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# Comparison of grizzly bear hair-snag and scat sampling along roads to inform wildlife population monitoring

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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.

**Keywords:** DNA inventory, genetic non-invasive sampling (gNIS), grizzly bear, population estimates and density, spatially explicit capture–recapture (SECR), species spatial distribution, systematic grid-based sampling, *Ursus arctos*, wildlife monitoring

Estimating population size and species presence or distribution in an area are critical components of wildlife monitoring required for species management (Nichols and Williams 2006, De Barba et al. 2010a) and recovery (Campbell et al. 2002). Genetic sampling of wild animals can distinguish between species to indicate geographical range and species distribution, and when combined with capture–mark–recapture methods, can identify individuals to estimate population

size, survival, recruitment and movement (Schwartz et al. 2007). Using blood or other tissues for genetic analyses is considered an invasive approach with challenges of capturing adequate numbers of animals (Ferreira et al. 2018), high costs (De Barba et al. 2010a, Sabino-Marques et al. 2018), risks in the field (Taberlet and Bouvet 1992, Gompper et al. 2006, Schwartz et al. 2007) and animal welfare and safety (Cattet et al. 2008, Lefort et al. 2019, Zemanova 2019).

Genetic non-invasive sampling (gNIS) has evolved as an alternative method to monitor wildlife populations (Höss et al. 1992, Taberlet and Bouvet 1992, Woods et al. 1999). This approach allows for extraction of genetic information from feces, hair, feathers or other sources (Beja-Pereira et al. 2009) without catching, handling, disturbing

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or even observing the animals (Taberlet et al. 1999). Many animals are eligible for gNIS (Beja-Pereira et al. 2009, Zemanova 2019), especially rare or threatened species where the feasibility and benefits of invasive sampling are low (Ferreira et al. 2018).

Recent studies have combined genetic data with spatially explicit capture–recapture (SECR; i.e. population models incorporating spatial parameters) to estimate population size and density (Boulanger et al. 2018, Roffler et al. 2019). Taking into account the spatial distribution of individuals and spatial detection information, SECR can utilize various DNA sources and sampling designs. However, the challenge of employing frequent, cost-effective approaches to monitoring wildlife populations remains (Efford and Boulanger 2019).

Several studies recommend non-invasive over invasive genetic methods for population estimates; for example, using fecal or hair sampling over live-trapping (Sabino-Marques et al. 2018, Croose et al. 2019), or fecal sampling over aerial surveys and ground observations (Solberg et al. 2006). Some research has explored differences between non-invasive methods (e.g. camera trapping versus hair or fecal sampling; Gompfer et al. 2006, Rodgers et al. 2014, Alldredge et al. 2019). Few studies have compared gNIS techniques directly (e.g. fecal sampling using detection dogs versus hair sampling; Mumma et al. 2015) or have used combined gNIS data sources (e.g. hair and fecal sampling along transects (Murphy et al. 2018) or grid-based hair-snag and opportunistic rub-tree hair sampling; Boulanger et al. 2008, Graves et al. 2012). As non-invasive approaches gain popularity, there is a growing need to further evaluate various gNIS methods.

Since the first DNA extractions from wild animal hair (Taberlet and Bouvet 1992, Woods et al. 1999), researchers have been developing techniques and applications to study a variety of wildlife using hair samples (Paetkau 2003, Beja-Pereira et al. 2009, Proctor et al. 2010). Hair is often collected through hair snares that, for example, use barbed wire or other adhesive alternatives at hair-snag sites or rub pads (Beja-Pereira et al. 2009, Mumma et al. 2015, Roffler et al. 2019). While hair samples provide high quality data that can be used to identify species and individuals, field protocols are time and cost intensive (Boulanger et al. 2006, Croose et al. 2019).

Collecting fecal DNA from wildlife originated alongside hair sampling (Höss et al. 1992, Kohn et al. 1999) and is considered a less invasive collection method (Lefort et al. 2019). Although success rates are generally lower for fecal DNA (Waits and Paetkau 2005, Mumma et al. 2015), laboratory advancements for some species and regions have led to reliable population inventories based on scat sampling (Beja-Pereira et al. 2009, Andreassen et al. 2012). Many studies collect scat opportunistically (Solberg et al. 2006, De Barba et al. 2010a), along transects (De Barba et al. 2010a, Murphy et al. 2018) or by dog searches (Mumma et al. 2015). With easy access and visibility, roads are practical features for collecting scat samples. However, potential biases can occur if animal densities near roads differ from areas without roads, especially when roads do not cover the entire sampling area. For species that use roads as travel corridors

and defecate along travel routes (e.g. canids), roads have been included within survey transects (Kohn et al. 1999, Gompfer et al. 2006, Murphy et al. 2018, Roffler et al. 2019). Roads and roadside vegetation attract bears (Roever et al. 2008, Graham et al. 2010); however, road transects, and specifically systematic grid-based fecal sampling, have yet to be conducted for bears.

