

Monitoring coyote population dynamics by genotyping faeces

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Abstract

Reliable population estimates are necessary for effective conservation and management, and faecal genotyping has been used successfully to estimate the population size of several elusive mammalian species. Information such as changes in population size over time and survival rates, however, are often more useful for conservation biology than single population estimates. We evaluated the use of faecal genotyping as a tool for monitoring long-term population dynamics, using coyotes (*Canis latrans*) in the Alaska Range as a case study. We obtained 544 genotypes from 56 coyotes over 3 years (2000–2002). Tissue samples from all 15 radio-collared coyotes in our study area had ≥ 1 matching faecal genotypes. We used flexible maximum-likelihood models to study coyote population dynamics, and we tested model performance against radio telemetry data. The staple prey of coyotes, snowshoe hares (*Lepus americanus*), dramatically declined during this study, and the coyote population declined nearly two-fold with a 1½-year time lag. Survival rates declined the year after hares crashed but recovered the following year. We conclude that long-term monitoring of elusive species using faecal genotyping is feasible and can provide data that are useful for wildlife conservation and management. We highlight some drawbacks of standard open-population models, such as low precision and the requirement of discrete sampling intervals, and we suggest that the development of open models designed for continuously collected data would enhance the utility of faecal genotyping as a monitoring tool.

Keywords: capture–mark–recapture, Cormack–Jolly Seber, coyote population dynamics, coyote, genetic tagging, non-invasive sampling, open-population models

Received 14 November 2004; revision accepted 2 February 2005

Introduction

Large predators are notoriously difficult to count because they tend to be rare, secretive, and wide ranging. Non-invasive genetic sampling in which genetic fingerprints are constructed from DNA extracted from hair or faeces, has been used to obtain short-term population estimates for several mammalian species (e.g. Kohn *et al.* 1999; Banks *et al.* 2002; Eggert *et al.* 2003). Boulanger *et al.* (2004) monitored grizzly bear (*Ursus arctos*) populations over time using DNA collected from hairs, but faecal DNA has not been previously used to track population trends. We used faecal genotyping to monitor the population dynamics

of coyotes (*Canis latrans*) in Alaska over 3 years (2000–2002). Snowshoe hares (*Lepus americanus*) are the staple prey of northern coyotes (Thurber *et al.* 1992; O'Donoghue *et al.* 1998) and the hare population declined approximately 10-fold during this study (Prugh 2004). We hypothesized that coyote population size and survival would decline in response to the snowshoe hare decline, as was found elsewhere in the north (Todd *et al.* 1981; O'Donoghue *et al.* 1997).

Accurate estimates of population parameters such as survival, recruitment, and abundance are crucial to the conservation and management of natural populations. Capturing, marking, and recapturing animals is commonly used to estimate these parameters (see Seber 1986; Pollock *et al.* 1990; Lebreton *et al.* 1992; Schwarz & Seber 1999). For species that are difficult to capture, collecting and genotyping shed DNA (commonly in the form of faeces and hairs)

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can be considered equivalent to capturing the animal that deposited the genetic sample. Mark–recapture models can then be used to estimate parameters such as population size and survival.

Mark–recapture statistical models generally fall under two categories: closed- and open-population models. Closed-population models, such as Petersen and Schnabel estimators, assume populations are closed to births, deaths, immigration, and emigration (Otis *et al.* 1978). Open-population models, such as Jolly Seber, Cormack–Jolly Seber, and Pradel models, do not assume population closure. Previous faecal genotyping studies used closed-population models to estimate abundance because sampling in these studies occurred over a relatively short time (e.g. Banks *et al.* 2002; Frantz *et al.* 2003).

All mark–recapture models assume that individuals are uniquely identifiable and that there is no error in their identification. When establishing individual identity through genotypes, however, there is always the chance of an identification error. Waits & Leberg (2000) found that typical levels of genotyping error (*c.* 5%) can lead to greatly inflated population estimates. Therefore, two preliminary goals of our study were to estimate the probability of failing to distinguish different individuals (probability of identity, Taberlet & Luikart 1999), and the probability of creating a new individual through genotyping errors. Additionally, we investigated factors that affected DNA amplification success rates, such as sample age and storage method, because long-term studies may store samples for several years prior to analysis.

The primary objective of this study was to evaluate the feasibility and utility of applying open-population models to long-term faecal genotyping data. We applied two open-population models to our 3-year faecal genotyping data set, and we used data from a concurrent radio telemetry study to evaluate model performance. One model allowed us to integrate faecal data and supplementary mortality data, and we used this model to evaluate the benefits of combining a faecal sampling scheme with a traditional radio telemetry study. Finally, we tested our hypothesis that coyote population size and survival would decline in response to food stress, using this example as a case study to show how faecal genotyping can be used to monitor wildlife populations over time.

Materials and methods

Sample collection

We collected a total of 1237 scats in the central Alaska Range (63°57'N, 147°18'W; Fig. 1) during November 1999–March 2000, January–March 2001, and January–March 2002. From these, we selected 850 scats for genetic analysis by random sampling without replacement and 834 of these scats were analysed (16 samples were contaminated during DNA extraction). We obtained tissue samples from 17 radio-collared coyotes (Arthur 2003) and five coyotes trapped for fur or found dead in our study area from 1998 to 2002. All genetic work, including scoring and analysis, was conducted by one person (LRP).

