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Article *in* Molecular Ecology Resources · February 2015

DOI: 10.1111/1755-0998.12390 · Source: PubMed

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Evaluating noninvasive genetic sampling techniques to estimate large carnivore abundance

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Abstract

Monitoring large carnivores is difficult because of intrinsically low densities and can be dangerous if physical capture is required. Noninvasive genetic sampling (NGS) is a safe and cost-effective alternative to physical capture. We evaluated the utility of two NGS methods (scat detection dogs and hair sampling) to obtain genetic samples for abundance estimation of coyotes, black bears and Canada lynx in three areas of Newfoundland, Canada. We calculated abundance estimates using program CAPWIRE, compared sampling costs, and the cost/sample for each method relative to species and study site, and performed simulations to determine the sampling intensity necessary to achieve abundance estimates with coefficients of variation (CV) of <10%. Scat sampling was effective for both coyotes and bears and hair snags effectively sampled bears in two of three study sites. Rub pads were ineffective in sampling coyotes and lynx. The precision of abundance estimates was dependent upon the number of captures/individual. Our simulations suggested that ~3.4 captures/individual will result in a < 10% CV for abundance estimates when populations are small (23–39), but fewer captures/individual may be sufficient for larger populations. We found scat sampling was more cost-effective for sampling multiple species, but suggest that hair sampling may be less expensive at study sites with limited road access for bears. Given the dependence of sampling scheme on species and study site, the optimal sampling scheme is likely to be study-specific warranting pilot studies in most circumstances.

Keywords: *Canis latrans*, CAPWIRE, hair snags, Newfoundland, scat detection dogs, *Ursus americanus*

Received 2 November 2014; revision received 8 February 2015; accepted 13 February 2015

Introduction

Large carnivore populations occur intrinsically at low densities (MacKay *et al.* 2008; Mondol *et al.* 2009) and have been further reduced by direct and indirect anthropogenic influences (Weaver *et al.* 1996). Managers are frequently tasked with monitoring population size and distribution to guide management actions for large carnivores. Traditional methods of large carnivore monitoring depend on capture and handling, which can be difficult, expensive and dangerous for both animals and handlers (Gompper *et al.* 2006). Noninvasive sampling techniques do not require physical capture or direct observation of target species and are often a viable alternative that can increase sampling success, reduce sampling cost and increase animal and handler safety (Waits 2004; MacKay *et al.* 2008; Kelly *et al.* 2012).

A myriad of noninvasive techniques are available to provide researchers with the ability to determine the distribution, abundance and population trends of carnivores (Long & Zielinski 2008). Track and scat surveys have a long history of use in determining carnivore occupancy (MacKay *et al.* 2008), and species detection has been improved through the use of scat detection dogs (Smith *et al.* 2003). Camera trapping has been used for occupancy and abundance estimation of species with distinct, individual markings (e.g. Mondol *et al.* 2009). With the advent of the polymerase chain reaction (PCR), species, sex and individual were able to be identified by genetic analysis of scat, hair, urine and saliva (Waits & Paetkau 2005) for the purpose of improving occupancy

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surveys (Gompper *et al.* 2006), evaluating genetic diversity and structure (Kohn *et al.* 1995), estimating species abundance (Kohn *et al.* 1999) and identifying diet items (Deagle *et al.* 2005).

Numerous studies have used NGS methods, and some have combined multiple sampling methods to estimate population parameters (De Barba *et al.* 2010a; Long *et al.* 2011; Reed 2011), but few have compared the effectiveness of multiple NGS techniques to sample multiple carnivore species. De Barba *et al.* (2010b) found that baited hair snags resulted in more samples and higher amplification success than sampling scat and hair opportunistically or along transects for brown bears (*Ursus arctos*); however, opportunistic sampling was less expensive than hair snags, while identifying a similar number of unique individuals. Other studies have shown that the collection of scats along trails or at bait sites provides a greater number of samples in comparison to hair collection and/or saliva collection from prey kill sites (Vine *et al.* 2009; Sugimoto *et al.* 2012), while Latham *et al.* (2012) demonstrated that optimal hair sampling methods may differ between bear species (*Ursus* spp.). Several additional studies determined that sampling with scat detection dogs provided the highest capture rates (Wasser *et al.* 2004 – bears), but was also the most costly (Harrison 2006 – bobcats (*Lynx rufus*); Long *et al.* 2007 – black bears (*Ursus americanus*), fishers (*Martes pennanti*) and bobcats). Our objective was to evaluate the utility of scat detection dogs and hair sampling to simultaneously provide viable, genetic samples for estimating the abundance of three large carnivore species across Newfoundland, Canada.

Determining predator abundance in Newfoundland has become a priority because of a declining caribou (*Rangifer tarandus*) population and a changing predator guild. Numbers of caribou in Newfoundland have declined >66% since 1998, and increased calf predation is considered a potential driver (Lewis & Mahoney 2014). Historically, gray wolves (*Canis lupus*), black bears and Canada lynx (*Lynx canadensis*) were Newfoundland's apex predators, but gray wolves were extirpated from the island prior to the 1930s (Lewis & Mahoney 2014). Beginning in the 1980s, coyotes (*Canis latrans*) colonized Newfoundland (Lewis & Mahoney 2014) and have become a major caribou calf predator (Mumma *et al.* 2014).