Hair-snag sites sampled systematically within a grid has become the standard of DNA hair sampling for bear populations in North America (Kendall et al. 2009, Proctor et al. 2010, Boulanger et al. 2018), while opportunistic scat sampling is employed for brown bears in Scandinavia (Bellemain et al. 2005, Andreassen et al. 2012, Schregel et al. 2012, 2018). In Alberta, Canada, grizzly bears *Ursus arctos* are elusive animals occurring in low densities (Boulanger et al. 2018). Since 2010 grizzly bears have been provincially designated as threatened (Festa-Bianchet 2010). The population status and ongoing developments in hair and fecal protocols make grizzly bears an excellent study species to explore various gNIS methods. The aim of our research was to compare hair and scat sampling approaches, develop a low-cost, low-effort approach to monitoring populations, and inform wildlife management – using a low-density population of grizzly bears in North America as our study species. Our specific objectives were to evaluate sampling and identification success, species level spatial distribution of detections, population estimates and project costs of each gNIS approach. While some studies have investigated hair and scat using traditional capture–mark–recapture methods (Wasser et al. 2004, De Barba et al. 2010b) this is, to our knowledge, the first direct comparison between hair-snag trapping and scat collection along roads using systematic grid sampling and SECR for any species.

## Material and methods

### Study area

The study area covers approximately 2450 km<sup>2</sup> within the foothills of the Rocky Mountains in Alberta, Canada (Fig. 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 3360 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 *Vaccinium* spp. species (Munro et al. 2006).