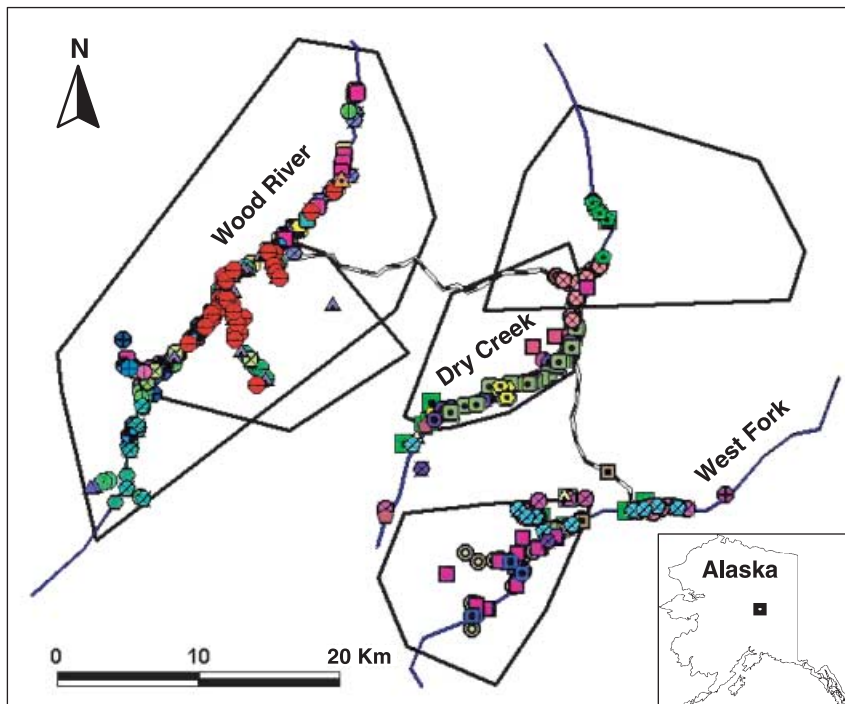


Fig. 1 Central Alaska Range study area (63°57'N, 147°18'W) showing major scat collection routes along the three main rivers (Wood River, Dry Creek, West Fork) and the two trails between river drainages (dashed lines). Point symbols represent locations of genotyped coyote faeces, with a different symbol for each of the 56 individuals. Polygons show composite home ranges of radio-collared coyote pairs, 2000–2002. Elevation increases to the south, and mountain peaks (2000–2600 m) occur between river drainages south of the connecting trails.

The study area encompassed approximately 1000 km² of mountains and foothills on the northern edge of the Alaska Range, and we established > 150 km of snowmobile trails along the three major river drainages (Fig. 1). Although trail routes were nonrandom due to topographical constraints, it is highly unlikely that any coyotes lived exclusively in the rugged, high-elevation areas between our trails. We searched for scats along the trails on a daily basis, and we also collected scats while following coyote tracks on foot. The study area was stratified and tracks were chosen from within these areas to ensure equal representation from each area and independence of samples between each monthly capture period. Distances travelled in search of scats were recorded as a measure of scat collection effort. We recorded the GPS location, estimated maximum age of the scat (based on travel and snowfall history), and our certainty level that the scat was from a coyote. Coyote scats could have been confused with those of grey wolves (*Canis lupus*), red foxes (*Vulpes vulpes*), lynx (*Lynx canadensis*), and dogs (*Canis familiaris*).

Sample storage, DNA preservation and extraction

Due to cold winter temperatures, faeces froze upon defecation. Collected scats were stored outdoors (average temperature = -14.7 °C) for 1–2 weeks before being flown to Fairbanks and stored at -30 °C. At the end of each field season, samples were prepared at the University of Alaska Fairbanks for DNA preservation. Approximately 100 mg of frozen faecal material was collected by scraping the surface of each sample with a scalpel and placing shavings into 2-mL vials.

Scats collected in the 1999–2000 and 2001 field seasons were stored in 1.5 mL DET buffer (20% DMSO, 0.25 M EDTA, 100 mM Tris, pH 7.5 and NaCl to saturation; Seutin *et al.* 1991). Samples from the 2002 field season were stored frozen in vials without the buffer. All vials were stored at -80 °C until DNA extraction. Scats from the 1999–2000 field season were stored for 45 months prior to DNA isolation, scats from the 2001 field season were stored for 33 months, and the 2002 season scats were stored for 14 months.

DNA samples were transported frozen to the Genetics Data Centre at the University of British Columbia for genetic analyses. We extracted DNA from faeces using QIAamp DNA Stool Mini Kits (QIAGEN) after centrifuging samples for 10 min and removing the storage buffer. Scats stored without the buffer were processed directly. Extracted DNA was eluted with 150 µL of the provided elution buffer. Negative controls were included in each batch of DNA isolation to monitor for contamination. DNA isolation and amplification were conducted in separate labs to minimize the risk of contaminating stock DNA with post-PCR products, and aerosol barrier tips were used for all procedures.

We examined four factors that we hypothesized could affect DNA amplification success rates: (i) supernatant

colour (which varied widely), (ii) storage method (buffer or not), (iii) storage time, and (iv) age of scat at the time of collection. For a subset of 142 samples, we recorded the colour of the sample after mixing with the ASL lysis buffer (QIAGEN protocol p. 22) on a scale of 1–5, with 1 being clear and 5 being black/brown. We compared the colour of successfully vs. unsuccessfully amplified samples using a chi-squared contingency test. For this test, data were combined into three categories: light (scale value 1 or 2), medium (3), or dark (4 or 5). We compared mtDNA amplification success rates of samples stored in buffer vs. no buffer, and of samples stored for 33 months vs. 45 months, using chi-squared tests on contingency tables. The effect of the estimated maximum age of a scat at the time of collection on amplification success was evaluated using logistic regression.

Species verification and microsatellite analysis

We screened each faecal DNA sample with a mitochondrial DNA test to ensure the isolated DNA was from a coyote. Briefly, ScatID primers (Adams *et al.* 2003) were used to amplify a section of the cytochrome *b* region of mtDNA, and the polymerase chain reaction (PCR) product was digested with *Taq*^α I restriction enzyme (New England Biolabs). Samples that did not amplify or showed noncoyote products were removed from the data set. For details of the species verification test, see Prugh & Ritland (in press).

Coyote microsatellite DNA was amplified for genetic fingerprinting in 10-µL reactions containing 2.5 µL of DNA extract (directly from kit extraction), 0.5 pmol forward IRD-labelled and reverse primers, 1 × reaction buffer, 1.5 mM MgCl₂, 1 unit of AmpliTaq polymerase (Roche), 0.2 mM dNTPs, and 1 mg/mL BSA. Negative controls were run with each batch of PCR. We amplified DNA in PTC-100 thermocyclers (MJ Research) using the following program: initial denaturation at 94 °C for 5 min; 35 cycles of 45 s at 94 °C, 45 s at 58 °C, and 45 s at 72 °C; final extension of 72 °C for 5 min. PCR products were visualized on 7% polyacrylamide gels using a LI-COR 4200 auto-sequencer. Gels were analysed using SAGA genotyping software (MX version, LI-COR).

