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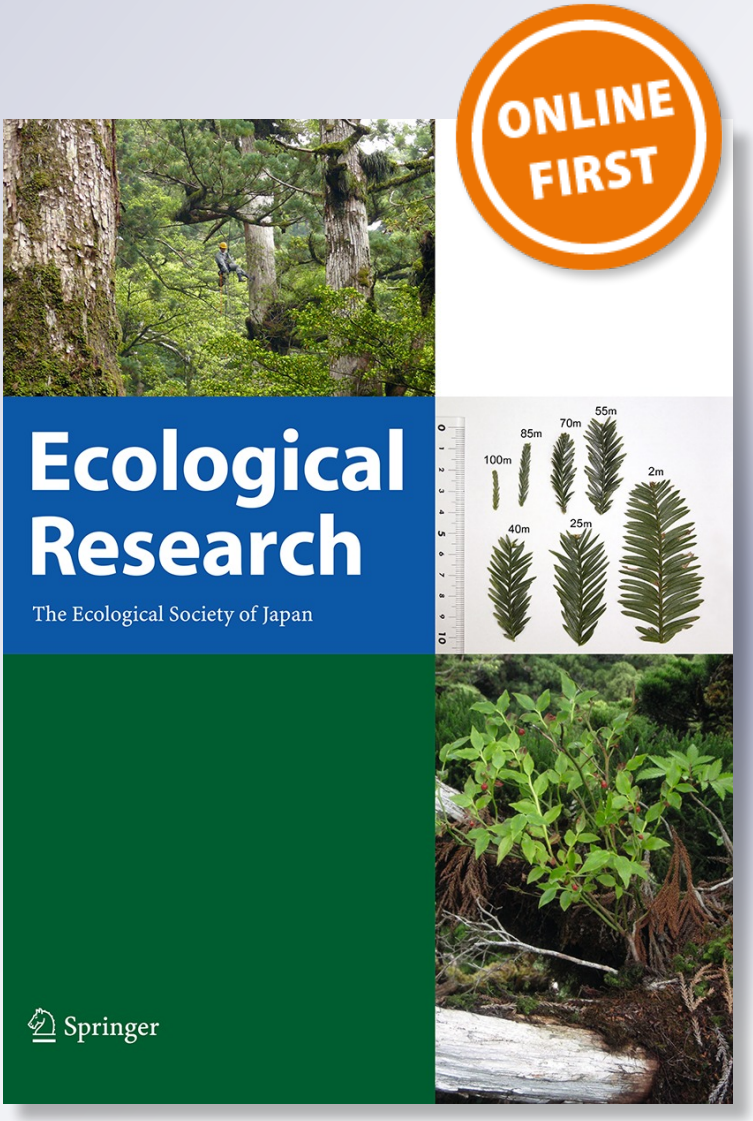

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ORIGINAL ARTICLE

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Abstract Monitoring the distribution and abundance of populations is an important component of efforts to meet management or conservation goals. Although the objectives for such studies are easy to define, cost-effective, precise, and accurate estimates are often elusive. We tested the efficacy and compared the cost-effectiveness of methods for estimating the number and recording the distribution of river otter (*Lontra canadensis*). We genotyped otter hair sampled using two noninvasive instruments and compared those results with a hypothetical study design based on DNA extracted from fecal matter. Patterns of distribution generated from DNA collected at latrine sites were then compared to observations of otter collected using VHF radiotelemetry. We achieved a high probability of genotyping river otter with a small number of hairs (i.e., 59.0 % probability of producing a genotype with 1 guard hair and >5 under hair samples) collected using wire body snares and knaplock hair snags. Body snares were more effective at collecting otter hair, but there was relatively little additional cost to using both sampling instruments. Genotyped hair resulted in a high multi-year recapture rate (61.9 %). Hair collection and genotyping was the most cost-effective method for monitoring populations of river otter ($168.50 US/datum) followed by radiotelemetry ($264.50 US/datum), and the extraction of DNA from fecal matter ($266.00 US/datum). However, the noninvasive techniques did not represent the full distribution and fine-scale movements of otter, as observed using radiotelemetry. There has been much recent reporting of the efficacy of fecal matter as a source of DNA for conducting mark–recapture population estimates for mesocarnivores. Our data suggested that collecting DNA in hair may be a more cost-effective and efficient approach.