### 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; Stenhouse et al. 2015).



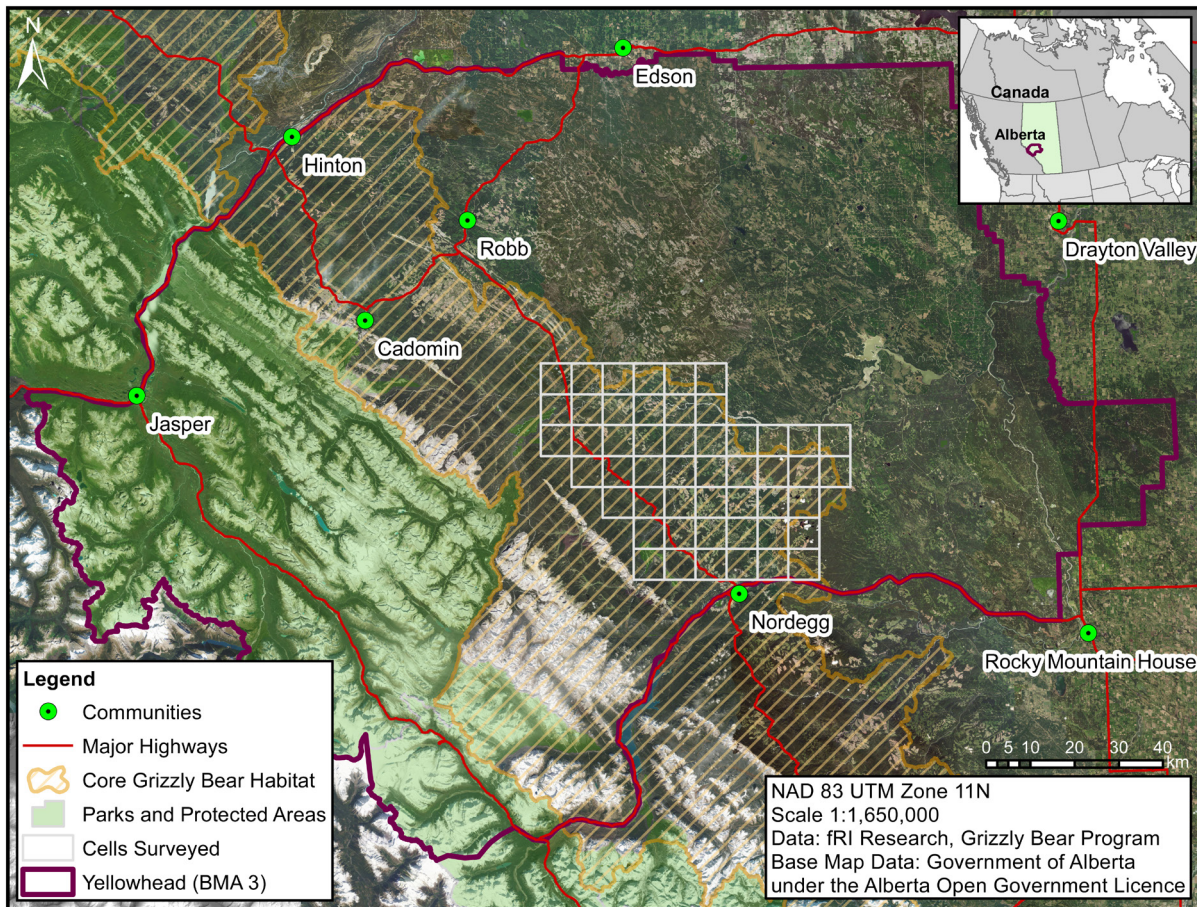


Figure 1. The study area within the Yellowhead Bear Management Area (BMA 3) of Alberta, Canada including the DNA inventory sampling grid, core grizzly bear habitat, parks and protected areas.

All sites were accessible by truck and a short hike (<1 km). Subsequent to hair-slug 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-slug 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-slug sites (Fig. 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<sup>-1</sup> for gravel roads and 20–50 km h<sup>-1</sup> 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<sup>3</sup> of scat, which we stored in uniquely bar-coded 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



