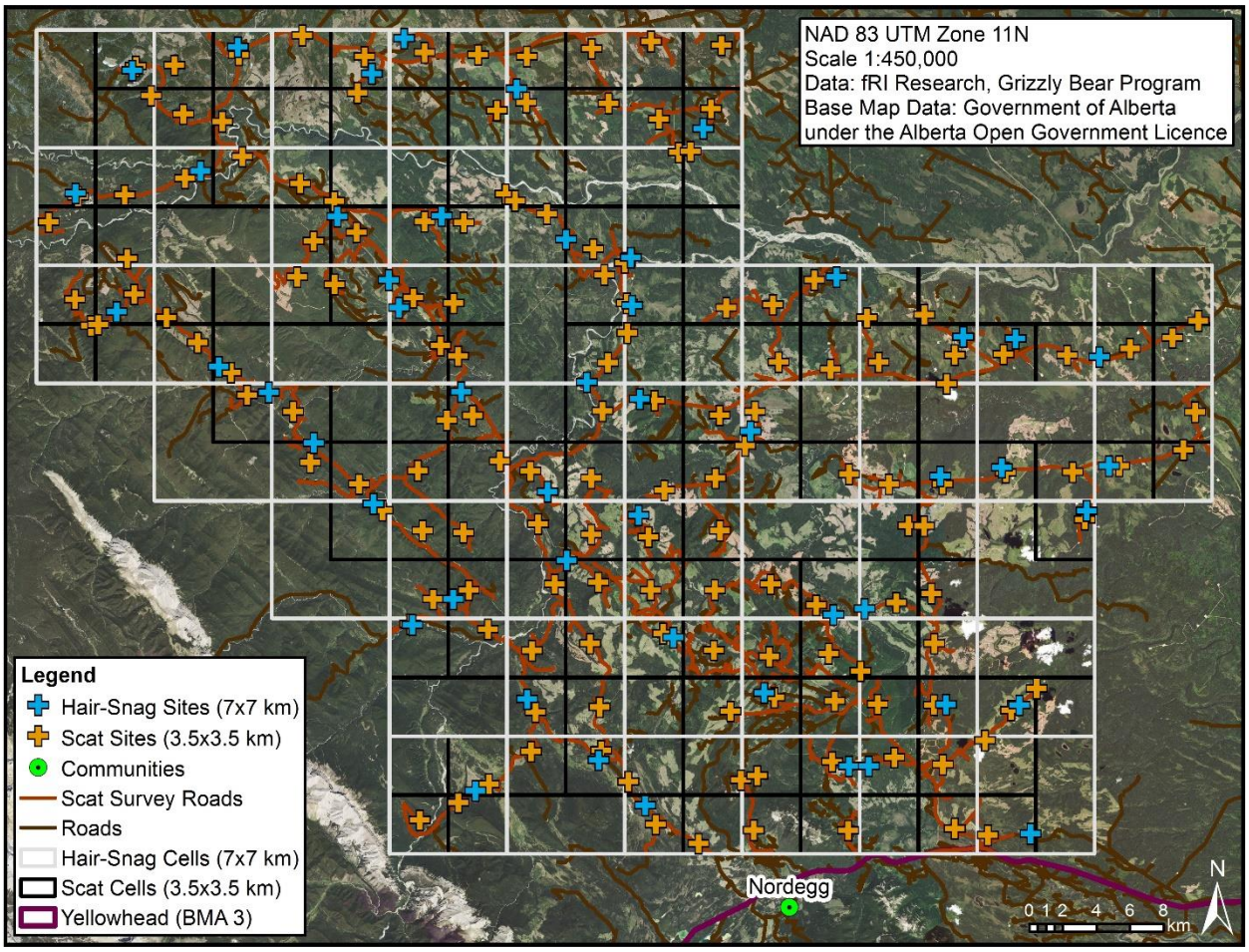


Figure 2.1 Study area and sampling grid

Hair-Snag Collection

We followed standard methods of barbed wire hair-snag collection for grizzly bears using spatial sampling designs (Woods et al. 1999, Proctor et al. 2010, Boulanger et al. 2018). We selected one fixed hair-snag site per 7×7 km cell within a 50-cell grid based on specific conditions (e.g., in high quality grizzly bear habitat) and human safety requirements (e.g., >200 m from roads; Boulanger and Efford 2014, Stenhouse et al. 2015) Figure 2.2. All sites were accessible by truck and a short hike (<1 km). Subsequent to hair-snag site set-up (session 0), we checked for hair and replenished non-reward, scent-lure bait piles every 14 days for four sampling periods (sessions 1–4). We collected, sub-selected and stored hair samples based on previous protocols (Stenhouse et al. 2015).



Scat Surveys

We conducted pilot surveys exploring bear scat collection along roads within BMA 3 during the fall of 2016. Crews found 0.02 suspected bear scat samples per km in low-density areas where few or no bears had been identified by hair-snag inventories (Sorensen et al. 2017). These results aligned with how grizzly bears use roads in spring and early summer, and roadside habitats in late summer and fall (Roever et al. 2008, Graham et al. 2010). Building on the pilot study, we designed our road surveys to mimic a low-cost, low-effort citizen science strategy similar to the Scandinavian approach where hunters collected scat samples for brown bear population estimates—although conducted opportunistically in late summer and fall (Bellemain et al. 2005, Andreassen et al. 2012, Schregel et al. 2012).

Our selected road network covered the same sampling grid that contained hair-snag sites (Fig. 2.2). We established a circuit of accessible gravel roads in each cell with occasional truck trails or unimproved roads. Roads behind locked gates, within forestry harvest blocks, in poor condition, or not existing in the government database were excluded. We tracked driving routes for navigation and circuit data (e.g., waypoints, date, time, etc.). Driving speeds were maintained between 50–80 km/h for gravel roads and 20–50 km/h for truck trail and unimproved roads.

Our scat surveys followed the hair collection schedule described above. During site set-up we assessed accessibility, selected roads and cleared off pre-existing scat. We drove the same circuits each session, collected all suspected bear scat and documented sample information (e.g., date, location, scat contents, exposure to sunlight, etc.). Although higher quality DNA generally occurs on outer layer of carnivore scat, environmental conditions and exposure time impact sample quality (Murphy et al. 2007, Stenglein et al. 2010, Wultsch et al. 2015). Because scat on roads is directly exposed to sunlight and ultraviolet radiation which damages DNA (Friedberg 2003), we sampled the inside layer. We collected 1 cm^3 of scat, which we stored in uniquely barcoded vials containing silica desiccant (adapted from Bellemain et al. 2005).