In 2009, we used scat detection dogs and hair sampling to collect samples across 3 study sites in Newfoundland for the purpose of estimating abundances of coyotes, black bears and Canada lynx. We wanted to identify the most efficient means and appropriate sampling intensity to simultaneously monitor these populations by answering the following questions. First, which method provides the greatest number of samples (both identified to species and individually identified) and the highest number of captures/individual for each species? Second, how many individually identified samples and captures/individual are necessary to precisely estimate population abundances? And finally, what is the total cost and the cost/individually identified sample for each method?

Materials and methods

Study site

The island of Newfoundland (111 390 km^2) is characterized by a cool, maritime climate and interspersed coniferous forest, windswept barrens and peatland (McManus & Wood 1991). Caribou are widely distributed and are the only native ungulate on Newfoundland. Three study sites (La Poile – LP, Middle Ridge – MR and Northern Peninsula – NP) ranging in size from ~500 to 1500 km^2 were selected following the delineation via telemetry data (Rayl *et al.* 2014) of four caribou calving grounds (La Poile – LP, Middle Ridge – MR, and the closely associated Northern Peninsula – NP and St. Anthony's herds – Fig. 1).

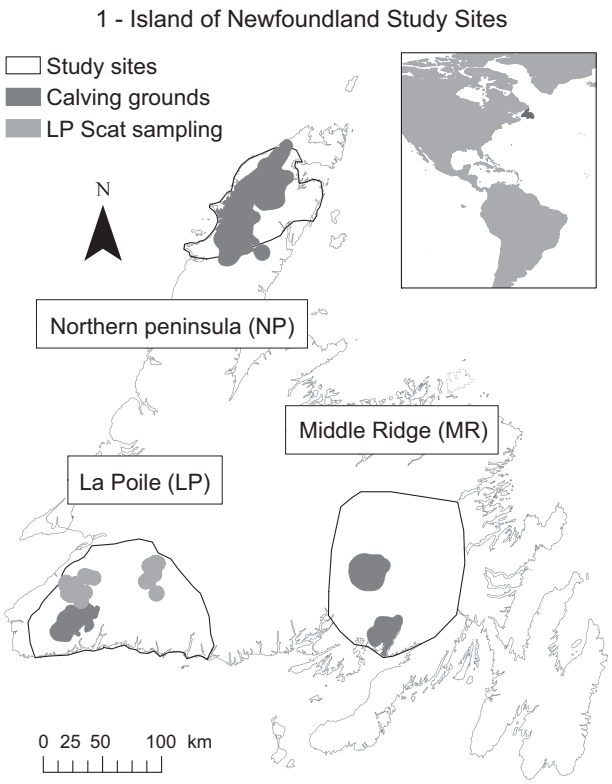

Fig. 1 The location of our three study sites and associated calving grounds. Scat sampling in La Poile (LP) is shown because it was primarily outside the LP calving ground.

Scat detection dog sampling

A trained scat detection dog (further details in Supporting information) was used to locate scats from coyotes, black bears and Canada lynx in the NP and LP. In the NP, 15 12 × 12 km grid cells were overlaid across the study site. The size of our grid cells (144 km^2) was based on the average home range size in Newfoundland of our target species (black bears – 391 km^2, coyotes – 110 km^2 and Canada lynx – unknown) in a manner similar to a previous study (Thompson *et al.* 2012), but was a compromise that likely influenced sampling success by altering sampling intensity between species. Although we did not know the average home range size for lynx in Newfoundland, we anticipated that it would be on the upper end of lynx home range sizes reported across mainland North America (8–738 km^2 – Poole 2003) as we also find with Newfoundland black bears and coyotes, and because snowshoe hare abundances were reduced in prior years and during the year of the study (Reynolds *et al.* 2010), which has been shown to result in two- to 10-fold increases in the size of lynx home ranges (Poole 2003). As a result of logistic constraints, 13 of the 15 grid cells were sampled 1–2 times in 2009 during June, July or August (Fig. S1, Supporting information). The scat detection dog team was flown via helicopter when grid cells were not accessible by roads. Scat sampling of the LP study site occurred in adjacent roaded areas, because of limited road access to the LP calving ground (Fig. 1). Locations were strategically chosen to provide similar sampling intensity and coverage as was achieved in the NP.

The scat detection dog was permitted to search freely, while the handler ensured coverage of a range of habitat types. Search length and time varied depending on the number of scats found and the weather, but generally consisted of a 2–6 h search spanning 5–10 km. Samples were placed in plastic bags using clean, latex gloves and frozen at the end of each day when possible. Prior to laboratory processing, scats were thawed and faecal material from multiple locations on the lateral surface of each scat (Stenglein *et al.* 2010a) was collected and placed in a 2-millilitre (ml) tube containing DETS buffer to prevent DNA degradation (Frantzen *et al.* 1998). Scats were given a sample ID, and electronic records were generated that included study site, grid cell, date and GPS coordinates.