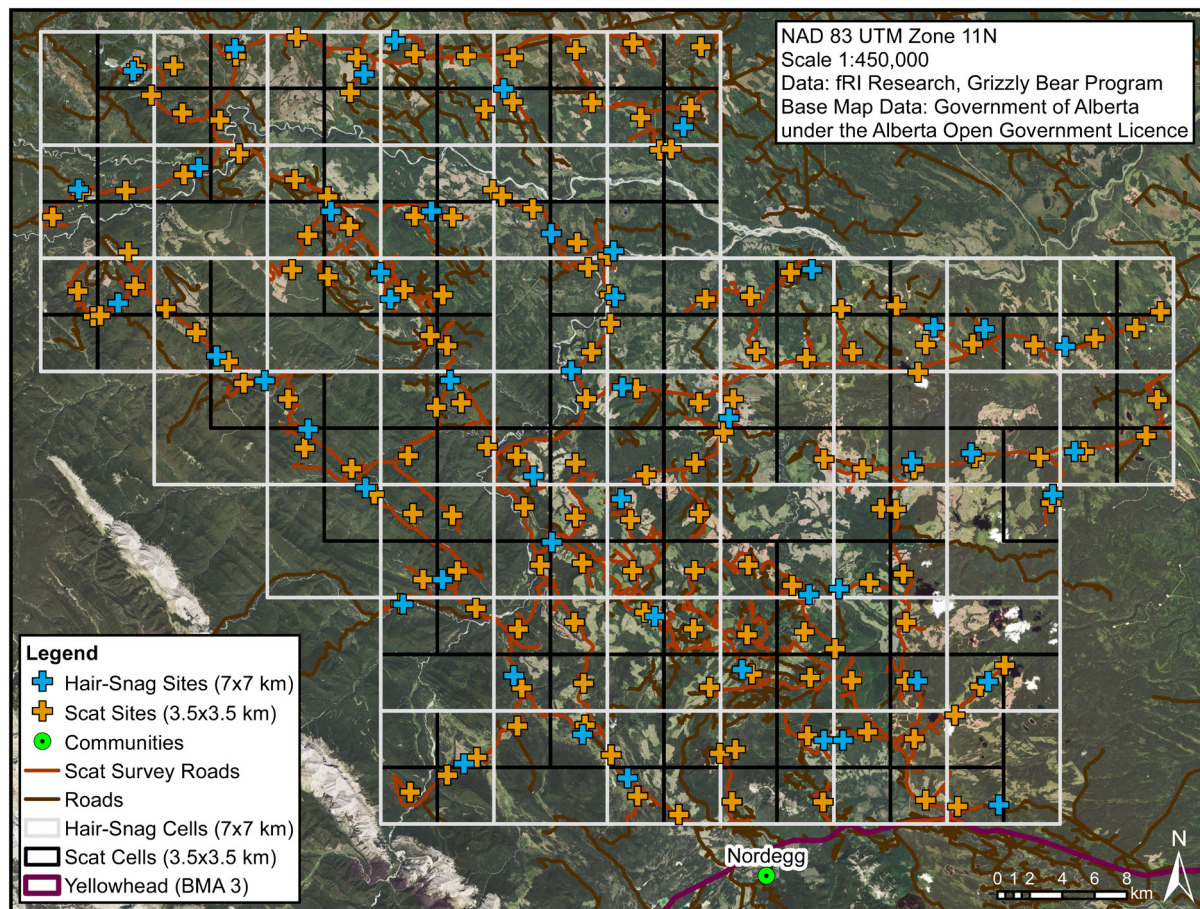


Figure 2. Roads systematically surveyed for grizzly bear scat in relation to the existing road network and spatially explicit capture–recapture (SECR) layout of hair and scat centroid-based detection sites for  $7 \times 7$  and  $3.5 \times 3.5$  km cell size, respectively, within the DNA inventory sampling grid in Alberta, Canada.

(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  $3.5 \times 3.5$  km cells (nested in the  $7 \times 7$  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).

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  $3.5 \times 3.5$  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:

$$CV_d = \sqrt{CV_n^2 + CV_a^2} \tag{1}$$

(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 (<www.r-project.org>) including *secr* ver. 3.2 (Efford 2019b) and *ggplot* ver. 3.3 packages (Wickham 2009).

## Results

### Scat survey search effort

The scat surveys covered approximately 3065 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 (Supplementary material Appendix 1 Table A1.1).

### Sampling and identification success

We found differences in species confirmation and unique individual identification success from hair and scat DNA (Table 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 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 (Supplementary material Appendix 1 Table A1.2, A1.3 for session specific details of each data type). 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 (Supplementary material Appendix 1 Table A1.4). 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). 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.

Our spatial datasets from hair-snap 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. 4).

### Population estimates

Model selection for hair-snap only data indicated that detection probabilities of grizzly bears at their home range center ( $g_0$ ) and spatial scale of grizzly bear movement ( $\sigma$ ) were associated with terrain ruggedness in the area surrounding sites (models H1 and H2; Table 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<sub>d</sub> = 54%) was due to the low number of bears detected on the sampling grid (CV<sub>n</sub> = 25%) and estimation of detection parameters (CV<sub>a</sub> = 47%). A CV<sub>n</sub>