Keywords Fecal DNA · Hair snag · Mark–recapture · Noninvasive · Population monitoring · River otter

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Introduction

Understanding spatio-temporal variation in the distribution and abundance of populations is essential to meet goals for conservation and management. Setting harvest quotas, conducting conservation assessments, and managing and monitoring the effects of human activities are examples of activities that require accurate and precise measures of the number and distribution of individuals (Piggott et al. 2006; Scheppers et al. 2007; Ruibal et al. 2010). Additionally, those populations should be reassessed over time to monitor for change that may be a function of conservation and management interventions, natural dynamics or alterations to the organism's environment. Although a straightforward objective, conducting a census or estimate of population parameters for many free-ranging species is challenging: the necessary data can be expensive to collect and precise and unbiased sampling is difficult to achieve (Boulanger et al. 2004; McKelvey and Schwartz 2004; Settlage et al. 2008). This is especially the case for low-density populations or arboreal, cryptic, fossorial, or aquatic species (Frantz et al. 2004; Bellemain et al. 2005).

Ecologists and biometricians have invested much effort in evaluating and developing effective sampling designs, techniques, and analytical methods for monitoring animal populations (Bremner-Harrison et al. 2006; Knapp et al. 2009; Sawaya et al. 2011). Modes of data collection for understanding the distribution of organisms are many, including Global Positioning System (GPS) collars, ground or aircraft-based surveys of the sign or presence of individuals, or georeferenced

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specimens from museum collections. Likewise, there are numerous methods for collecting the necessary data and estimating population abundance and associated parameters (Seber 2002). Individuals can be counted directly by observing identifiable marks such as natural variation in morphology and coloration or indirectly through genetic signatures. Where individuals have no obvious or practical differentiating feature, one can use temporary or indelible markers including radio transmitters, coded tags, and skin or fur pigments. In many cases, methods for directly measuring or estimating population parameters are also applicable to the collection of data that represent animal distribution (Ovaskainen 2004). Although there is a wide choice in technique, efficacy and cost-effectiveness can vary considerably. For long-term monitoring of population change, one must consider both the feasibility of the technique for the given system and species, as well as the costs of data collection and ultimately the precision and accuracy of results (Settlage et al. 2008).

The North American river otter (*Lontra canadensis*) is a challenging species to monitor (Mowry et al. 2011). Typically occurring at low densities, this species inhabits aquatic and near-shore forested habitats that are difficult to census. Individuals are indistinguishable by sight and typically they avoid contact with humans. In contrast to these enumeration challenges, groups or single otters repeatedly visit and mark easily identified shoreline latrines (Ben-David et al. 2005; Crowley et al. 2012).

The presence of fecal material at latrine sites allows one to monitor the spatio-temporal distribution of otter populations across lake, river and marine systems (Crowley et al. 2012). Also, fecal matter contains viable samples of DNA that can be used to measure the use of latrines by individual otters identified to sex and perhaps age (Guertin et al. 2010; Pauli et al. 2011). Alternatively, predictable occurrence of otters at latrines allows for the efficient deployment of snares and the collection of DNA in hair (Depue and Ben-David 2007). In comparison to the extraction of DNA from fecal matter, hair sampling and analysis has received relatively little attention in recent studies, but may provide a higher amplification rate and lower cost per sample.

Noninvasive fecal and hair sampling is less harmful for the study animal and potentially more cost-effective and reliable relative to the capture and marking of animals with passive tags or active radio transmitters (Mills et al. 2000; Stricker et al. 2012). However, both noninvasive and invasive techniques allow the identification of individuals and provide similar data for conducting mark–recapture estimates of population status. Researchers have contrasted techniques for collecting DNA from carnivores and methods for generating population estimates using a range of models or forms of noninvasive data collection (Bellemain et al. 2005; Arrendal et al. 2007; Mowry et al. 2011; Sawaya et al. 2011; Stricker et al. 2012). However, few researchers have considered the efficacy of the method in combination with cost-effectiveness (but see Harrison 2006).

We provide a comparative analysis of several methods for monitoring the distribution and abundance of river otter populations. First, we assess the efficacy of two noninvasive techniques for sampling DNA from hair and provide a population and distribution estimate from those data. We compare the genotype success rate and costs to a sampling protocol based on DNA collected in fecal matter. The process and success of extracting and amplifying DNA in fecal matter is now well reported (Arrendal et al. 2007; Guertin et al. 2010; Mowry et al. 2011) thus, we report metrics of the relative cost-effectiveness for our system only. Second, we contrast the animal location data gained from sampling and genotyping hair samples with those data gained from the use of radio transmitters. We provide not only a quantitative analysis and discussion of the advantages and disadvantages of these techniques for understanding the ecology and population status of otter, but also guidance on cost-effectiveness. This is a consideration that is seldom reported, but essential when developing a long-term monitoring protocol.