Hair sampling

We overlaid 5 × 5 km grid cells across the LP (20 cells), MR (44 cells) and NP (22 cells) study sites. A single transect was placed in each cell using a random starting location and orientation that was subsequently adjusted for some cells to permit reasonable access by field personnel and increase the probability of capture by selecting nearby locations containing game trails. Our transects were 600 m long and contained bear hair snags at each end and a coyote rub pad and a Canada lynx rub pad at 150, 300 and 450 m (Fig. 2 – further description in Supporting information).

Study sites were accessed using helicopters, and snags and pads were set up in mid-May and checked and rebaited in late June, early August and late September with a final check in mid-October of 2009. Recovered samples were placed in paper envelopes using clean, latex gloves and labelled with the study site, grid cell, transect number, station type, and sample number and date. Paper envelopes were placed collectively in plastic bags filled with silica desiccant to prevent DNA degradation until laboratory processing (Roon *et al.* 2005).

DNA extraction and species identification

We extracted samples in a DNA laboratory dedicated to samples (i.e. hair, scat and saliva) containing low quantities of DNA or likely to be degraded to limit the occurrence of contamination using the Qiagen QIAamp DNA stool mini kit (Qiagen Inc., Valencia, CA, USA) for scat samples and the Qiagen DNeasy tissue kit for hair samples. We used up to 10 follicles for hair extractions when available and used a negative control in all scat and hair batches to monitor for contamination.

We used a mitochondrial DNA (mtDNA) control region fragment analysis test (Mumma *et al.* 2014) adapted from the methods of De Barba *et al.* (2014), which combined previously developed primers (Murphy *et al.* 2000; Dalén *et al.* 2004; Onorato *et al.* 2006) to identify each sample to species. This test could not detect Canada lynx, so we tested all failed samples using

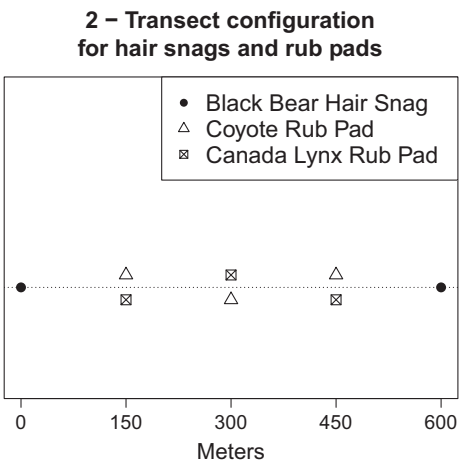

Fig. 2 The transect configuration for hair sampling.

mtDNA primers developed for Iberian lynx (*Lynx pardinus*) by Palomares *et al.* (2002).

Nuclear DNA individual and sex determination

For coyotes, we combined nine microsatellite loci (FH2001, FH2054, FH2088, FH2137, FH2611, FH2670, FH3725, C09.173 and Cxx.119 – Breen *et al.* 2001; Guyon *et al.* 2003; Holmes *et al.* 1994) based on the methods of Stenglein *et al.* (2010a) with two sex-determining loci (DBX6 and DBY7 – Seddon 2005) to form a single canid PCR multiplex (Mumma *et al.* 2014).

We developed two black bear PCR multiplexes (Mumma *et al.* 2014). Black bear multiplex 1 included six microsatellite loci (G10C, G10M, G10P, G10X, CXX20 and Mu23 – Paetkau *et al.* 1998; Taberlet *et al.* 1997; De Barba *et al.* 2010b; Ostrander *et al.* 1993) and a sex-determining locus (Ennis & Gallagher 1994). Black bear multiplex 2 included five microsatellite loci (G1A, G10B, Mu15, Mu50 and Mu59 – Paetkau *et al.* 1998; Taberlet *et al.* 1997; Bellemain & Taberlet 2004). Additional PCR and allele scoring details for species and individual identification can be found in Mumma *et al.* (2014).

We combined nine felid microsatellite loci (FCA096, FCA275, F85, FCA043, F124, FCA132, FCA082, FCA0008 and F53 – Menotti-Raymond *et al.* 1999) and a sex-determining locus (Amel – Pilgrim *et al.* 2005) into a single multiplex to individually identify Canada lynx samples (further details in Supplementary information). We determined allele sizes for species and individual identification using an Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA, USA) and associated GENEMAPPER 3.7 software.

We tested all samples in duplicate for their first respective PCR multiplex. We dropped samples that failed to amplify at ≥4 loci. Black bear samples that amplified at ≥4 loci were tested in duplicate for the second bear multiplex. We required each allele to be detected twice for heterozygotes and three times for homozygotes to obtain a consensus genotype. We ran up to six PCR replicates for each multiplex and dropped samples that failed to achieve a consensus genotype at ≥6 loci for coyotes and ≥9 loci for black bears. We selected our minimum number of consensus loci for coyotes and black bears to assure that all samples had a probability of identity siblings (PID_{sibs}) (Waits *et al.* 2001) value of less than 0.01 regardless of which loci were complete using the software GENALEX6 – Peakall & Smouse 2006. This means that less than 1/100 comparisons of first degree relatives would result in identical genotypes for our minimum number of consensus loci and was used to avoid false recaptures in the data set. When all loci were completed for an individual, our estimated PID_{sibs} values for black bear and coyote were <0.001. We did not establish a minimum number of completed loci for lynx or calculate PID_{sibs} values because we only identified 1 individual.