Probability of identity and genotyping error rates

The probability of identity (P_{ID}) is dependent upon the number of loci used to construct the genotype, the heterozygosity of the loci, and the relatedness of individuals within the population (Waits *et al.* 2001). DNA was amplified from our coyote tissue samples ($n = 22$) at 11 microsatellite loci, and we used these allele frequencies to calculate heterozygosity and P_{ID} . We calculated P_{ID} for unrelated individuals as a lower bound ($P_{ID-RAND}$) and for siblings as an upper bound (P_{ID-SIB}) using equations from Waits *et al.* (2001). Based on our P_{ID} values (see Results), we used our

Table 1 Genotyping error rate estimates for coyote faecal samples collected in the Alaska Range. Five replicate PCRs were conducted for 45 samples at five loci. \overline{PD}_j^* is the allelic dropout rate calculated using eqn 1, \overline{PD}_j is the allelic dropout rate calculated with eqn 2, \overline{PF}_j is the false allele rate (eqn 3), and P_{ERROR} is the total estimated error rate when homozygotes were replicated three times and heterozygotes were replicated twice (eqn 6)

Locus	\overline{PD}_j^*	\overline{PD}_j	\overline{PD}_j SE	\overline{PF}_j	\overline{PF}_j SE	$(\overline{PD}_j)^3$	$(\overline{PF}_j)^2$	P_{ERROR}	P_{ERROR} 95% CI*
FH2001	0.102	0.086	0.020	0.015	0.010	0.0006	0.0002	0.0009	0–0.002
FH2137	0.030	0.019	0.019	0.014	0.008	0.0000	0.0002	0.0002	0–0.001
FH2140	0.072	0.061	0.019	0.014	0.008	0.0002	0.0002	0.0004	0–0.001
FH2159	0.013	0.008	0.008	0.032	0.029	0.0000	0.0010	0.0010	0–0.015
FH2235	0.071	0.049	0.021	0.023	0.015	0.0001	0.0005	0.0006	0–0.005
FH2096†	0.058	0.045	0.018	0.020	0.014	0.0002	0.0004	0.0006	0–0.005
Total						0.0012	0.0026	0.0038	0–0.029

*Negative lower confidence intervals were set to 0.

†No estimates were obtained for this locus, so the average of the other five loci was used in order to estimate total error. An alternative method of calculating error from the final data indicated that FH2096 did have fairly 'average' error rates.

best six loci for individual identification (all tetra repeats: FH2137, FH2159, FH2140, FH2235, FH2096, FH2001; http://www.fhrc.org/science/dog_genome/).

The genotyping error rate was determined by replicating PCR amplification five times for 45 faecal samples at five loci. A consensus genotype was constructed by examining the five replicates, and each replicate was compared to the consensus genotype to determine the per-replicate, per-locus error rate. Error due to allelic dropout and false alleles were recorded separately. The mean per-replicate probability of allelic dropout at locus j was calculated in two ways:

$$\overline{PD}_j^* = \frac{D_{jk}}{A_{\text{het}_j}} \quad (\text{eqn 1})$$

$$\overline{PD}_j = \frac{D_{jk}}{A_j} \quad (\text{eqn 2})$$

where D_{jk} is the number of amplifications showing a missing allele at locus j in replicate k , A_{het_j} is the number of consensus genotypes that are heterozygous at locus j , and A_j is the total number of consensus genotypes at locus j (after Broquet & Petit 2004). The per-replicate probability of obtaining a false allele at locus j was calculated as:

$$\overline{PF}_j = \frac{F_{jk}}{A_j} \quad (\text{eqn 3})$$

where F_{jk} is the number of amplifications showing a false allele at locus j in replicate k .

We adopted the comparative multiple tubes approach developed by Frantz *et al.* (2003) to reduce our final genotyping error rate. Based on this protocol, we replicated PCR at least twice for heterozygous samples and three times for homozygous samples. Samples were replicated a maximum of five times at each locus and were included in the data set if they had consensus genotypes at four or more loci. After

employing the replication protocol, the probability of allelic dropout in the final multilocus genotype was:

$$P_D = \sum_j^I \overline{PD}_j^3 \quad (\text{eqn 4})$$

Notice that we used allelic dropout eqn 2, which uses the total number of positive amplifications (heterozygotes and homozygotes) as the denominator. Although homozygotes have zero probability of showing allelic dropout, including these genotypes accounts for their occurrence in the data set and facilitates calculation of the total error probability. We show the alternative calculation (eqn 1, using heterozygotes only) in Table 1 (see Broquet & Petit 2004).

The probability of a false allele in the final multilocus genotype was:

$$P_F = \sum_j^I \overline{PF}_j^2 \quad (\text{eqn 5})$$

The total probability of obtaining an erroneous multilocus consensus genotype (i.e. individual) was:

$$P_{\text{ERROR}} = P_D + P_F \quad (\text{eqn 6})$$

These calculations differ somewhat from the formulae in Bonin *et al.* (2004), but results were nearly identical and our approach facilitated the calculation of confidence intervals.

We grouped identical and near-identical multilocus genotypes to look for potential errors by sorting in Excel (Microsoft) and using the program GIMLET (Valiere 2002). Samples that only differed by one or two alleles from other samples were examined for scoring inconsistencies by aligning all replicates side by side using SAGA genotyping software (LI-COR). Samples without matching genotypes were subjectively evaluated to determine whether they were likely to be unique individuals or erroneous genotypes. Criteria such as band intensity, clarity, and repeatability

were used to visually scrutinize questionable samples, and poor quality samples (e.g. faint bands that were difficult to score) were removed from the data set.

Sex identification

We determined the sex of each coyote by choosing one faecal sample per individual and amplifying a 104-bp region of the SRY gene on the Y chromosome with primers designed specifically for canids (5'-CTCGCGATCAAA-GGCGCAAGAT-3' upstream and 3'-TTCGGCTTCTGT-AAGCATTTTC-5' downstream; Meyers-Wallen *et al.* 1995b). With this method, only DNA from males amplified. We therefore amplified the 177-bp canine *HPRT* gene as an internal control to distinguish between females and failed reactions (5'-GTAATGATCAGTCAACGGGGGAC-3' upstream and 3'-CCAGCAAGCTTGCAACCTTAACAA-5' downstream; Meyers-Wallen *et al.* 1995a). The PCRs contained the same reagents as our species verification test (Prugh & Ritland in press), and the thermocycler program was the same as it was for our microsatellite reactions.