Methods

Study area

The research was conducted in central British Columbia, Canada, on a 17,000-ha portion of crown land contained within the John Prince Research Forest (Fig. 1). The research forest is bordered by two large lakes, Tezzeron and Pinchi, and several major tributaries, as well as numerous wetlands and smaller water bodies. Tezzeron Lake's shoreline stretches for 82 km (area = 8,079 ha), while the perimeter of Pinchi Lake is 67 km (area = 5,586 ha). The mean depth of Tezzeron and Pinchi Lakes are 11.2 and 23.9 m, respectively. Shoreline topography varies considerably along both lakes, but the area surrounding Pinchi Lake is generally steeper with more rocky outcrops. We focused animal capture and radio tracking as well as the monitoring of latrine sites on Tezzeron and Pinchi Lakes and associated tributaries. The collection of hair samples was restricted to latrine sites located on Tezzeron Lake. With the exception of a non-operational mercury mine on Pinchi Lake and forestry operation in upland areas, neither lake has significant human development. Over the course of the study, we were unaware of any trapping activity resulting in the loss of otters.

Animal capture, marking, and relocation

We identified locations of latrine sites on Pinchi and Tezzeron Lakes as well as tributary streams that were fish-bearing and navigable by canoe or kayak (1 km from lake-stream confluence). Two complete surveys of all shorelines were conducted in 2007. We chose to

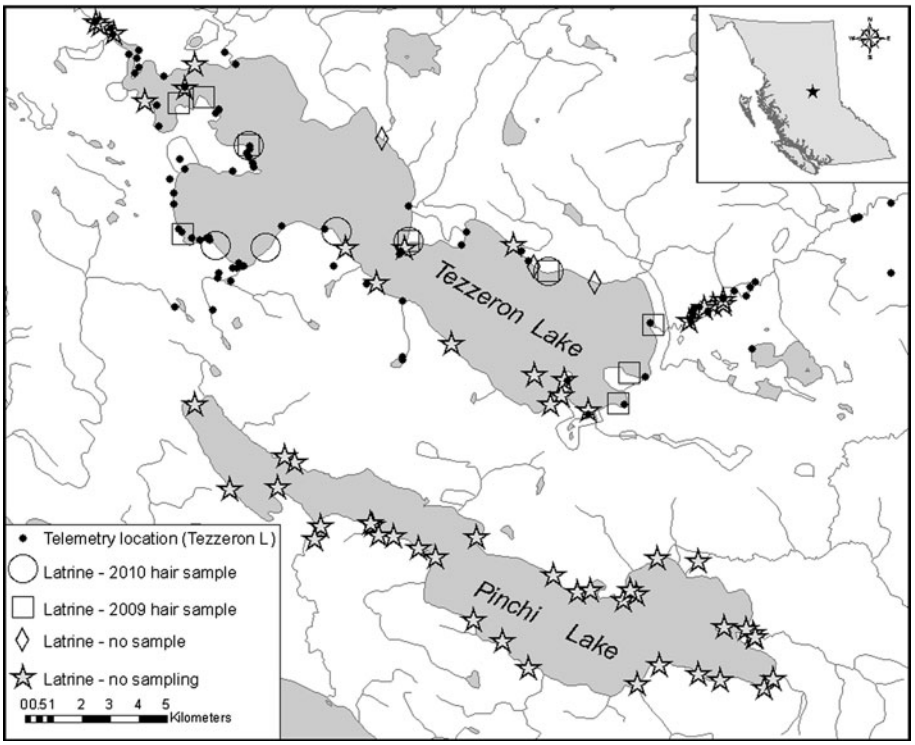

Fig. 1 Location of Tezzeron and Pinchi Lakes in central British Columbia, Canada (see *inset map*). Telemetry locations for river otter on Tezzeron Lake and latrine sites active during the study period (2007–2010) are marked as well as latrine sites where body snares or knaplock hair snags collected hair that was successfully genotyped

conduct surveys during three distinct time periods, late spring, summer, and early fall, to account for variation in prey availability. In 2008, we randomly selected and intensively surveyed two hundred 200-m segments of shoreline along the Tezzeron and Pinchi Lake systems. This intensive survey was a test of detection accuracy and allowed us to determine if the majority of the active latrine sites were being monitored. We located a total of 73 latrine sites across 155 km of shoreline, including immediate tributaries. Sixty-seven and six latrine sites were found in 2007 and 2008, respectively. Only two new latrine sites were found in areas previously surveyed in 2007. The other four latrine sites were found in areas not surveyed in 2007 (Crowley et al. 2012).