We used the software GENALEX6 (Peakall & Smouse 2006) to match completed genotypes. We also evaluated replicate PCRs for samples that matched at all but one or two loci to determine whether mismatches could be attributed to allelic dropouts or false alleles. We analysed individuals that were only detected once using the software RELIOTYPE (Miller *et al.* 2002) to evaluate the reliability of the final consensus genotype. We required consensus genotypes to be ≥95% reliable and retested samples until we achieved our threshold to reduce the likelihood that single captures were the result of genotyping error. We also calculated error rates (frequency of allelic dropouts and false alleles) for hair and scat samples across species and study site by comparing consensus genotypes from randomly selected individually identified samples to their first 2 PCR replicates. As additional replicates are often necessary to establish consensus genotypes for low-quality samples, we only evaluated the first 2 PCR replicates of each sample to avoid a positive bias in error rates that would result from the increased number of PCRs conducted on low-quality samples in comparison to high-quality samples.

Abundance estimation and simulations

We used hair and scat samples to generate black bear and coyote abundance estimates via the package CAPWIRE (Pennell *et al.* 2013) in program R (R Core Team 2014). Program CAPWIRE and the corresponding R package CAPWIRE were developed specifically for the analysis of noninvasive genetic samples by allowing multiple samples from a single individual collected within the same sampling session to be analysed (Miller *et al.* 2005). Simulations have shown that CAPWIRE performs as well or better than other abundance estimators when populations demonstrate capture heterogeneity and are <200 individuals (Miller *et al.* 2005). CAPWIRE requires that all individuals are correctly identified, all samples are independent, and the population is closed during the period of sample deposition and collection.

We designed our previously discussed genetic protocols to satisfy the assumption that all individuals were identified correctly. For scat sampling, we assumed that all samples were independent and assumed that the population was closed during deposition and collection. Scat degradation studies suggest a low probability of obtaining a complete genotype after 30 days for wolf and brown bear scats (Murphy *et al.* 2007; Santini *et al.* 2007). We expected similar to more extreme degradation patterns for coyote and black bear scats in our study due to Newfoundland's damp climate, which is likely to

exacerbate degradation (Murphy *et al.* 2007). Therefore, we assumed that successfully identified scats were deposited no earlier than 30 days prior to our initial sampling periods in the LP and NP, which was 11–12 weeks from the date of our last sampling periods. An insufficient number of scat samples were collected in the LP for black bears and in the LP and NP for Canada lynx to permit abundance estimation. An additional limitation that may have caused some bias in our abundance estimates from both scat and hair sampling was the potential for an increasing trend in the probability of detection for young of the year as a result of a gain in mobility as summer progressed.

To maintain our assumption of independence for hair samples, we only included one sample per individual per snag per session. However, we did consider black bear hair samples collected in the same session from different hair snags to be independent and even considered samples to be independent when they were collected from opposite hair snags along the same transect, because very few individuals were detected at both snags of a single transect in the same session (13/189 opportunities). Similar to the approach taken by Robinson *et al.* (2009), we combined hair collection sessions to increase our average number of captures/individual to the frequency (≥1.7) recommended by Stenglein *et al.* (2010b) when using CAPWIRE. In the MR, we combined the first three sessions, but eliminated the fourth session to limit our sampling period to ~16 weeks, which is comparable to the duration of other studies that assumed population closure (Boersen *et al.* 2003; Boulanger *et al.* 2008). In the NP, we used all four sessions to maximize our number of recaptures, which extended the duration of our study to ~20 weeks and increased the likelihood of violating our assumption of closure. Hair snags failed to provide a sufficient number of samples to estimate abundance of black bears in the LP and coyotes and Canada lynx across all study sites.

We ran models under the assumption of an equal capture (ECM) rate for all individuals and two innate rates (TIRM) of capture within the population and used likelihood ratio tests to determine the best supported model. Following preliminary analyses, we parameterized the MR black bear model using a maximum population size of 200 individuals and set the maximum population size to 100 individuals for coyote and black bear models using scat or hair in the LP and NP study sites. We estimated 95% confidence intervals for all population size estimates using 1000 bootstraps.

Following abundance estimation, we performed simulations to estimate the number of samples necessary to achieve a < 10% CV (Sokal & Rohlf 1995). We used the parameters (abundance, # of individuals with the lower capture rate, # of individuals with the higher capture rate and the ratio of capturability between the two classes) estimated under the TIRM, which was the best supported model for all of our analyses, to generate 100 theoretical capture histories at five different sampling intensities of each population for which abundance was estimated. We then averaged the estimates of abundance and 95% confidence intervals and determined the coefficient of variation for each set of capture histories.

Following our abundance estimates and simulations, it became evident that the CV (%) was related to the average number of captures/individual, but the nature of this relationship was affected by the size of the population being estimated. Therefore, we ran a linear regression in R (R Core Team 2014) for our four lower abundance estimates (23–39 individuals) between the average number of captures/individual and the corresponding CV (%) to estimate a recommended number of captures/individual to achieve a CV of <10% for populations <39 individuals.