Although our primers were designed for canids, these regions of the genome are highly conserved among mammals and it may be possible to amplify DNA from prey remains in coyote faeces. Therefore, the wrong sex could be recorded if a female coyote ate a male mammalian individual. To test the accuracy of sex identification from our coyote faeces, we amplified five scats each from two male and two female radio-collared coyotes for which we had multiple faecal genotypes. An additional 18 faecal genotypes matched coyote tissue genotypes, and we compared the result of faecal DNA sexing to the known sex for these samples.

Estimation of population parameters

To apply mark-recapture models to our data set, we divided each winter into 'capture sessions' such that each month represented a different sampling interval. We did not estimate survival or population size in November or December 1999 because lack of snow limited faecal collections to the Dry Creek drainage during these months. A capture history was constructed for each coyote by recording whether or not it was 'captured' during each interval. Only faecal samples were counted as captures; radio locations of collared coyotes were not included. For the Burnham joint live and dead encounters model, we also recorded whether the animal was found dead (either opportunistically or with radio telemetry) during the interval. These capture histories were imported to the program MARK for analysis (White & Burnham 1999). We evaluated the accuracy of the models by comparing the estimated survival rates of the radio-collared coyotes to their true survival rates. True survival was calculated by dividing the number of collared

coyotes known to have died by the number of collared coyotes known to have been in the study area during each interval.

The first model we considered was the Cormack-Jolly Seber (CJS) model, which estimates apparent survival (ϕ) and recapture rates (p) of coyotes. Apparent survival (ϕ) is the probability that an individual is alive and in the study area. Recapture rates are calculated for all time periods except the first, because there are no possible recaptures in the first interval. Survival estimates are calculated for all intervals except the last, because they represent the chance of surviving to the next interval. We used the CJS model as an example of how faecal genotyping data can be used in an open-population model without supplementary mortality data. We then used the Burnham joint live and dead encounters model, which allowed us to include mortality data from radio telemetry and opportunistic encounters in order to estimate real survival (S), site fidelity (F), recapture rates (p), and the probability of recovering dead animals (r).

In both models, parameter estimates were obtained using a general linear equation:

$$\ln\left(\frac{\theta}{1-\theta}\right) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \dots + \beta_x X_x + \varepsilon \quad (\text{eqn 7})$$

where θ is the parameter of interest (e.g. ϕ , p , S), X_s is the value of the s th covariate, β_s is the slope of the s th covariate (obtained through maximum likelihood), and ε is the error (Lebreton *et al.* 1992). We used the following covariates to model heterogeneity: sex, location, sampling effort, year, and whether or not a coyote was radio collared. Location was recorded as the river drainage (Wood River, Dry Creek, or West Fork) where the majority of a coyote's scats were collected.

Within each model type (CJS or Burnham), models with different combinations of covariates were ranked and weighted according to Akaike information criterion (AIC_c; Burnham & Anderson 2002). On the basis of the recommendations of Lebreton *et al.* (1992), we began with the fully parameterized model (excluding interactions because of the limited data set). We then checked for lack of fit using a bootstrapping goodness-of-fit test in MARK before analysing reduced parameter models. When we had a final set of model results, we used model averaging to obtain parameter estimates and standard errors (Buckland *et al.* 1997; Burnham & Anderson 2002).

These open-population models do not produce estimates of population size directly within MARK. Therefore, we used the estimated recapture rates (\hat{p}) from the CJS model to derive estimates of coyote abundance using a simple Horvitz-Thompson-type estimator developed by McDonald & Amstrup (2001). This estimator is:

$$\hat{N}_t = \sum_{s=1}^n \frac{I_{st}}{\hat{p}_{st}} \quad (\text{eqn 8})$$

where \hat{N}_t is the estimated population size at time t , I_{st} is '1' if animal s was captured during time t and '0' if it was not, and \hat{p}_{st} is the maximum-likelihood estimate of the recapture rate of animal s at time t . The approximate variance of N_t is:

$$\text{var}(\hat{N}_t) = \sum_{s=1}^n \left(\frac{I_{st}(1-\hat{p}_{st})}{\hat{p}_{st}^2} + \frac{I_{st}\sigma_{p_{st}}^2}{\hat{p}_{st}^3} + \frac{I_{st}(1-\hat{p}_{st})\sigma_{p_{st}}^2}{\hat{p}_{st}^4} \right). \quad (\text{eqn 9})$$

Results

Factors affecting amplification success

Samples stored at -80°C in the DET buffer had higher mtDNA amplification success rates (83.6%, $n = 495$) than samples stored at -80°C without buffer (65.9%, $n = 407$), despite the fact that the buffered samples were stored 19–31 months longer prior to DNA extraction ($\chi_1^2 = 38.3$, $P < 0.001$). Of the scats stored in buffer, samples that were stored for 33 months had higher amplification success than samples stored for 45 months (91% vs. 79%, respectively, $n = 493$, $\chi_1^2 = 13.9$, $P < 0.001$).

Samples with lighter supernatant colour had higher amplification success rates than darker samples; success rates were 90%, 64%, and 48% for light, medium, and dark supernatants, respectively ($n = 142$; $\chi_2^2 = 17.1$, $P < 0.001$). All samples in this trial were stored at -80°C without buffer for 14 months prior to extraction.

We found a weak, and perhaps biologically unimportant, effect of scat age (estimated based on snowfall and travel history) on amplification success. The probability of successful amplification decreased slightly as the age of scats at the time of collection increased (logistic regression $\chi_1^2 = 4.9$, $P = 0.02$, $n = 834$). The mean estimated age of successfully amplified scats was 13.3 days (95% CI = 12.1–14.6), vs. 16.4 days (95% CI = 14.0–18.8) for unsuccessfully amplified scats.

Probability of identity and genotyping error rates

The average heterozygosity of our six loci was 0.74 (range = 0.68–0.79). The probability of identity using all loci was 0.0002 for unrelated individuals ($P_{\text{ID-RAND}}$) and 0.005 for siblings ($P_{\text{ID-SIB}}$). We included multilocus genotypes with 4–6 loci, so the maximum probability of obtaining the same genotype for different individuals (i.e. $P_{\text{ID-SIB}}$ for our four least heterozygous loci) was 0.033. Of the 544 multilocus genotypes in the final data set, 26 samples were genotyped at 4 of 6 loci, 78 were genotyped at 5 of 6 loci, and 440 were genotype at all six loci, for a weighted average P_{ID} range of 0.0004–0.0067.