Between September 2007 and May 2008, we conducted four trapping sessions. We set between two and five #3 softcatch leg-hold traps at latrine sites recently occupied by otter (Blundell et al. 1999). Occupancy was determined by fresh fecal matter, tracks, or disturbance of substrate. Traps were checked at a minimum of every 12 h.

Trapped otter were handled with a noose pole and transported to a veterinary facility in a cage constructed from 40-cm diameter PVC pipe (Serfass et al. 1996). Sedated animals were implanted with an intraperitoneal Advanced Telemetry Systems M1250B radio transmitter ($30 \times 112 \times 30$ mm; $\sim$100 g) (Hernandez-Divers et al. 2001). Each animal was further marked with a PIT tag. Following the implant procedure, otters were placed in the transport tube and monitored for recovery from the anesthetic and then returned to the location of capture. Telemetered otter were located at a minimum of once per week throughout the year. The majority of animal locations (83 %) were calculated using triangulation or measured directly using line-of-site referencing and a GPS while the remainder were collected using aerial telemetry. Location accuracy for terrestrial methods was conservatively estimated as $\pm$15 m.

Noninvasive hair sampling

Between June 2009 and October 2010, we conducted five systematic hair sampling sessions at latrine sites on Tezzeron Lake. At the beginning of each year, we visited and assessed all known latrines for use by otter. Where latrines had recent activity, we established two types of hair collection instruments: body snares and knaplock hair snags.

We set 3–5 wire breakaway body snares at each active latrine (Depue and Ben-David 2007). Snares were constructed from 1.6-mm aircraft cable that was frayed manually. The locking device for each snare was a #1 (32-mm) paperclip. Each snare was secured to the substrate using a stake or nearby tree and suspended by twig or low shrubs. After the snare tightened around the otter's body, the paperclip would bend and the wire loop would disengage, releasing the animal and snagging a sample of guard hair or under hair in the frayed wire (see http://www.youtube.com/watch?v=zT48AYW72HY). This trap had the advantage of collecting hair from only one animal.

The knaplock hair snag consisted of a 15-cm length of knaplock carpet anchor (i.e., aluminum tack strip). At

each site, we deployed 3–5 traps by securing knaplock strips against exposed root substrate. As otter pulled their bodies and tails over the root, the sharp edges of the knaplock snagged guard hair or under hair.

Each of the five hair-sampling sessions included between 9 and 11 ($\bar{X}$ = 10, SD = 0.71) days of continuous monitoring. During a session, latrines were visited every second day and snares and snags were checked closely for hair samples. When hairs were collected, a snare or snag was sanitized with a propane torch.

Genetic analysis

DNA extraction and microsatellite analysis were conducted by Wildlife Genetics International (Nelson, BC, Canada). Genotyping of individual otter from hair samples involved a two-phase process. First, 12 high-quality hair samples collected in 2009 were used to test 17 readily available and common microsatellite loci (i.e., markers) for mustelids (e.g., Davis and Strobeck 1998). For this and subsequent analyses, DNA was extracted using QIAGEN's DNeasy Tissue Kits. Results of this initial screening were insufficient for genotyping: five markers amplified >1 alleles, but the mean heterozygosity was too low for the accurate identification of individuals (mean H_E for 7 markers <0.68; Paetkau 2003; Table 1). We investigated four additional markers published by Beheler et al. (2004, 2005). Although these markers amplified well and were variable, the mean H_E of 0.65 was again low. Whereas 5–7 markers are often sufficient for the identification of individuals, we compensated for low variability by using all nine variable markers (Paetkau 2004).