Lab Methods

We followed laboratory sub-selection criteria for hair samples based on protocols that maximize the number of individual grizzly bears identified using the fewest samples (Proctor et al. 2010, Stenhouse et al. 2015). We sent hair samples with grizzly bear characteristics—hair that was not pure black—to Wildlife Genetics International, Nelson, Canada, for genotyping to identify species, gender and individual bears. DNA extracts were analyzed using eight short tandem repeat (STR) markers (G10B/G10H/G10J/G10M/G10P/G1A/G1D/X-Y) and an additional 13 for full genotypes (CPH9/CXX110/CXX20/G10C/G10L/G10U/G10X/MSUT2/MU23/MU50/MU51/MU59/REN145P07). Samples went through cleanup passes and error checking following established protocols (Paetkau 2003).

We have been working to standardize field protocols, validate procedures and ensure reliable genetic identification from scat samples since 2012 in collaboration with the DNA lab at the Norwegian Institute of Bioeconomy Research, Ås, Norway. All scat samples collected underwent species-specific mitochondrial DNA-based tests to distinguish between grizzly bear and black bear (*Ursus americanus*). We analyzed fecal DNA extracts using the same STR markers as hair. Individuals identified using hair and scat were compared with known grizzly bears from a provincial reference database.

Species Spatial Distribution

We explored the spatial distribution of detections within the study area defined by detection or non-detection of a species within a given cell (MacKenzie et al. 2018). Detection was determined by the presence of one or more grizzly bear hair or scat samples within a cell during any session.

Spatially Explicit Capture-Recapture

SECR methods (Efford 2004, Efford and Fewster 2013) use multiple detections of animals at unique detector sites within a sampling session to model animal movements and detection probabilities. Using this information, we estimated the detection probabilities of grizzly bears at their home range center, the spatial scale of movements around the home range center, and bear density. This method assumes home ranges can be approximated by a circular symmetrical distribution of use; however, recent work suggests it is relatively robust to deviations from circularity if sampling is systematic (Efford 2019a). We

used the actual shape and sampling grid configuration while estimating home range, scale of movements and density, thus accounting for study-area size and configuration effects on the degree of population closure violation and subsequent density estimates.

SECR can be applied to transect and area searches (Efford 2011), where transects with discrete endpoints are most suited. The branching, circuitous nature of roads challenges transect SECR detector implementation. To circumnavigate this issue, we considered a cell-based approach where the mean centroid of roads in 3.5x3.5 km cells (nested in the 7x7 km cells used for hair-snag sampling) acted as the SECR detector. Using the centroid of roads meant the detector fell near roads. We reduced multiple scat samples per cell and sampling session for individual bears to a single detection event. Unique detections were assigned a centroid location based on the mean location of the original samples in each cell. This approach allowed for relative independence between scat detectors and minimized the distance between road cell centroids and actual roads. The smaller cell design resulted in fewer redundant samples and reduced the difference between scat detection locations and road cell centroids (mean=0.9 km, min=0.1 km, max=2.0 km, n=17). The final layout illustrates that scat and hair-snag sampling was conducted primarily in the vicinity of roads and that the scat sites fell systematically on roads (Fig. 2.2).

We conducted SECR analyses with hair, scat, and combined hair+scat data to compare population estimates between various methods and determine if scat data alone could provide a reliable estimate. Model selection for hair focused on sex-specific differences in scale of movement and detection (using sex as a covariate) and the effect of site placement on bear detection using previously defined canopy cover and terrain ruggedness site covariates (Boulanger et al. 2018). For scat-only model selection, we considered the kilometers of roads driven in each 3.5x3.5 cell as a site covariate. For the hair+scat analysis, scat sites were entered as point detectors with covariates used to test for differences in detection between each sampling method. We defined a systematic grid of points delineating the total possible area that bears could have encountered the DNA sampling grid (i.e., a SECR mask) using a 40 km buffer around the grid (Boulanger et al. 2018). Within the mask, we spaced points at 3 km intervals and used these points to estimate density. This spacing optimized computation time with minimal changes in estimates compared to tighter mask point intervals.

The precision of SECR estimates is primarily related to the number of bears on the sampling grid and the number of recaptures during sampling (Efford and Boulanger 2019). It is indexed by the coefficient of variation (CV_d), which is the standard error of an estimate divided by the estimate. One central question in study design is whether precision of estimates is limited by the number of bears on the sampling grid or estimation of detection parameters, which relates to recaptures and the complexity of detection models. To explore this question, we dichotomized estimate precision into binomial variation caused by the number of bears detected on the sampling grid (CV_n) in contrast to the variance caused by estimation of effective sampling area and related detection parameters (CV_a). These two components add up to the CV of the density estimate using the equation:

$$\mathrm{CV_d} = \sqrt{\mathrm{CV_n}^2 + \mathrm{CV_a}^2} \quad (1)$$

(Huggins 1991, Borchers and Efford 2008, Efford 2019b).

We report abundance estimates as the average number of bears on the grid at one time (i.e., expected population size; Efford and Fewster 2013) which is simply the density estimate times the area of the sampling grid. Analyses were conducted using R software (R Core Team 2009) including *secr v 3.2* (Efford 2019b) and *ggplo*t *v 3.3* packages (Wickham 2009).

Results

Scat Survey Search Effort

The scat surveys covered approximately 3,065 km of roads per session. We drove on average 48% of the total kilometers of roads within cells (11–92% per cell). Many roads were inaccessible as they consisted of truck trails, unimproved and winter roads. We collected on average 0.08 (SD=0.15) suspected bear scat samples per kilometer of road surveyed and from these samples, we confirmed 0.05 (SD=0.10) grizzly bear scat samples per kilometer of road surveyed.**Error! Reference source not found.**

Sampling and Identification Success

We found differences in species confirmation and unique individual identification success from hair and scat DNA (Table 3.1). The success rate of identifying species was 18% higher for scat sampling compared with hair sampling, while the success of identifying individual grizzly bears was 86% higher for hair sampling compared with scat sampling. Hair sampling identified almost two times more individuals than scat sampling. Even with our sub-selection protocol minimizing black bear hair samples prior to genetic analysis, we found a lower proportion of grizzly bear versus black bear samples for hair compared with scat.

Table 3.1. Sample numbers and success rate comparisons between hair and scat approaches, from collection to individual bears identified in the DNA inventory.