Sampling costs

We estimated the sampling cost (Tables S1 & S2, Supporting information) and cost/individually identified and independent sample (hereafter referred to as III sample) across study sites and collection methods. We were more interested in the cost/III sample than cost/sample, because our goal of abundance estimation was reliant on the samples that were individual identified and considered independent. As sampling was concurrent for all species, we determined the cost/III sample by dividing the total sampling cost over the number of III samples for each species in each study site and by dividing the total sampling cost over III samples for all species in each study site. Our scat detection dog sampling was performed by a graduate student, so we used rates provided by an independent conservation dog company (Find It Detection Dogs) to estimate the cost of conducting a comparable level of sampling (Table S1, Supporting information). As hair sampling was performed by a combination of Newfoundland provincial biologists and graduate students, we estimated costs for hair sampling using only provincial biologists (Table S2, Supporting information). We also report total laboratory costs and laboratory costs/III sample for each method.

Results

Sampling and molecular identification success

Scat detection dogs located 185 and 193 samples in the LP and NP (Table 1). Ninety-five per cent of these samples were successful for species identification (Table 1). Fifty-eight coyote samples, 94 black bear samples and

Table 1 Number of samples and success rates by study site [La Poile (LP), Middle Ridge (MR) and Northern Peninsula (NP)], sample type (scat or hair) and species [coyote, black bear (B. Bear) and Canada lynx (C. Lynx)]. (III Samples = individually identified and independent samples, IND = individual)

Study site	Sample type	Total samples	% Identified to species[†]	Species	No. of samples	# IND identification (%)	III samples[‡]	No. of unique INDs	Captures/ IND
LP	Scat	185	95%	Coyote	94	71 (76%)	71	19	3.7
				B. Bear	58	19 (33%)	19	15	1.3
				C. Lynx	1	0	NA	NA	NA
LP	Hair	18	39%	Coyote	0	NA	NA	NA	NA
				B. Bear	7	5 (71%)	3	2	1.5
				C. Lynx	0	NA	NA	NA	NA
MR	Hair	679	68%	Coyote	8	3 (38%)	2	2	1.0
				B. Bear	453	273 (60%)	171	99	1.7
				C. Lynx	0	NA	NA	NA	NA
NP	Scat	193	95%	Coyote	62	53 (86%)	53	22	2.4
				B. Bear	87	25 (29%)	25	13	1.9
				C. Lynx	6	1 (17%)	1	1	1.0
NP	Hair	141	63%	Coyote	0	NA	NA	NA	NA
				B. Bear	89	50 (56%)	25	16	1.6
				C. Lynx	0	NA	NA	NA	NA

[†]Unidentified scats failed to amplify or were nontarget species.
[‡]All scat samples considered III samples, but hair samples from the same individual and session were only considered III samples if collected from different hair snags.

one Canada lynx sample were identified in the LP, and 62 coyote, 87 black bear and 6 Canada lynx samples were identified in the NP (Table 1). Coyote samples had higher individual identification rates (76% – LP, 86% – NP) than black bear samples (33% – LP, 29% – NP) (Table 1). False allele and allelic dropout rates were similar across study sites and loci and were 0.007 and 0.06 for black bears and 0.006 and 0.03 for coyotes, respectively. We did not estimate error rates for lynx because of the limited number of samples collected. No individual lynx were identified in the LP, and only 1 of the 6 NP lynx samples were individually identified (Table 1). The average number of captures/individual was 3.7, 1.3, 2.4 and 1.9 for LP coyotes, LP black bears, NP coyotes and NP black bears, respectively (Table 1).

Eighteen, 679 and 141 hair samples were collected in the LP, MR and NP. Eight hundred and thirty-five of these samples were collected at hair snags, and only three of these samples total (one bear and two samples that failed species identification) were collected from rub pads. Thirty-nine per cent of hair samples were successfully identified to species in the LP vs. 68% and 63% in the MR and NP, respectively (Table 1). Five hundred and forty-nine of the hair samples identified to species were from black bears with eight remaining samples from the MR attributed to coyotes (Table 1). Individual identification success rates from hair samples were 71%, 38%, 60% and 56% for LP black bears, MR coyotes, MR black bears and NP black bears (Table 1). False allele and allelic dropout rates were similar across study sites and loci and were estimated at 0.04 and 0.002, respectively, across all 3 study sites of individually identified bear hair samples. We did not estimate error rates for coyotes, because of the limited number of samples collected. Once we accounted for sample independence, the number of III samples was 3, 2, 171 and 25, and the average number of captures per individual was 1.5, 1.0, 1.7 and 1.6 for LP black bears, MR coyotes, MR black bears and NP black bears, respectively (Table 1).