The average per-locus, per-replicate dropout rate (PD) was 0.045 (95% CI = 0.006–0.084), and the average per-locus, per-replicate false allele rate (PF) was 0.018 (95% CI = 0.01–0.04; Table 1). Using the modified multiple tubes approach

(after Frantz *et al.* 2003), the average number of replicates conducted per sample, per locus was 2.8 (SE = 0.11) including failed reactions and 2.4 (SE = 0.07) excluding failed reactions. The probability of obtaining a false six-locus genotype after replication (P_{ERROR}) was 0.004 (95% CI = 0–0.029; Table 1). On the basis of these data, we could expect anywhere from 0 to 16 erroneous genotypes in our sample of 546 genotypes.

We matched identical and near-identical genotypes to find samples that may have been erroneous using the logic that samples without matching genotypes were the most likely ones to contain errors. Of the 546 samples, 27 had no matching genotypes. Two of these samples appeared to be of poor quality (see criteria in Methods) and were removed from the data set. Eleven of these samples differed from another genotype at only one locus. We assumed these samples had genotyping errors and assigned them the identity of the closely matching genotype to reduce the possibility of falsely identifying new individuals and thereby inflating our population estimates. We decided that the remaining 14 unmatched samples were unique individuals rather than genotyping errors. One sample matched the genotype of a radio-collared coyote, and the others were of high quality and differed from other genotypes at three or more loci.

Sex identification

All faecal samples from coyotes of known sex matched the correct sex ($n = 34$ samples from 11 males and 7 females). This included five replicate scats each for two male and two female coyotes. In addition, we analysed three replicate scats from three coyotes of unknown sex, and the replicates showed 100% agreement for each coyote. In total, 38 tests assigned the correct sex and none were incorrect.

Coyote population dynamics

We identified 56 unique individuals from the 544 scats in our final data set, 24 are females and 32 are males (Supplementary material). The number of scats per individual ranged from one to 49 (Fig. 2), indicating considerable capture heterogeneity among individuals. All 15 radio-collared coyotes that were present during scat collection and for which we obtained tissue or blood samples (we did not obtain a sample from one coyote) matched ≥ 1 faecal genotype. All tissue samples from coyotes that were either fur-trapped or found dead in the study area during scat collection ($n = 4$) matched the genotype of ≥ 1 faecal sample as well. Therefore, the chance of 'capturing' a coyote present in our study area through faecal genotyping was very high. Moreover, we were able to non-invasively identify almost four times as many coyotes with faecal genotyping as we were by physically capturing animals in the radio telemetry study.

Table 2 Top 10 Cormack–Jolly Seber models run in program MARK to calculate maximum-likelihood estimates of the apparent survival rates (ϕ) and recapture rates (p) of coyotes in the Alaska Range, 1999–2002. Twenty-two models were tested and ranked according to their AIC_c values. Models included the following covariates: whether a coyote was radio collared (collar), sampling effort (effort), sex, location, or year (models including year and location were not among the top 10 models). Some models included time variation (t), where the parameter (ϕ or p) was estimated during each time interval

Model	AIC_c	ΔAIC_c	AIC_c weights	Model likelihood	No. of parameters
$\phi(\text{collar}) p(\text{collar} + t)$	307.4	0.0	0.55	1.00	13
$\phi(\text{collar}) p(\text{collar} + \text{effort})$	309.0	1.6	0.24	0.44	5
$\phi(\text{collar}) p(\text{collar} * \text{effort})$	311.2	3.8	0.08	0.15	6
$\phi(\text{collar}) p(t)$	311.8	4.5	0.06	0.11	12
$\phi(\text{collar}) p(\text{effort})$	313.3	5.9	0.03	0.05	4
$\phi(\text{sex} + \text{collar}) p(\text{sex} + \text{collar} + t)$	314.8	7.4	0.01	0.02	17
$\phi() p(\text{effort})$	316.0	8.6	0.01	0.01	3
$\phi(\text{collar}) p(\text{collar})$	317.3	9.9	0.00	0.01	4
$\phi() p(\text{collar})$	317.6	10.2	0.00	0.01	3
$\phi(\text{collar} + t) p(\text{collar} + t)$	318.8	11.4	0.00	0.00	22

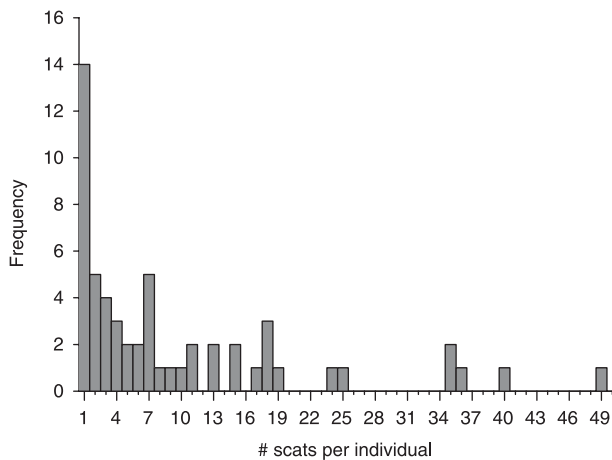


Fig. 2 Number of scats collected per coyote in the Alaska Range, 1999–2002. A total of 544 faeces were genotyped from 56 coyotes.

The Cormack–Jolly Seber model. The bootstrapping goodness-of-fit test showed that our fully parameterized CJS model fit the data reasonably well ($P = 0.15$, $\hat{c} = 1.15$). We then examined reduced-parameter models and found that the best model included ‘radio collar’ as a covariate for both apparent survival (ϕ) and the recapture rate (p), and the recapture rate varied over time (Table 2). The top five models included radio collaring, sampling effort, or both as covariates. The number of parameters included in the models (3–24, Tables 2 and 3) was often high compared to the number of individuals in the analysis (56); models with > 6 parameters in this study should be considered with caution.