	Hair Sampling Technique	Scat Sampling Technique
Samples Collected	958	183
Samples Sent to the Lab*	94	183
Samples Visually Excluded from Analysis	11	-
Samples Analyzed	83	183
Samples Identified as Bear Species	80% (66/83)	98% (179/183)
Samples Determined Black Bear	46% (38/83)	20% (37/183)
Samples Determined Grizzly Bear	34% (28/83)	78% (142/183)
Grizzly Bear Samples Identified to Individual	100% (28/28)	14% (20/142)
Individual Grizzly Bears Identified	14	8

* Note hair samples were sub-selected and only those showing grizzly bear characteristics underwent genetic analysis

The temporal distributions of sampling and detections varied between techniques. The number of grizzly bear detections for hair increased in later sessions, while detection numbers for scat decreased across

the sampling period. The number of individual bears detected by scat showed a similar decreasing pattern, while the numbers identified by hair were highest during the middle sessions. Equal numbers of females and males were identified by each method with seven and four of each sex from hair and scat, respectively. Using combined data, we identified 18 unique bears (eight female and ten male). Ten bears were identified by hair sampling, four by scat sampling, and four by both sampling methods.

Species Spatial Distribution

Although hair and scat collection covered the same area, scat sampling detected grizzly bears in grid cells where hair sampling showed non-detection, with almost twice the number of cells indicating grizzly bear presence (Fig. 3.1). Grizzly bears were detected by both techniques in 22% of cells, only by scat sampling in 34% of cells, and only by hair sampling in 8% of cells. There was non-detection by both methods in the remaining 36% of cells. A more comprehensive assessment of distribution by estimating individual bear home range centers using SECR methods is given in a subsequent section.

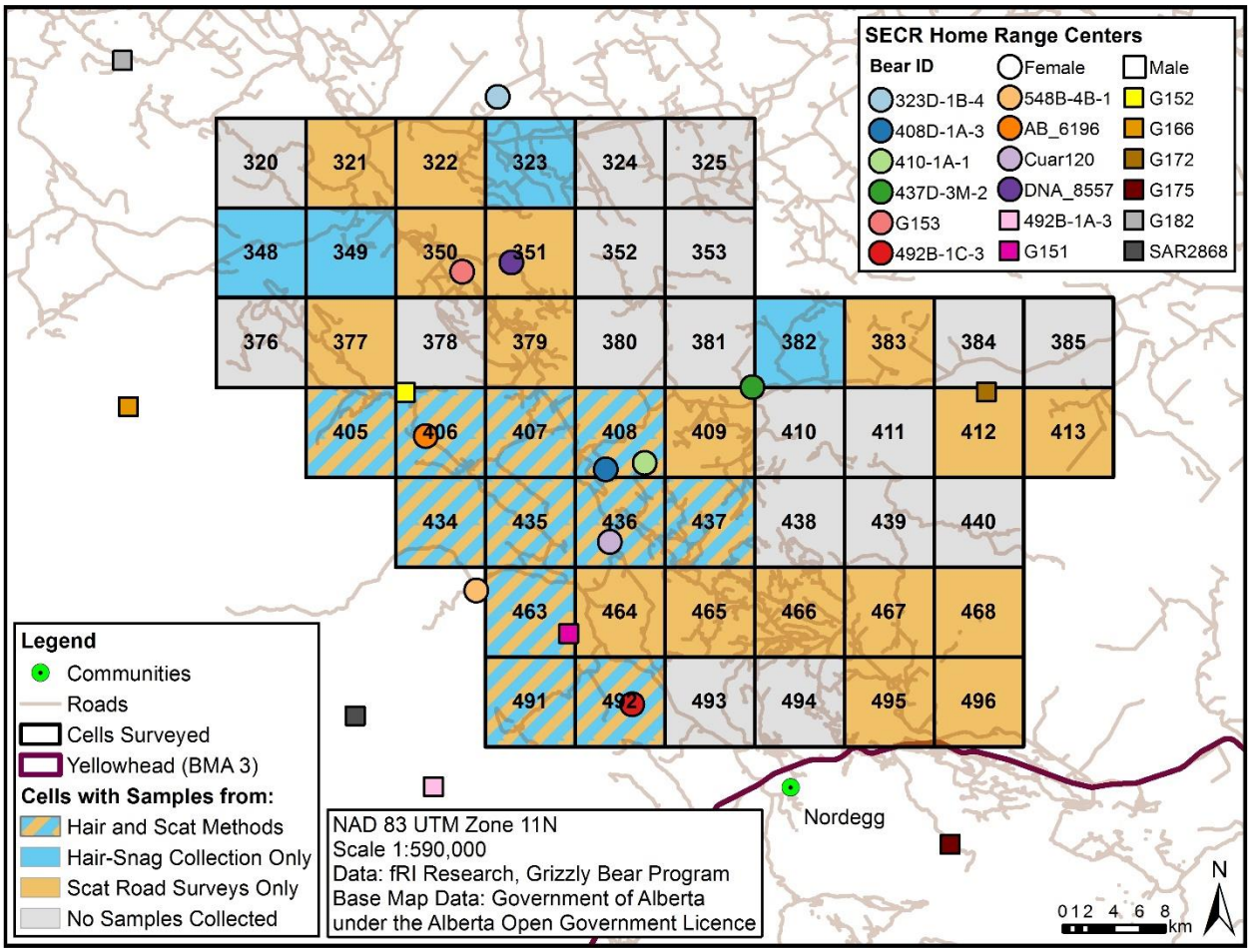


Figure 3.1 Species distribution

Our spatial datasets from hair-snag and scat each separately indicated higher recapture numbers and movements of male bears, primarily in the western portion of the grid for both techniques and in the east for scat sampling. Spatial redetections of females were limited for both data types in comparison to

males. Spatial detections using combined hair+scat data were enhanced compared to hair and scat only datasets, with additional recaptures as well as higher coverage for males (Fig. 3.2).