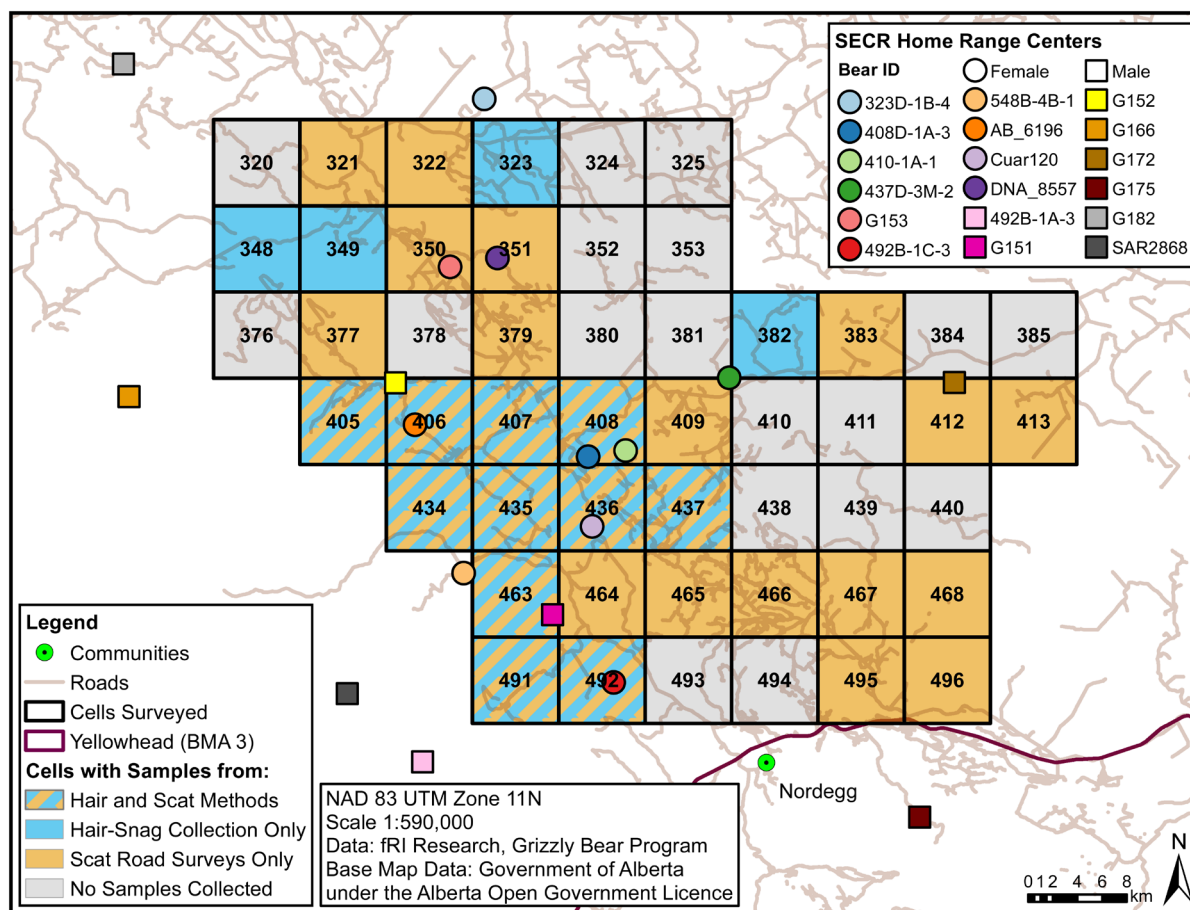


Figure 3. Cells with grizzly bears identified by hair, scat or both sampling methods, as well as estimated home range centers for individual grizzly bears from the spatially explicit capture-recapture (SECR) analysis of combined hair+scat data (model HS1; Table 2) within the DNA inventory sampling grid in Alberta, Canada.