Coyotes that were radio collared had higher survival and recapture rates than uncollared coyotes (Fig. 3; likelihood ratio tests, survival: $\chi^2_1 = 4.87$, $P = 0.03$; recapture: $\chi^2_1 = 4.75$, $P = 0.03$). Sex differences in survival and recapture rates were minimal, and models including this covariate

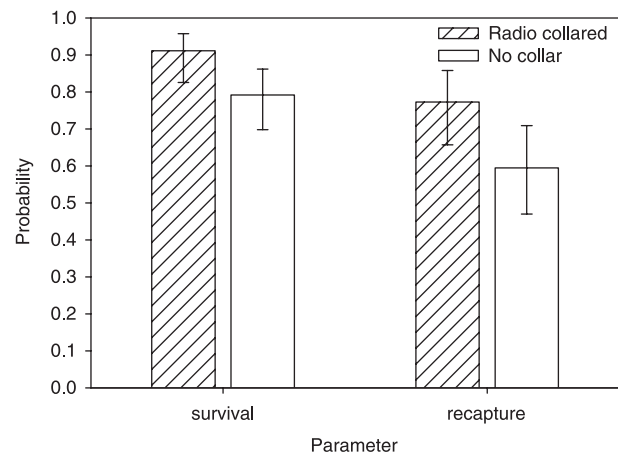


Fig. 3 Estimated apparent survival (ϕ) and recapture (p) rates of radio-collared and uncollared coyotes in the Alaska Range, 2000–2002. Error bars represent 95% confidence intervals. These maximum-likelihood estimates were obtained using the Cormack–Jolly Seber model ‘ $\phi(\text{collar}) p(\text{collar})$ ’ in program MARK.

were given little weight (Table 2). Likewise, location and year had minimal effects on survival and recapture rates and were therefore absent from the top 10 models listed in Table 2 (out of 22 models tested). Recapture rates varied from 0.30 to 0.92, and sampling effort explained approximately 32% of this variation.

Estimates of population size derived from these recapture rates showed an increasing population that reached a peak of 35 coyotes in January 2001 and declined to 20 coyotes by March 2002 (Fig. 4). The uncertainty surrounding these estimates was high (Fig. 4). On average, 16.9% of this variation was attributable to model selection uncertainty. Radio-collared coyotes accounted for 23–50% of the population at any given time during the study.

Table 3 Top 10 Burnham joint live and dead encounter models run in program MARK to calculate maximum-likelihood estimates of survival (S) and recapture rates (p) of coyotes in the Alaska Range, 1999–2002. Twenty-two models were tested and ranked according to their AIC_c values. Models included the following covariates: whether a coyote was radio collared (collar) and sampling effort (effort). Some models included time variation (t), where the parameter (S or p) was estimated during each time interval. For all models, we fixed the parameter for site fidelity (F) at 1 and the dead animal recovery probability (r) at 1 for radio-collared coyotes. All models estimated r for uncollared coyotes, with no time variation, in addition to the parameters listed

Model	AIC_c	ΔAIC_c	AIC_c weights	Model likelihood	No. of parameters
$S(\text{collar}) p(\text{collar} + t)$	347.0	0.0	0.51	1.00	14
$S(\text{collar}) p(\text{collar} + \text{effort})$	349.0	2.0	0.19	0.37	6
$S(\text{collar}) p(t)$	350.4	3.3	0.10	0.19	13
$S(\text{collar} + t) p(\text{collar} + \text{effort})$	350.9	3.8	0.08	0.15	16
$S(\text{collar}) p(\text{effort})$	352.3	5.3	0.04	0.07	4
$S() p(\text{collar} + \text{effort})$	352.9	5.9	0.03	0.05	5
$S(\text{collar} + t) p(\text{effort})$	353.1	6.0	0.03	0.05	14
$S(\text{collar} + t) p(\text{collar} + t)$	353.7	6.6	0.02	0.04	24
$S() p(t)$	355.29	8.24	0.01	0.02	12
$S(t) p(\text{collar} + t)$	356.0	8.9	0.01	0.01	23

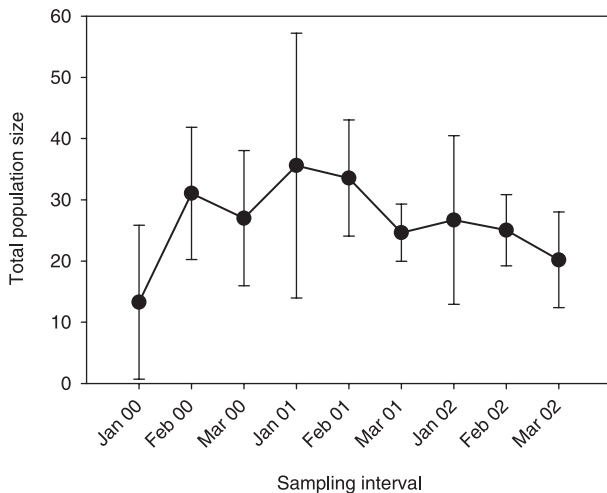


Fig. 4 Total estimated coyote population size in the Alaska Range study area during each sampling interval. Error bars represent 95% confidence intervals.

Burnham joint live and dead encounters model. Our fully parameterized Burnham model was a good starting point for evaluating reduced-parameter models ($P = 0.21, \hat{c} = 1.08$). We did not include sex, location, or year as covariates in the Burnham model because these factors were found to be unimportant in the CJS model. As with the CJS model, the Burnham model with the most support included radio collaring as a covariate for survival (S) and recapture rates (p), and recapture rates varied over time (Table 3). We examined survival trends by using estimates from the most supported model that allowed survival to vary over time [$S(\text{collar} + t) p(\text{collar} + \text{effort})$, Table 3]. Survival declined in 2001, when the coyote population size was highest, but it increased in 2002 (Fig. 5). The confidence intervals for

estimated survival rates included the true survival rates of radio-collared coyotes in all time intervals except two, and in both cases true survival was 1 (Fig. 5a). Compared with estimates from the equivalent CJS model, the Burnham survival estimates were more precise (average Burnham CV = 14%, CJS = 17%) and deviated less from true survival rates (Burnham sum-of-squares = 0.05, CJS SS = 0.16).

The Burnham model also estimated site fidelity (F) and the probability of finding dead animals (r). We fixed r at 1 for radio-collared coyotes, and our estimated probability of finding dead uncollared coyotes was 0.11 (SE = 0.06). Due to our limited data set, the models could not estimate both survival (S) and site fidelity (F). When survival was held constant, F was estimated as 0.98 (SE = 0.03) for collared coyotes and 0.87 (SE = 0.05) for uncollared coyotes. Our radio telemetry data agree: 2 of the 16 radio-collared coyotes emigrated during the study, which is a per-interval site fidelity rate of 0.99. In order to reduce the number of estimated parameters in our models, we fixed F at 1 for all coyotes. Therefore, survival rates (S) in our Burnham models were actually apparent survival rates ($S \cdot F$). Estimates of recapture rates from the Burnham model were nearly identical to those from the CJS model, as were the derived population size estimates and variation.