Abundance estimates and simulations

We were able to use scats to generate abundance estimates (TIRM) for coyotes in the LP and coyotes and black bears in the NP and used hair sampling to generate abundance estimates for black bears in the NP and MR. We estimated a population size of 24 (7.5% CV) coyotes for the LP area sampled via scat detection dogs (71 samples), which was consistent with simulations that indicated a III sample size of ~75 would result in an estimate with a < 10% CV (Fig. 3A). An abundance estimate of 32 (19.2% CV) was determined for NP coyotes using scat sampling, and we estimated that ~100 III samples would be necessary to achieve our desired level of precision (<10% CV) (Fig. 3B). Twenty-three (28% CV) and 39 (39.6% CV) black bears were estimated using scat and hair samples, respectively, for the Northern Peninsula, and we estimated that ~75 and ~100 III samples would

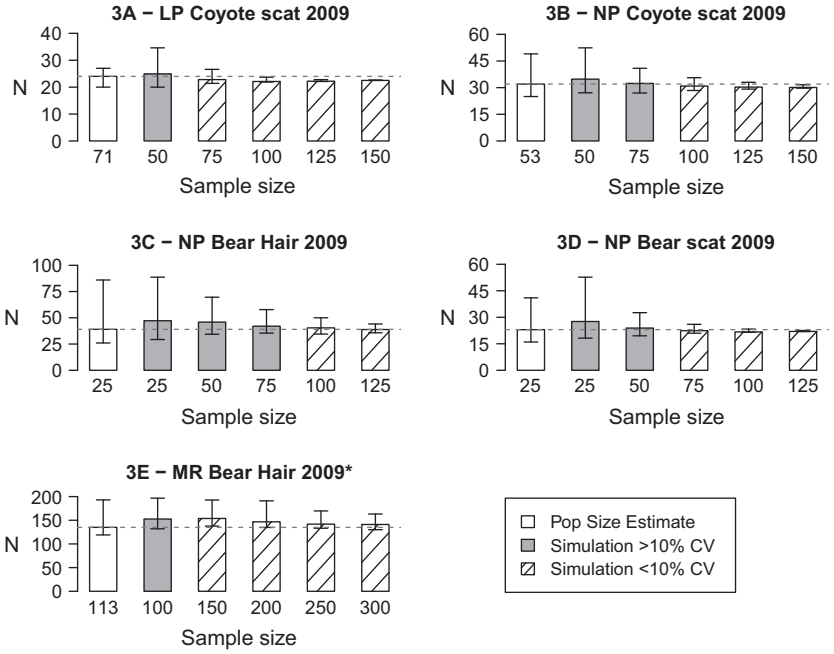

Fig. 3 Abundance estimates and simulations for coyotes and black bears in the LP (La Poile), MR (Middle Ridge) and NP (Northern Peninsula). *Only three sessions in the MR were used to satisfy the closure assumption, thereby creating a mismatch between the number of hair samples indicated in Table 1 and the number used in the MR black bear abundance estimate.

be necessary to reduce estimates below a 10% CV (Fig. 3C,D). One hundred and thirty-five (14% CV) black bears were estimated for the MR using hair samples from the first three sampling sessions, and ~150 III samples would be necessary to achieve a < 10% CV (Fig. 3E). In our plot of captures/individual vs. CV, our regression suggested a CV of <10% could be achieved with ~3.4 captures/individual for populations between 23 and 39 individuals (Fig. 4).

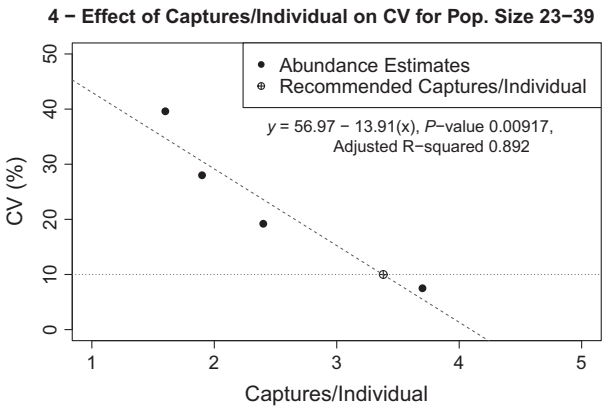

Fig. 4 A regression to determine the estimated number of captures/individual necessary to achieve a 10% CV using the number of captures/individual and the estimated CV corresponding to the abundance estimates of our four smaller populations.

Sampling costs

The total estimated cost of scat sampling was $16 363 (USD) and $28 182 for the LP and NP (Table 2). We estimated a per III sample cost for scat samples of $230, $531, $861 and $1,126 for LP coyotes, NP coyotes, LP black bears and NP black bears, respectively (Table 2). Our cost/III sample for scat from all species was $182 for the LP and $352 for the NP (Table 2). Our costs for hair sampling were $86 382, $166 235 and $86 340 for the LP, MR and NP study sites (Table 3). We estimated a per III sample cost for hair samples of $972 and $3454 for MR and NP black bears, respectively (Table 3). The cost per III sample for hair from all species was $961 for the MR and $3454 for the NP (Table 3). The total laboratory costs (and the cost/III sample) for all samples across study sites was $4809 (and $28.29) for scat and $6,195 (and $30.52) for hair.