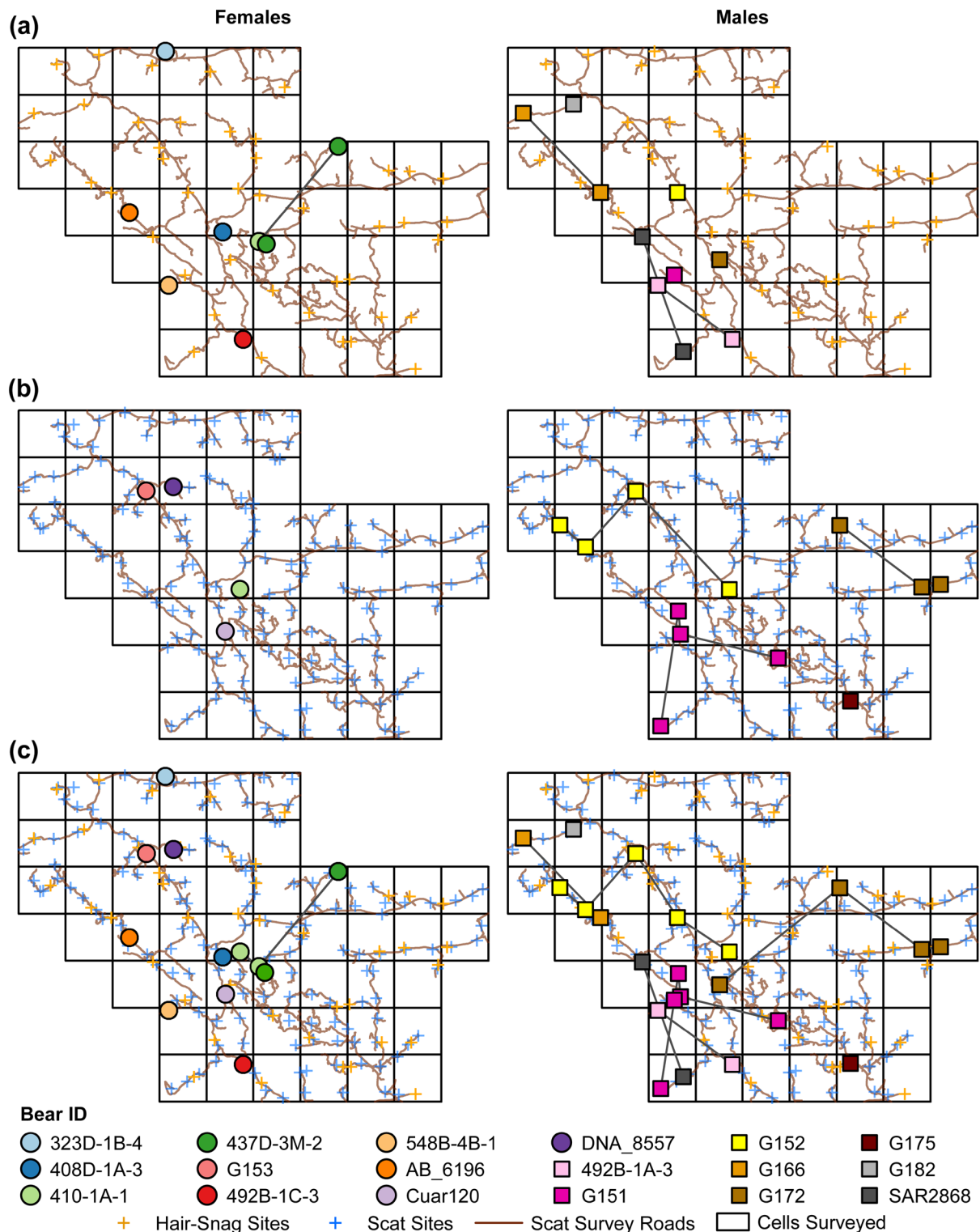


Figure 4. Spatial detections and redetections of female and male grizzly bears using (a) hair-snag sites, (b) road detection scat sites and (c) hair-snag and scat sites on the DNA inventory sampling grid in Alberta, Canada. Actual sequences of movement are approximate.

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  $\sigma$  (model S1) due to lack of spatial recaptures for female bears compared to multiple spatial recaptures for

male bears (Fig. 4). A model with sex-specific  $g_0$  with constant  $\sigma$  was also supported (model S2; Table 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



Table 2. Model selection for hair-s snag, scat and hair + scat analyses. Sample size adjusted Akaike information criterion ( $AIC_c$ ), the difference in  $AIC_c$  between the model and the most supported model ( $\Delta AIC_c$ ),  $AIC_c$  weight ( $w_i$ ), the number of model parameters (K) and log-likelihood (LL) are given. Baseline constant models are italicized for reference with covariate models.

Model	Detection ( $g_0$ )	Scale ( $\sigma$ )	$AIC_c$	$\Delta AIC_c$	$w_i$	K	LL
Hair-s snag models							
H1	TRI <sup>a</sup>	constant	160.29	0.00	0.31	3	-75.9
H2	constant	TRI	160.61	0.32	0.27	3	-76.1
H3	TRI + bk <sup>b</sup>	constant	163.31	3.02	0.07	4	-75.4
H4	TRI	sex	163.86	3.56	0.05	4	-75.7
H5	TRI + b <sup>c</sup>	constant	163.91	3.70	0.05	4	-75.7
H6	constant	sex + TRI	164.14	3.84	0.05	4	-75.8
H7	sex + TRI	constant	164.22	3.93	0.04	4	-75.9
H8	TRI	TRI	164.32	4.02	0.04	4	-75.9
H9	TRI + sex	sex	168.89	8.59	0.00	5	-75.7
H10	session	constant	170.98	10.69	0.00	5	-76.7
H11	constant	session	171.35	11.06	0.00	5	-76.9
H12	CC <sup>d</sup>	constant	166.27	5.97	0.02	3	-78.9
<i>H13</i>	<i>constant</i>	<i>constant</i>	<i>165.00</i>	<i>4.71</i>	<i>0.03</i>	<i>2</i>	<i>-80.0</i>
H14	bk	constant	166.84	6.55	0.01	3	-79.2
H15	constant	bk	167.34	7.04	0.01	3	-79.5
H16	constant	trend	167.47	7.18	0.01	3	-79.5
H17	constant	sex	167.62	7.33	0.01	3	-79.6
H18	sex	constant	168.05	7.76	0.01	3	-79.8
Scat models							
S1	constant	sex	197.99	0.00	0.55	3	-93.0
S2	sex	constant	198.61	0.62	0.41	3	-93.3
S3	trend	constant	205.03	7.04	0.02	3	-96.5
S4	roadkm <sup>e</sup>	constant	205.54	7.55	0.01	3	-96.8
S5	constant	roadkm	206.34	8.35	0.01	3	-97.2
S6	<i>constant</i>	<i>constant</i>	<i>207.28</i>	<i>9.28</i>	<i>0.01</i>	<i>2</i>	<i>-100.4</i>
S7	session	constant	232.75	34.76	0.00	5	-96.4
S8	constant	session	233.54	35.55	0.00	5	-96.8
Hair + scat models							
H + S1	constant	Sex × HS <sup>f</sup>	382.91	0.00	0.86	5	-184.0
H + S2	HS	sex	387.90	4.99	0.07	4	-188.4
H + S3	sex × HS	constant	389.41	6.50	0.03	5	-187.2
H + S4	HS × TRI	sex	390.39	7.48	0.02	6	-185.4
H + S5	HS × TRI	sex × HS	391.05	8.14	0.01	8	-179.5
H + S6	constant	HS	396.52	13.61	0.00	3	-194.4
H + S7	sex	constant	397.16	14.25	0.00	3	-194.7
H + S8	constant	sex	397.21	14.30	0.00	3	-194.8
H + S9	HS	constant	397.82	14.91	0.00	3	-195.1
H + S10	HS	constant	398.24	15.33	0.00	5	-191.6
H + S11	sex	sex	398.60	15.68	0.00	4	-193.8
H + S12	scat <sup>g</sup> × road	constant	398.99	16.08	0.00	5	-192.0
H + S13	HS × sex	HS × sex	399.05	16.14	0.00	8	-183.5
H + S14	HS	HS	399.79	16.88	0.00	4	-194.4
<i>H + S15</i>	<i>constant</i>	<i>constant</i>	<i>407.82</i>	<i>24.91</i>	<i>0.00</i>	<i>2</i>	<i>-201.5</i>