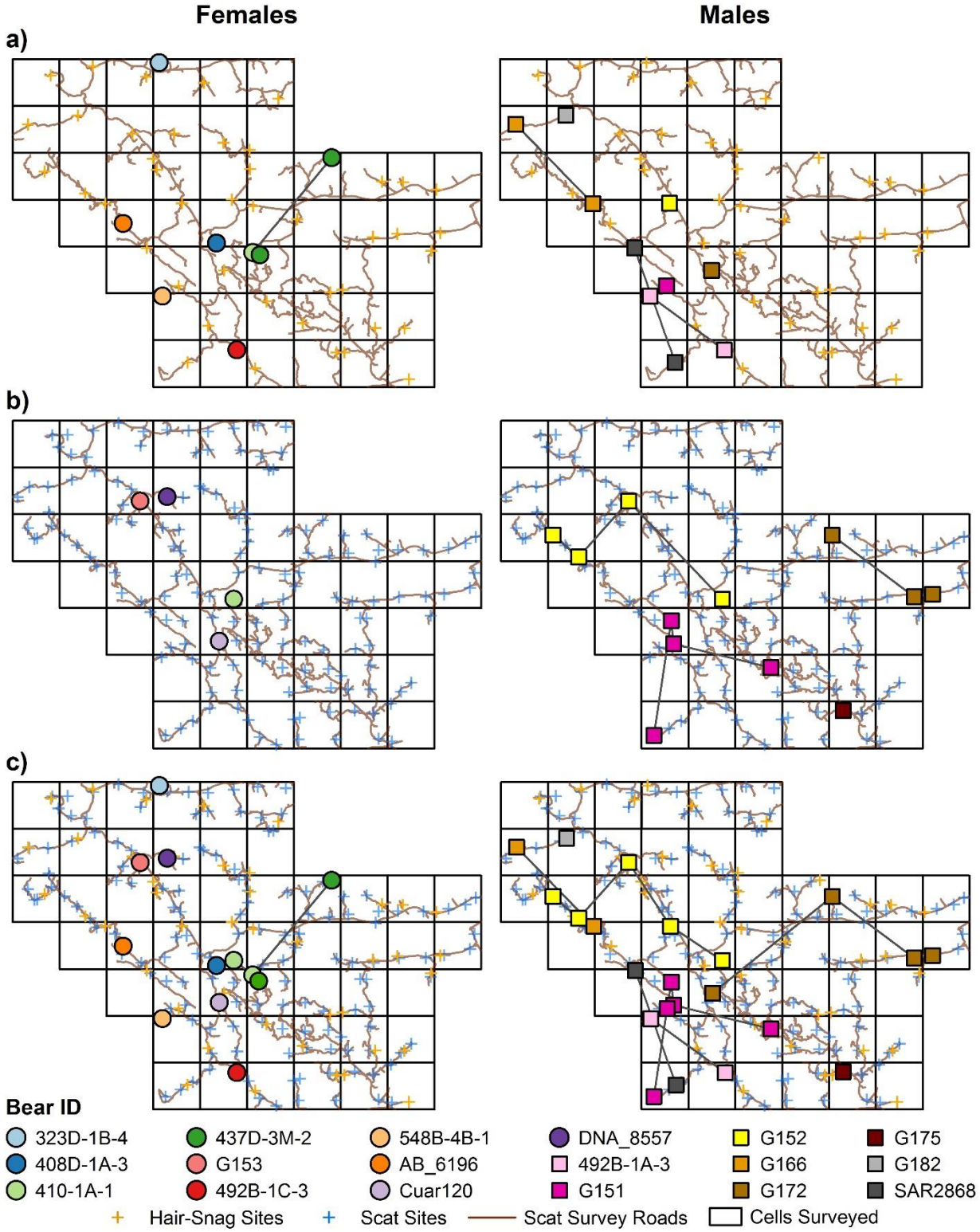


Figure 3.2 Spatial detections of grizzly bears from hair and scat samples

Population Estimates

Model selection for hair-snag only data indicated that detection probabilities of grizzly bears at their home range center (g_0) and spatial scale of grizzly bear movement (σ) were associated with terrain ruggedness in the area surrounding sites (Models H1 and H2; Table 3.2). We found increased rates of detection in areas of higher terrain ruggedness. The average number of bears estimated from the most supported model was 22.7 (SE=12.2, CI=8.4–60.9, CV=54%). Low precision of the overall estimate (CV_d=54%) was due to the low number of bears detected on the sampling grid (CV_n=25%) and estimation of detection parameters (CV_a=47%). A CV_n of 25% means CV_d would be 25%, if detection parameters were known with certainty; therefore, a limiting factor in precision of estimates is the low sample size of bears detected.

Scat only model selection indicated constant g_0 with sex-specific σ (Model S1) due to lack of spatial recaptures for female bears compared to multiple spatial recaptures for male bears (Fig. 3.2). A model with sex-specific g_0 with constant σ was also supported (Model S2; Table 3.2). Abundance estimates were only possible from Model S3 as estimates of abundance from sex-specific detection models had unrealistic standard errors presumably due to small sex-specific sample sizes (four females and four males detected) as well as a lack of spatial redetections for female bears. The Model S3 estimate, which pooled sex-specific detection parameters and had temporal trends in g_0, was 5.1 bears (SE=2.0, CI=2.4–10.8, CV=40%). This estimate corresponds to bears that use roads enough to have a non-zero probability of depositing scats.

Model selection for combined hair+scat data revealed constant g_0 with sex-specific σ relative to hair-snag sites (Model H+S1; Table 3.2), showing much higher support compared to other candidate models. Detection function plots show similar detection rates at home range centers for both methods, but greater scale of movements for hair sampling. Male and female bears exhibited non-zero detection probabilities at distances up to 35 and 12 km, respectively, from home range centers for hair, with 25 and 5 km for scat (Supplementary Material Appendix 1, Fig. A1.1). The population estimate from Model H+S1 was 23.4 (SE=9.4, CI=11.0–49.8, CV=40%), a result close to the hair-snag only estimate, but with higher precision. The abundance estimate for the sampling grid translates to a density estimate of 9.6 (CI=4.4–20.0) bears per 1000 km^2. Precision due to the number of bears detected (CV_n) was 26%, similar to hair-snag sampling alone (CV_n=25%). The precision due to detection (CV_a) was reduced to 30% from 47%, indicating the addition of scat data improved estimate precision. SECR estimates of bear home range centers using Model H+S1 revealed five of the detected bears had home range centers outside the grid (Fig. 3.1).

Cost Comparison

Based on our field costs in 2018, we estimated an approximate 30% cost reduction for scat sampling relative to hair sampling (Supplementary Material Appendix 2, Table A2.1). Cost savings stemmed mainly from minimal labour for scat sampling, with lower salary, accommodation and food expenses.

Discussion

Our research findings illustrate the value of sampling scat along roads in relation to hair-snag sampling by comparing the two concurrently conducted, grid-based gNIS approaches. We examined each method's ability to monitor wildlife through SECR population size estimates and species spatial distribution. Extending beyond our case specific results, we demonstrate the utility of comparative studies and speak to potential applications for other species and regions.