Discussion

Applying open-population models to our 3-year faecal genotyping data set provided insights into the response of coyotes to changes in food supply. We identified nearly four times as many coyotes with faecal genotyping as we did by physically capturing coyotes in the radio telemetry study, and we had ≥ 1 genotyped faecal sample for every coyote known to be in the study area ($n = 19$). Supplementary

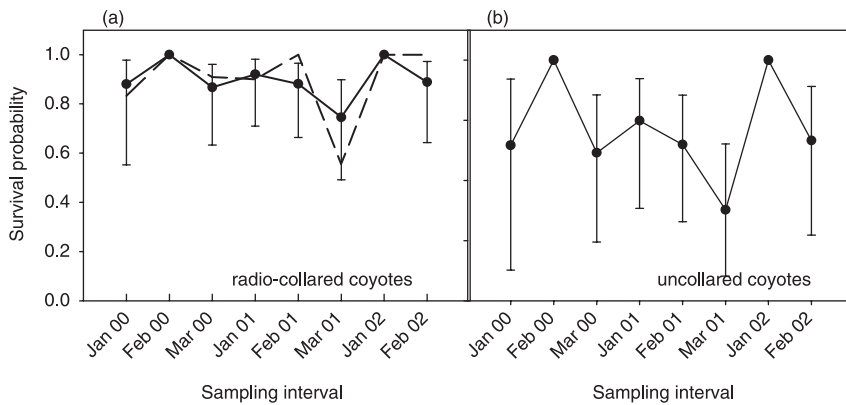


Fig. 5 Maximum-likelihood estimates of the survival rates of (a) radio-collared coyotes and (b) uncollared coyotes in the Alaska Range during each sampling interval. Estimates were obtained from the Burnham joint live and dead encounter model 'S(collar + t) p(collar + effort)' in program MARK. Error bars represent 95% confidence intervals. The dashed line (a) is the true survival rate of radio-collared coyotes.

radio telemetry data was useful when estimating survival rates but did not increase the accuracy or precision of population estimates. When necessary precautions are taken to ensure data accuracy and appropriate mark-recapture models are used, faecal genotyping can be a practical technique for long-term wildlife monitoring.

Amplification success

Freezing at standard temperatures (-20 to -80 °C) is probably not a sufficient storage method for long-term preservation of faecal DNA. Mitochondrial DNA amplification success rates were 18% higher for samples stored frozen in DET buffer compared with samples stored frozen without buffer despite the fact that buffered samples were stored approximately 2 years longer. Frantz *et al.* (2003) also had higher success rates with badger faeces stored in DET buffer vs. frozen. Other storage methods, such as drying samples with silica beads or storage in ethanol (or both), have also been used to preserve DNA (Goossens *et al.* 2000; Murphy *et al.* 2002; Nsubuga *et al.* 2004). Our amplification success rates were high (80–90%) with faeces that had been stored in DET buffer for 3 or 4 years. To our knowledge, these are the longest storage times for faecal DNA reported in the literature, and we were therefore pleased to have such high amplification success rates. However, success rates were 12% lower with samples stored in buffer for 4 years vs. 3 years. Thus, the shelf life of faecal DNA, though potentially quite long, is not indefinite.

The scats in our data set varied considerably in the length of time they could have been in the field prior to collection (1–90 days), but this variable did not have a strong impact on amplification success. The cold winter temperatures and constant snow cover during our study may have reduced the activity of bacteria in the faecal material and prevented contact with organisms in the environment that degrade DNA.

One of the challenges of amplifying DNA from faeces is the presence of PCR inhibitors, such as polysaccharides

from food residue (Monteiro *et al.* 1997). In fact, PCR inhibitors may pose more of a problem for amplification than DNA quality or quantity. We found that supernatant colour, which may be an indicator of inhibitor level, had a stronger impact on amplification success rates than age of sample or storage method. The success rates from dark scats, which likely have high levels of PCR inhibitors, were 42% lower than success rates from light scats. Research is needed to determine whether supernatant colour is related to PCR inhibitors and what factors cause these levels to vary among faeces.

Reliability of sex determination

Unlike Murphy *et al.* (2003), who found that sex determination from captive grizzly bear faeces was unreliable, our sex test using coyote faecal DNA was 100% accurate. The primers we used were more specific and coyote digestive processes might degrade prey DNA more effectively than bear digestion. Williams *et al.* (2003) also found that these primers accurately sexed coyote samples even in mixtures with high concentrations of sheep DNA.

Genotype reliability

In this study, we were more concerned about falsely identifying individuals (genotyping error) than about failing to identify unique individuals with matching genotypes (the shadow effect; Mills *et al.* 2000). Several studies have shown that genotyping error is a more serious problem than the shadow effect for population estimation. Mills *et al.* (2000) showed that the closed population $M_{h-jackknife}$ CAPTURE model can produce relatively unbiased population estimates despite the presence of shadow effect, whereas Waits & Leberg (2000) found that this model can produce extremely biased estimates with common levels of genotyping error. Indeed, Creel *et al.* (2003) found that Yellowstone wolf population estimates from faecal genotyping were up to five-fold higher than true numbers.

Our total per-replicate, per-locus error rate (6.3%) was lower than most faecal genotyping studies, which have reported error rates of 1–48% (Broquet & Petit 2004). After employing the comparative multiple tubes method to reduce error (Frantz *et al.* 2003), our mean error rate was 0.4%, which should produce fairly unbiased population estimates based on simulations (Waits & Leberg 2000). However, we found that it was important to consider the uncertainty surrounding error rate estimates, because the number of genotypes in our data set that were likely erroneous (13) was closer to upper end of our 95% confidence interval (0–16 expected erroneous genotypes) than it was to the mean (2 expected errors).