Discussion

Evaluating multiple sampling methods is important to ensure efficient use of resources when monitoring populations. Similar to other studies (Harrison 2006; Long *et al.* 2007), our results suggest that scat sampling is more effective for sampling multiple species. However, our results do not point to a single optimal sampling method, but instead suggest that the most effective

Table 2 Total cost ($USD) and cost/sample for scat detection dog sampling in the La Poile (LP) and Northern Peninsula (NP) for coyotes, black bears and Canada lynx. (NA = cost/sample not recorded due to minimal sampling, III Samples = individually identified and independent samples)

Category	Item	LP Scat costs†	NP Scat costs†
Transportation	Roundtrip Travel to NF	2993	3233
	Roundtrip Ferry Costs	597	597
	Travel Costs within NF	507	507
	Helicopter	NA	11 559
Staff	Roundtrip Travel Per Diem	2800	2800
	Camper Per Diem	900	900
	Hotel + Per Diem	615	615
	Scat Dog Team Cost	7800	7800
	Dog Insurance	150	150
	Total	16 363	28 162

Sample Info	Species	LP samples	NP samples
III Samples	Coyote	71	53
	Black Bear	19	25
	Canada Lynx	0	1
	All	90	80
Cost/III Sample	Coyote	230	531
	Black Bear	861	1126
	Canada Lynx	NA	NA
	All	182	352

†Based on estimates from 'Find It Detection Dogs' for one dog and handler (See Supplemental Information for itemized descriptions).

Table 3 Total cost ($USD) and cost/sample estimates for hair sampling in the La Poile (LP), Middle Ridge (MR) and Northern Peninsula (NP) for coyotes, black bears and Canada lynx. (NA = cost/sample not recorded due to minimal sampling, III Samples = individually identified and independent samples)

Category	Item	LP hair costs†	MR hair costs†	NP hair costs†
Transportation	Helicopter	74 765	149 529	74 765
	Gas	251	109	210
Staff	Salary	5612	7808	5612
	Lodging	3137	4531	3137
	Per Diem	1603	2231	1603
Supplies	Lures	115	229	115
	Posts	276	552	276
	Barbed Wire	233	466	233
	Bait	368	736	368
	Misc. Supplies	22	44	22
	Total	86 382	166 235	86 340

Sample Info	Species	LP Samples	MR Samples	NP Samples
III Samples	Coyote	0	2	0
	Black Bear	3	171	25
	Canada Lynx	0	0	0
	All	5	173	25
Cost/III Sample	Coyote	NA	NA	NA
	Black Bear	NA	972	3454
	Canada Lynx	NA	NA	NA
	All	NA	961	3454

†Based on four sessions and two biologists (See Supplemental Information for itemized descriptions).

sampling method will be highly specific to species and study site. In our study, scat detection dogs successfully sampled coyotes and black bears, while hair sampling was only successful in generating abundance estimates for bears in two of three study sites, and neither method was successful in sampling lynx (Tables 1 and 2).

There are a variety of reasons that might explain the differences in sampling success between methods and species. Detection is likely, in part, a function of the relationship between home range size, which differed between our species, and sampling scheme. For scat sampling, the increased size of black bear home ranges should have increased the number of capture locations that fell within each bear's home range in comparison to coyote home ranges, but perhaps more importantly may have changed (increased or decreased) the proportion of the home range being sampled; however, understanding the interaction between home range size and detection was not extremely clear given the plethora of additional considerations. For example, the detection of coyote scats may have been increased as a result of sampling in close proximity to roads, which coyotes often use for travel (Larrucea *et al.* 2007).

Despite the potential bias introduced by sampling close to roads, a similar number of black bear and coyote scats were collected in the LP and NP, but the number of III bear samples was reduced because of lower individual identification success rates for black bear scats (Table 1). Another study did not indicate higher amplification success rates for canid scats in comparison to bear scats (Broquet *et al.* 2007). Potential reasons for the trend we observed could be differences in diet (Murphy *et al.* 2003) or chemical composition between species, either of which could lead to inhibiting DNA amplification or increasing degradation (Huber *et al.* 2002; Murphy *et al.* 2003). Alternatively, the morphology of scats could explain these differences (Murphy *et al.* 2003), as a firm coyote scat may be more prone to slough off the epithelial cells necessary for molecular identification than a softer, less formed bear scat.

For lynx, amplification success rates were of little concern because so few samples were collected, which might be explained by a biological or methodological cause.

Lynx densities in Newfoundland may be lower than black bear and coyote densities and/or may be lower in the areas accessible to roads (Vashon *et al.* 2008) where scat sampling occurred. Alternatively, there may have been lower rates of detection for lynx as a result of there being a limited number of positive lynx scats available during dog training. The limited success of rub pads may also have been the result of a methodological cause. In fact, McDaniel *et al.* (2000) deployed rub pads in fall and winter when food was scarce and used different lures, which were applied more frequently. Moreover, these explanations may explain the failure of rub pads to effectively sample coyotes, in addition to not using a natural substrate as the rubbing object (Ausband *et al.* 2011).

Although hair sampling was successful for black bears, we found differences in the number of samples collected between study sites (Table 1). A larger number of samples were collected in the MR in comparison to the LP and NP, which we speculated was the result of higher black bear densities in the MR as demonstrated by the higher MR abundance estimate (Fig. 3) even after accounting for the greater number of hair snags in the MR. The lower number of samples collected in the LP and NP also corresponded to a lower number of captures/individual.