Population size estimates are a common requirement for monitoring programs that, if designed well, provide high quality information regarding wildlife populations (Nichols and Williams 2006). Given our methodology and the present level of genotyping success for grizzly bears in North America, our key finding is that grid-based barbed wire hair-snag sampling retains its position as a more accurate method for measuring grizzly bear population size within a small population. In areas where densities are relatively low (e.g., species distribution edges or expansion areas), higher detection and redetection rates compensate for lower numbers of individuals. In our study, hair sampling detected and redetected more individual bears. Scat sampling identified bears not detected by hair-snags, but the addition of these bears to hair data did not substantially change population estimates—suggesting hair-snag sampling still targeted the majority of bears in the area. While our scat approach successfully collected an adequate number of samples along roads, it was difficult to acquire individual genetic profiles from scat.

Both sampling methods identified individuals and their gender; however, the success rate for individual identification was much lower for scat (14%) compared with hair (100%). Bearing in mind differences in methods and season of collection, our scat sampling success rate was also lower than rates for brown bears in Italy (17–53%; De Barba et al. 2010a) and Sweden (55–80%; Kindberg et al. 2011), and for other species in Canada (black bear 29–33% or coyote 76–86%; Mumma et al. 2015). Lower individual success can be related to the execution of genetic analyses (Waits and Paetkau 2005); however, laboratory control measures were taken and repeated sample extraction did not improve success. A more likely determinant of our low success is sample quality, which is highly dependent on field conditions and sampling techniques.

Sample quality can be affected by diet (Murphy et al. 2003), precipitation (Brinkman et al. 2010, Wultsch et al. 2015, Roffler et al. 2019), temperature, humidity and sample age (Murphy et al. 2007, Brinkman et al. 2010). Spring and early summer bear diets in the interior of western Canada largely consist of grasses and forbs (Munro et al. 2006), contents which in Scandinavia produce lower success rates compared with scat containing only berries (but see Murphy et al. 2003). Scat collected in spring and autumn have higher success rates compared to summer in Scandinavia (Bellemain et al. 2005)—likely a combined factor of weather conditions and diet. We speculate that sun exposure and UV radiation, which degrades DNA (Friedberg 2003), plays a key role in our individual identification success because of the extreme environments found on road surfaces, especially during the summer months. We tried to

mitigate this impact by sampling inside scat layers, which contain fewer DNA cells, more moisture and higher susceptibility to microorganism degradation, but that are protected from UV exposure (Stenglein et al. 2010, Wultsch et al. 2015). Without direct comparisons between layers, it is difficult to determine which factors had the strongest impact on individual success given our field conditions. To optimize scat collection, further research could examine how UV radiation affects DNA quality by comparing individual success rates between inside and outside layers of solar impacted scat. Additional adjustments in scat field protocols (e.g., season or sample extraction location) and continued developments in genetic profiling would likely improve success rates. With our current genotyping success, scat was unable to provide reliable population estimates, but it did improve estimate precision.

Estimate precision and low variance help determine trends and statistical differences in population sizes over time. Incorporating scat with hair data improved the precision of estimates by 14% (CV=54% for hair only versus CV=40% for hair+scat). Our results parallel comparisons of hair-snag and rub tree sampling using traditional mark-capture for grizzly bears conducted in Montana (Boulanger et al. 2008). Rub tree estimates alone were lower than hair-snag estimates and the joint use of rub tree and hair-snag data increased overall population estimate precision. Similarly, integrating hair rub pads and scat transect data improved population density estimate precision using SECR methods for coyotes (*Canis latrans*) in Louisiana (Murphy et al. 2018). Combining techniques comes with challenges of required resources, but could be a way to address estimate precision issues when monitoring small populations and low-density areas.

The overall estimate precision from our top model was limited by the relatively small number of bears estimated (23.4 from the hair+scat model) on the sampling grid—indicated by the CV_n of 25%. Eighteen bears of the population estimate were detected by hair and scat. The remaining five bears were likely partial residents within the study area, which is another factor affecting detection in our analysis. Increasing the sampling grid size and subsequently the size of bears vulnerable to detection would be the best approach to offset lower precision. An inventory conducted for the entire Yellowhead BMA in 2014, which includes and surrounds our study area, estimated a grizzly bear density of 7.5 bears per 1000 km^2 (CI=5.7-9.9, CV=14%) using hair-snag sampling with 7x7 km grid cells. Precision of the 2014 estimate was better than both our hair-snag only and hair+scat estimates (CV=14% versus 54% and 40%, respectively), likely due to the larger grid size and therefore larger sample size of bears detected (n=66; Stenhouse et al. 2015). Further simulations could indicate the best approach to assess relative precision and potential bias in variance estimates due to summarizing scat samples at different scales as well as hair-snag sampling across a range of study area configurations. Depending on the study area, expanded sampling grids may fall into regions without roads, to which our scat sampling protocol is limited to. The scat approach also faces potential road bias for detecting individuals (e.g., those crossing or traveling along roads).

Spatially explicit models assume sampling is representative of the overall landscape. Our sampling grid encompassed an area where all cells were accessible by road to enable a controlled comparison of hair-snag and scat sampling protocols. Our methods potentially caused bias against bears that avoid roads (Graham et al. 2010), especially for scat sampling which did not use any attractant. While allowing for

variable spatial sampling effort, SECR models assume that bears in the sampling area have a non-zero detection probability if they encounter sampling sites. In this context, the assumption is that all bears will traverse roads and potentially deposit scat. If some bears avoid roads (e.g., females) they have no chance of being part of the bear population sampled by scat and will negatively bias estimates. In contrast, the intensive sampling design (7x7 km cells) for hair snares compared to the estimated scale of movement (up to 35 km) indicates that a high level of bias with hair-snag sampling is improbable—likely an effect of scent-lures drawing bears into hair-snag sites (Boulanger et al. 2004a). Scat sampling along roads still detected male bears from up to 25 km. The large scale of movement relative to the sampling grid, and having estimated home range centers occur outside of the grid, mitigated the effect of sampling near roads. Even with potential biases, the road survey approach demonstrated advantages for determining where grizzly bears occurred within the study area.