The equations we used to calculate genotyping error rate assume that the chance of having an error in one replicate is independent of the chance of having an error in a second replicate. This may not be true for low quality samples, which could explain why we found more errors than expected. Creel *et al.* (2003) highlighted the importance of considering variation in sample quality and recommended using a matching approach to reduce bias. We found that matching near-identical genotypes and carefully scrutinizing samples without matches were useful means of removing potentially erroneous genotypes. Unfortunately, the evaluation of sample quality is subjective and therefore complicates estimation of the probability of having erroneous genotypes in the final data set.

Population dynamics of coyotes

The coyote population in our study area declined nearly two-fold during the snowshoe hare decline, with a 1^{1/2}-year time lag. The hare population peaked in our study area in summer 1999 and winter 2000 (Prugh 2004), whereas the coyote population peaked in 2001. Likewise, coyotes in the Yukon tracked the snowshoe hare population in a classic predator–prey cycle with a 1-year time lag and a four-fold change in density during the hare decline (O'Donoghue *et al.* 1997). The estimated survival rates of coyotes decreased after the peak in coyote numbers but then recovered, which is a mortality pattern that has been documented in northern lynx populations (Poole 1994; O'Donoghue *et al.* 1997).

Utility of open population models

We found that faecal genotyping data could be used in open-population models to study coyote population dynamics. The basic structure of the Cormack–Jolly Seber model as implemented in the program MARK facilitates modelling of capture heterogeneity in a biologically relevant manner (Lebreton *et al.* 1992; White & Burnham 1999). The model selection process highlighted factors that affected coyote survival and recapture rates, such as radio collaring and

sampling effort, while factors such as sex, year, and location were shown to have little impact on these parameters.

Differences in survival, site fidelity, and recapture rates between collared and uncollared coyotes probably occurred because we attempted to radio collar resident adults rather than juveniles, and the telemetry data showed that home ranges of collared coyotes were within the boundaries of our study area (Fig. 1). Nearly all juveniles in the population were uncollared, and juvenile coyotes tend to have lower survival and higher dispersal rates than adults (Windberg 1995; Crête *et al.* 2001). Recapture rates were probably lower for uncollared adult coyotes because they were more likely to be transient or to have home ranges extending beyond the edges of our study area. In effect, radio collaring may have been a proxy for age or resident status in our models.

The modified CJS model (Burnham joint live and dead encounters model) allowed us to include mortality data gathered opportunistically and through radio telemetry, which increased the accuracy and precision of the estimated survival rates but had no impact on estimates of recapture rates or population size. The estimated survival rates of collared coyotes were close to the true survival rates during each interval, with slight underestimations occurring when true survival was 1. Survival estimates from the Burnham model were more accurate and precise than the CJS model, and we therefore recommend supplementing faecal surveys with radio telemetry when possible if survival estimation is a critical component of the study. If the primary goal is to track changes in population size over time, however, telemetry data may be unnecessary.

Confidence intervals surrounding our survival and population estimates were wide, and it was therefore difficult to make strong inferences about trends. There were often numerous parameters in the models because we had nine time intervals and were interested in changes over time, and this increased uncertainty. The usefulness of open-population models may therefore be limited to faecal genotyping studies with relatively large sample sizes.

Other models may also be used to examine population changes over time with faecal genotyping data. In an analysis not reported here, we examined the coyote population trajectory using the Pradel model, which is an open mark–recapture model that estimates the population rate of change, λ (Pradel 1996; Boulanger *et al.* 2004). Results were quite similar to those derived from the CJS models, both in terms of the population trajectory and precision (Prugh *et al.*, unpublished data). Closed-population estimators tend to be more precise, but using these models to estimate the size of open populations can lead to inflated estimates because recapture rates will be biased low (Baker 2004). Since genotyping error can also inflate estimates, researchers using faecal data in mark–recapture models

should be particularly cautious about violating the assumption of population closure (Boulanger & McLellan 2001). Bayesian techniques may be able to handle sparse data more effectively than traditional models, but most Bayesian models assume population closure (Gazey & Staley 1986; Garthwaite & Hope 1995; Ananda 1997). The joint recovery/recapture open-population model developed by King & Brooks (2002), which combines CJS and Bayesian techniques, may be particularly promising for faecal genotyping data sets.

While considerable attention has been given to the impact of genotyping error on population estimates (and rightly so), the usefulness of faecal genotyping will increase if more attention is focused on the development and appropriate use of population models. In particular, there is a need for a robust open-population model designed for data that are collected continuously. Many carnivore studies collect scats continuously over field seasons of several months in which births, deaths, immigration, and emigration may occur. The covering of faeces by snow or the risk of DNA degradation in summer can make it more practical to collect scats continuously than during discrete intervals, particularly as it is necessary to have relatively large samples. Furthermore, animals deposit scats continuously regardless of the researcher's collection schedule. It is unclear how the division of such data into arbitrary capture intervals affects the estimation of population parameters. Mark-recapture models for continuous data have been developed for closed populations (Wilson & Anderson 1995), but none currently exist for open populations. Rarefaction analysis, which is appropriate for continuously collected data, does not allow for capture heterogeneity and therefore can produce biased results (Boulinier *et al.* 1998; Eggert *et al.* 2003), and it is not appropriate for open populations. Capture heterogeneity is ubiquitous and presents a major challenge for both closed- and open-population estimation (Pledger & Efford 1998), so any model used to estimate population parameters needs to adequately model capture heterogeneity.

Acknowledgements

Funding for the genetics work was provided by the Alaska Department of Fish and Game (ADF & G), the UBC Genetics Data Centre, the American Wildlife Research Foundation, and a Grant-In-Aid from the American Society of Mammalogists. Fieldwork was funded by ADF&G in cooperation with the Alaska Chapter of the Foundation for North American Wild Sheep, and by the Natural Sciences and Engineering Council of Canada. L. Prugh was supported by a National Science Foundation graduate fellowship during fieldwork and a Welder Wildlife Fellowship (contribution # 633) during lab work. Invaluable field assistance was provided by T. Heakes and J. Bernier. Housing in the field was generously provided by the Buzbys. Many thanks to K. Blejwas, P. Groves, A. Miscampbell, and the Burnells for materials, lab space, advice, and assistance.

Supplementary material

The following material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC2533/MEC2533sm.htm>

Table S1. Genotypes of the 56 coyotes identified in a sample of 544 genotyped faeces collected in the Alaska Range, 2000–2002. Allele sizes are shown for each locus, with '0' recorded for missing data. For each individual, the sex, number of scats collected, number of years present, and whether or not it was radio collared were recorded.

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