When evaluating our abundance estimates and simulations, it was evident that the number of captures/individual impacted the precision of abundance estimates. Our regression between the number of captures/individual and the CV demonstrated a decrease in the CV resulting from an increase in the number of captures/individual and predicted that ~3.4 captures/individual would result in a 10% CV (Fig. 4). However, this recommendation appeared highly dependent upon the size of the population. When comparing black bear abundance estimates using hair samples from the MR and NP, we have a similar number of captures/individual (Table 1) and a 1.2-fold increase for the size of the MR confidence interval (Fig. 3), but we find a nearly threefold decrease in the CV as a result of the much larger MR population size. This makes intuitive sense, because the CV is a normalized measure of dispersion; therefore, we would expect the CV to decrease for a set number of captures/individual as population size increases.

To maximize the number of captures/individual and ensure precise abundance estimates, researchers using NGS must maximize both the number of samples and their amplification success (Solberg *et al.* 2006). One possibility to increase the number of samples would be to add additional sampling sessions, while a method to increase amplification success would be to decrease the time between sessions. The long duration between sessions coupled with Newfoundland's wet environment likely increased DNA degradation (Stetz *et al.* 2015). Our individual identification success rates ranged from 56 to 71% for black bear hair samples. However, this does not account for the 281 (34%) hair samples that failed species identification. As nearly all the hair samples identified to species were from black bears (Table 1), individual identification success rates may have been as poor as 40% (328 individually identified black bear/830 potential black bear samples). Other studies have reported a wide range (14–99%) of hair genotyping success for bears (Roon *et al.* 2003; De Barba *et al.* 2010b). For scat sampling, decreasing the time between sessions would only be effective if the same areas/paths were searched in each session, which should serve to remove older scats and improve the likelihood that scats collected were deposited since the last session and not degraded.

Although MR hair snags produced the largest number of III samples of any species and study site, the cost/III sample for black bears in the MR was similar to the cost/III sample for black bear scats and more expensive than coyote scats collected in the LP and NP as a result of the substantially higher total cost of hair sampling in comparison to scat sampling (Tables 2 and 3). However, this cost was almost entirely driven by helicopter support, which was required for hair sampling across all study sites. In contrast, scat sampling only occurred in areas where most sampling locations could be accessed via roads. Furthermore, only 2 h/day were charged to scat sampling in the NP when helicopter support was necessary (Table S1, Supporting information), because helicopters were already being used for additional research in the area. If helicopters were hired for scat detection dog sampling alone, additional costs would have accrued during the 4- to 8-h window between transporting and retrieving the scat detection dog team to and from sampling locations. In the NP, where only one sampling location required helicopter support, scat detection dog sampling would remain the best option for sampling multiple species and coyotes regardless of whether or not helicopter costs could be buffered by other research activities. In the more remote MR study site, helicopter costs could become prohibitively expensive if not buffered by other research activities; however, our results indicated that these costs may be necessary to sample for coyotes given the lack of hair sampling success.

In this study, we evaluated multiple NGS methods to determine the optimal approach for sampling three large carnivore species for the purpose of abundance estimation. We found that the optimal sampling method was dependent on species and study site differences, which limits the ability of our findings to generalize across systems. However, we think our study can guide other researchers by illuminating the need to consider species biology and study site attributes when designing

sampling approaches, but also recognize that pilot studies will often be necessary for researchers to identify the most effective sampling methods for their study systems.

Acknowledgements

This study was funded by the Newfoundland and Labrador Department of Environment and Conservation (Sustainable Development and Strategic Science and the Institute for Biodiversity, Ecosystem Science and Sustainability) and the Safari Club International Foundation. We thank Rob Otto, Truman Porter, Steve Gullage, Tyler Hodder, Jason McGinn, Peter Tremblett, Frank Norman, Keith Lewis, Andrew Mouland, Nathaniel Rayl, and the Newfoundland and Labrador Department of Environment and Conservation (Sustainable Development and Strategic Science and the Institute for Biodiversity, Ecosystem Science and Sustainability) for field support. We thank Rachael Toldness, Jennifer Adams and the Waits Lab Group, along with the Laboratory of Ecological, Evolutionary, and Conservation Genetics for support and Jon Horne, Janet Rachlow, and Dennis Murray, Colleen Soulliere, Bruce Deagle, and three anonymous reviewers for manuscript edits.

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M.A.M performed field and laboratory work and wrote the manuscript. C.Z. (and his dog Marvin) performed scat sampling and C.Z. provided manuscript input and edits. S.P.M. administered monetary support for the research and contributed to field sampling design. T.K.F. provided manuscript input and edits. L.P.W. guided research efforts and provided manuscript input and edits.

Data accessibility

Genotypes, capture histories and R code for CAPWIRE simulations can be found in the DRYAD database (doi:10.5061/dryad.n90hg).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1 Additional details describing scat detection dog training, hair snags and rub pads, and lynx individual identification and PCR conditions.

Table S1 A list of itemized descriptions for cost estimates displayed in Table 2 of the main text by study site

Table S2 A list of itemized descriptions for cost estimates displayed in Table 3 of the main text by study site

Fig. S1 The scat detection dog tracks across the Northern Peninsula study site.