Monitoring species presence, spatial distribution and expansion areas requires species level identification and ideally gender. The success rate of identifying species and their sex using scat was higher than for hair (98% and 80%, respectively). While some published research fails to explicitly state DNA extraction rates (e.g., Gompper et al. 2006), these results provide valuable information, which is notably species and source dependent. Our species identification rates align with comparable results for other species (e.g., 95% for black bear, coyote and lynx (*Lynx canadensis*) samples combined; Mumma et al. 2015). Scat sampling additionally covered more ground in the study area, surveying many kilometers of roads per cell compared to one scent-lure baited hair-snag site. Detectability was higher for scat with almost twice the number of cells indicating grizzly bear presence, including crucial areas where hair sampling showed non-detection (i.e., the eastern edge of the study area, which is the known limit of the population). While combined hair and scat data provided complimentary results, scat sampling alone was still well suited for determining species distribution—with higher species identification success and a broader coverage within cells. While full genotyping success to the individual level enables accurate and more precise population estimates, species identification alone may be adequate depending on the specific objectives of the monitoring program. Therefore, particular management goals for wildlife monitoring may impact which population measures and corresponding methods are appropriate.
Efficient use of limited resources is important to both researchers and managers interested in conserving wildlife populations. Monitoring objectives and study design need to be considered in conjunction with available resources and budgets. We found that hair sampling was more resource intensive (30% higher cost), as standard hair-snag methods require additional staff for time-consuming protocols compared to our road survey methods. The intended use of data (e.g., population estimate or species distribution) affects budgets and can indicate the appropriate method required and respective costs. Monitoring programs could also consider adapting scat survey methods as a citizen science approach to further reduce field costs (Kindberg et al. 2011)—directing the bulk of required resources to laboratory, analysis and report preparation costs. In addition, citizen involvement could help develop and expand long-term genetic databases while boosting the feasibility of recurrent monitoring.
Our findings demonstrate the potential of systematic scat surveys along roads. With improved individual identification rates, for use in other areas, or for species where scat success rates are already higher, scat sampling could serve as a stand-alone DNA inventory method. As with hair sampling, genetic information from fecal DNA gathered long-term can be used to monitor the survival of individual bears,

population level survival rates, and assess the use of landscapes through time (e.g., Boulanger et al. 2004b). Scat sampling along roads could equally explore the spatial distribution of black bears and with adaptations could be applied to other species known to defecate on roads (e.g., canids; Kohn et al. 1999) or a combination of species. Pooled resources applied for multiple species could additionally assist wildlife managers in meeting their monitoring and conservation objectives. Although the best methods are sometimes species specific (Mumma et al. 2015), finding a practical single sampling method for multiple species (e.g., scat sampling along roads) could maximize resource and cost efficiency.

Additional New Findings

During the 2018 field season and the 2019 spring fRI Grizzly Bear Research Collaring program we captured and radio collared grizzly bears for other research activities within BMA 4. Some bears were captured within the 2018 DNA sampling grid while some bears (n=2) (Figure 8) were captured outside this sampling grid in the agricultural areas east of the green zone within the BMA. When animals were captured within the agricultural zone we were able to work directly with landowners who had reported sightings of grizzly bears on their properties. In these cases efforts were made to capture these bears near the sighting location and most importantly these bears were released at the capture site to allow detailed information to be collected on the movements, behavior and home ranges of these eastern edge bears.

Figure 8 G363 a few days following capture in agricultural zone along the eastern edge of BMA 4 in 2019

Figure 5 shows the MCP home ranges of research bears in BMA 4 for the period 2017-2019. The two agricultural zone bears in this figure are G361 and G363 and the home ranges of these bears indicates

that in 2019 they did have home ranges that did overlap portions of the 2018 DNA sampling grid (Figure 6) and thus did have the opportunity to be detected within the population inventory effort in 2018. However these bears were not identified by the hair snag methods used in the 2018 inventory.

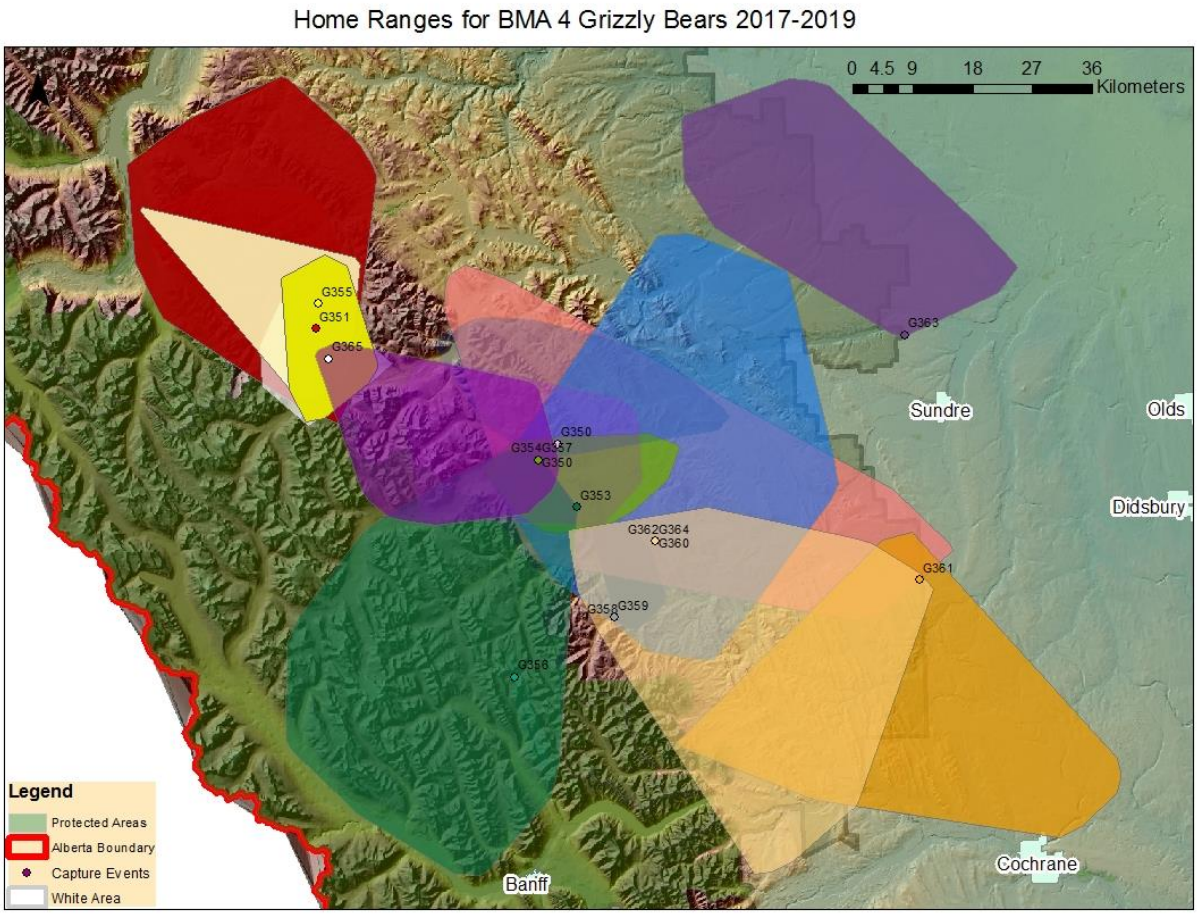


Figure 5. MCP home ranges for research grizzly bears in BMA 4 2017-2019.