<sup>a</sup> Terrain ruggedness index, <sup>b</sup> trap-specific behavioural response where detection parameters change for a site once a bear is detected, <sup>c</sup> bear-specific behavioural response where detection parameters change for a bear after initial detection, <sup>d</sup> canopy cover covariate, <sup>e</sup> hair-s snag site, <sup>f</sup> scat site and <sup>g</sup> km of roads driven.

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  $\sigma$  relative to hair-s snag sites (model H + S1; Table 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-s 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<sup>2</sup>. Precision due to the number of bears detected (CV<sub>n</sub>) was 26%, similar to hair-s snag sampling alone (CV<sub>n</sub> = 25%). The precision due to detection (CV<sub>d</sub>) 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).

## 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-snap 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-snap 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-snap 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-snap 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-snap estimates and the joint use of rub tree and hair-snap 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<sup>2</sup> ( $CI = 5.7–9.9$ ,  $CV = 14\%$ ) using hair-snap sampling with  $7 \times 7$  km grid cells. Precision of the 2014 estimate was better than both our hair-snap 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 ( $7 \times 7$  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 (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 (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.

## Conclusions

While hair-snag sampling retains its position as the standard for grizzly bear population estimates with superior individual identification rates, scat sampling holds great promise. 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 wildlife monitoring efforts in North America. 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.

## Data accessibility

Genetic and field data are publicly available in Zenodo (Phoebe et al. 2020). Because grizzly bears are a threatened species in Alberta, precise location data has been excluded.

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**Author contributions** – GS and IP conceived the ideas; AS, HGE and KG contributed in project development; GS, IP and JB designed methodology; IP and field technicians collected the data; IF and SBH conducted scat sample genetic analyses; KG managed genetic databases; IP and JB analyzed the data; JB conducted SECR analysis; IP led the writing of the manuscript. All authors contributed critically to the drafts.

**Conflicts of interest** – We have no conflict of interest.

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Supplementary material (available online as Appendix wlb-00697 at <[www.wildlifebiology.org/appendix/wlb-00697](http://www.wildlifebiology.org/appendix/wlb-00697)>). Appendix 1. Supplementary results: Table A1.1. Scat sampling search effort results, Table A1.2. Hair and scat sample counts and individuals identified per session, Table A1.3. Summary statistics of sampling for SECR analysis, Table A1.4. Details of individual bears identified in our study, and Figure A1.1. Detection function plots for male and female bears. Appendix 2. Sampling approach cost comparison: Table A2.1. Comparison of project costs.