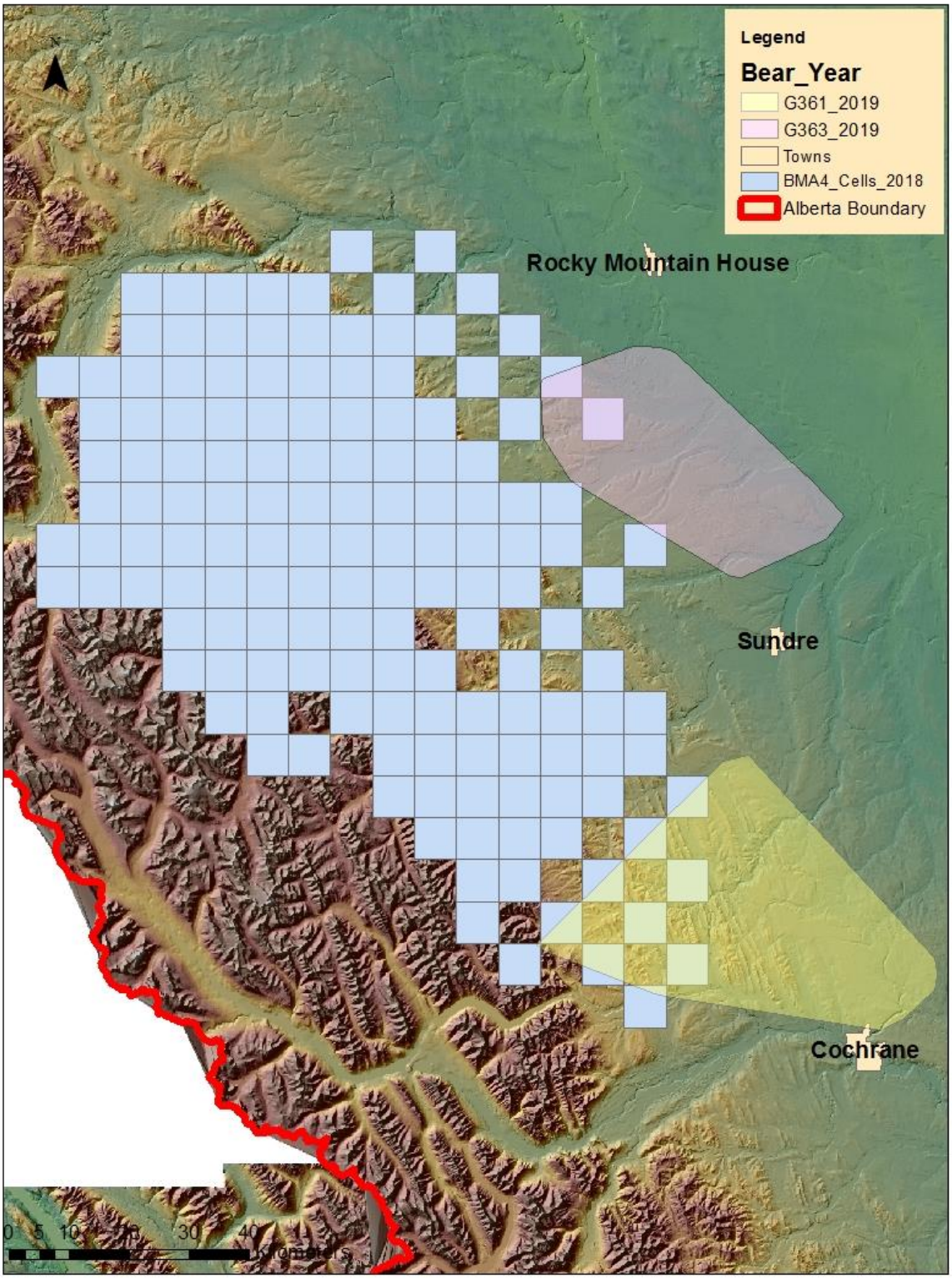


Figure 6. DNA grid overlap with agricultural zone bears MCP's in 2019

It is also interesting to note that a sample of hair that was collected by a landowner where G361 was captured (7 km NW of Sundre) was found to be from our research bear G352 (adult male – age 7 in 2018) who was a research capture (too large to collar) in 2018 and was also detected in the 2018 population inventory work (Figure 7). Although these samples sizes are small they do provide information suggesting that although large DNA sampling grids may not cover all habitats where bears are possibly observed given the large home ranges and movements of grizzly bears they do provide a statistical possibility of detection, which is required within inventory efforts. These data also show the value of local residents and citizens collecting biological samples from bears (hair or scat) that may be seen along more eastern edges of recognized grizzly bear range to add to the existing genetic database and thus allow ongoing monitoring of grizzly bear survival, habitat occupancy, and reproduction over time.

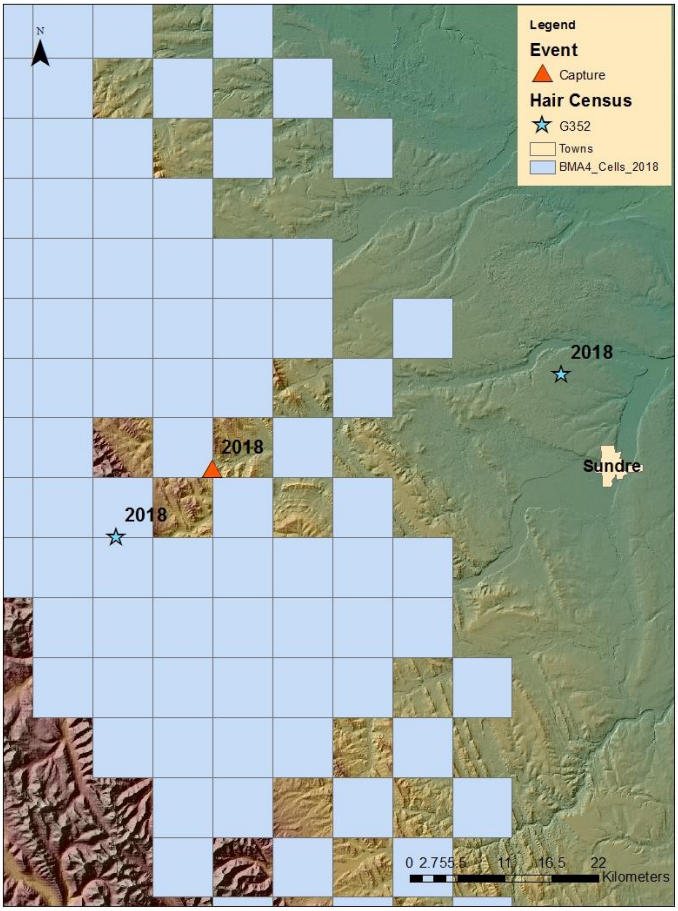


Figure 7. Bear G352 showing multiple sampling locations in 2018.

Application (extension) and Recommendations

Exploring the Mystery of Low Grizzly Bear Individual Identification Success from Scat DNA

The use of grizzly bear fecal DNA has come a long way in Alberta through collaboration between the Grizzly Bear Program and program partners. Over almost a decade, our team has worked closely with Scandinavian researchers to examine the application of citizen scat collection in Alberta to monitor our bear populations. With our laboratory colleagues at the Norwegian Institute of Bioeconomy Research (NIBIO), we developed a process to identify black bears (a species not found in Europe), refined sampling techniques to improve DNA extraction rates, expanded the number of extractable markers for grizzly bears and standardized results from different genetics labs to allow for direct comparison between scat (NIBIO), hair (Wildlife Genetics International) and tissue (Alberta Environment and Parks). We can now identify parent offspring relations from fecal DNA using 21 STR markers and one sex-specific marker. However, the quality of fecal DNA collected still remains an issue as we have yet to see high success rates in these advanced analyses.

The goal of our latest research project was to determine if a novel systematic scat sampling approach along roads could be a cost effective alternative to the standard barb wire hair-snag DNA inventory method. Our scat approach was effective from the sample collection perspective. We found an abundance of scat samples on roads, with lower costs for staff and equipment. However, our individual success rates from grizzly bear scat samples was only 14% (# of samples identifying individuals/# of grizzly bear samples), which is lower than rates for brown bears in Sweden or Italy (55–80%, Kindberg et al. 2011 and 17–53%, De Barba et al. 2010, respectively) and other species in Canada (black bear 29–33% or coyote 76–86%, Mumma et al. 2015).

Although it is difficult to parse out the reason behind the low individual identification success rate, looking back to our research results from past years provides some additional insight. In 2014, we conducted walking scat transects mainly within forested habitat and the individual identification success rate was 32% (Table 1). We completed a sweep survey, driving most accessible gravel roads only once within a study area in 2016, with a 12% success rate. Expanding our driving techniques to parallel hair-snag inventories in 2018, we established road circuits that were systematically driven, sampled and cleared every two weeks, with the 14% success rate. Note that there were major differences between projects in terms of time of year, area searched and method of searching. Pooling our samples together from over the years, we can tease out potential effects on the individual success rate (# of individual samples/# of grizzly bear samples by feature and season). The samples collected on gravel roads in the summer had a 14% success rate, those collected on roads (including old roads) in the fall had 22% success, while samples found on other features (cutblocks, pipelines, seismic lines, forest floor, etc.) in the fall had a 32% success rate (Table 2).

Working towards successful individual identification from scat, citizen scat collection, and scat DNA inventories, we suggest further research be specifically designed to compare different collection conditions. With replication, we would recommend that citizen science projects collect grizzly bear scat

in the fall off of roads. However, the challenge with this approach would be finding enough scat piles. To continue progress with the bountiful systematic road surveys, we recommend increasing the frequency of scat collection to minimize effects of potentially quickly degrading fecal DNA on roads or further exploring field methods for the collection, preservation and storage of scat samples to extract adequate DNA for individual identification.

With continued efforts, we hope to solve the mystery of the low success in identifying individual grizzly bears using fecal DNA. We envision a time where successful scat sampling methods can pave the way for additional long-term grizzly bear population monitoring in Alberta.

Table 1: Individual success rates of identifying grizzly bears from scat by research project.

Sampling Approach	Year & Months	Features Sampled	# Samples Collected	# GB Samples	# Individual Samples	Success Rate
Walking Transects	2014: Aug – Oct	Cutblocks, wellsites, gravel roads, pipelines, seismic, forest off trail	130	63	20	32%
Single Road Sweep	2016: Sep – Oct	Gravel roads	62	41	5	12%
Systematic Road Surveys	2018: Jun – Jul	Gravel roads	183	142	20	14%

Table 2: Individual success rates of identifying grizzly bears from scat by feature and season.

Features & Season	# GB Samples/Features & Season	# Individual Samples	Success Rate
Other features in fall	40	11	28%
Roads in fall	64	14	22%
Roads in summer	142	20	14%

We believe that the value and utility of grizzly bear scat collection efforts by those working in grizzly bear habitat would prove valuable in monitoring local bear populations over time. To ensure scientific rigor this type of collection effort should be organized and coordinated by a single group who would distribute collection kits, arrange sample pick up, deliver samples to laboratories for analysis and then analyze DNA results in relation to known bears within the BMA. It is very important to recognize that millions of dollars and countless hours of research time has gone into compiling a massive grizzly bear genetic database for all the 7 BMA's in the province. To gain the maximum value from this investment it will be important to continue to gather genetic samples (hair or scat) from local bear populations over time. The techniques and results that our research team has presented provide a framework for one important aspect of grizzly bear monitoring on a shared, complex and multi-use landscape. It would be possible to have a program of this nature organized and delivered at the local level with groups such as

Bearsmart but analysis and data management would need to be delivered by groups/organizations with experience and background such as fRI Research or AEP.

Over time these results would serve to provide important information of grizzly bear survival, distribution, occupancy and reproduction which are all important metrics of grizzly bear recovery. A program of this type would require some annual funding to succeed and of course the active participation of those working, recreating and living in provincial grizzly bear range.

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