Table 1 Summary of marker variability (heterozygosity = H_E) for samples of river otter hair collected from central British Columbia, Canada

Marker/locus	Alleles	H_E	n
MP0055	3	0.67	15
MP0114	5	0.75	15
MP0175	1	0	6
MP0197	1	0	6
MP0144	1	0	6
MP0182	1	0	6
MP0059	0	NA	6
MP0273	1	0	6
MP0085	1	0	6
MP0227	5	0.78	15
MP0247	2	0.4	15
MP0263	1	0	6
Lut-604	3	0.63	15
Ma-2	1	0	6
Ma-9	1	0	6
Ma-7	1	0	6
MP0120	0	NA	6
RIO11[a]	3	0.48	15
RIO13[a]	5	0.72	15
RIO07[a]	4	0.57	15
RIO18[a]	6	0.83	15

Analysis was conducted using 23 high-quality samples (~10 guard hair or 30 hairs consisting of under hair). Sample size (n) was number of individuals (i.e., unique multilocus genotypes) identified for each marker. We used nine markers with $H_E > 0$ to identify individual otters. Markers that did not amplify are denoted as having 0 alleles

[a]Published by Beheler et al. (2004, 2005)

During the second phase of the project, we used the nine variable markers (Table 1) to identify individual otters in the remaining and subsequent samples of hair. During genotyping, samples were removed from the analysis if they had low confidence genotype scores for >4 of the nine markers. Marginal samples with inconclusive results were reanalyzed using a greater volume of DNA per reaction. This reanalysis resulted in most marginal samples receiving 9-locus, high confidence genotype scores. Finally, an error-checking procedure was used to re-analyze mismatching markers in similar genotypes (Paetkau 2003; Kendall et al. 2009). Here, data entry and amplification errors were identified and corrected. Following this process, there were no individuals differing on <3 loci suggesting that the probability of identifying a unique, but incorrect genotype was low. Once genotyping was complete, an individual was assigned to each unique multilocus genotype.

We used an additional marker (ZFX/ZFY) to identify the sex of the sampled otter. For individuals identified in the collection of 2009, one sample was selected for this analysis. As a more robust process, all samples from individual otter collected in 2010 were analyzed for sex.

Data analysis

Efficacy: hair sampling

We used a two-sample t test with unequal variances to identify statistical differences in the total number of hair samples (guard hair and under hair) collected using body snares and knaplock hair snags. We assessed the total number of hair samples for each collection instrument, not number of hair samples that were successfully genotyped. We used logistic regression to explore sampling factors related to the successful identification of a high confidence 9-locus genotype for the sampled otter hair. We related the success of genotyping to four variables: number of under hair, represented as a categorical variable incremented in five hair increments, with a minimum of 0 and a maximum of 20 hairs; number of guard hairs with a root; and trap type, body snare (1) or knaplock snag (0). We also fitted a variable for year that ultimately captured seasonal effects as sampling months differed between 2009 and 2010.

We used Akaike's Information Criterion for small sample sizes (AIC_c; Anderson et al. 2000) to identify the most parsimonious logistic regression. The model with the lowest AIC_c score and the highest AIC weight ($AIC_c w_i$) was chosen as the best model to explain the variation in genotype success. We used the area under

the curve (AUC) of the receiver operating characteristic (ROC) to evaluate the predictive ability of the most parsimonious model. Swets (1988) suggested that models with AUC scores between 0.7 and 0.9 have good predictive power and scores >0.9 have high power. We had insufficient sample size to withhold a percentage of the observations for model testing. Thus, we used a bootstrap routine where each record was sequentially removed from the model building process and the withheld record's probability of producing a successful genotype was calculated independent of the training data. We used these independent probabilities to generate the ROC test.

Analysis of abundance

We used the CAPWIRE method (Miller et al. 2005) to generate a population estimate for the otters monitored on Tezzeron Lake. This method is based on an urn model and was developed for the non-systematic collection of DNA. We produced a population estimate with independent (n = 59) and the full set (n = 69) of capture samples. Independent captures did not include multiple hair samples of the same individual at a latrine within a sampling interval. The estimate based on the full set of captures was meant to mimic opportunistic sampling protocols with no temporal boundaries as was the intended use of CAPWIRE. For both data sets, we produced population estimates for the summer (2009) and autumn (2010) sampling periods. Stratification of data recognized an open population across, but not within sampling years. We tested for the simple even capture model and the two innate rates model, where capture heterogeneity occurs between two segments of the population.

Efficacy: analysis of distribution

We used independent data consisting of a set of radiotelemetry locations and geographically referenced genotyped hair samples to generate metrics describing the spatial extent (i.e., distribution) of the monitored otter. These data sets were independent—the telemetered animals were not necessarily represented in the sample of genotyped otter. We assumed that frequent radiotelemetry data would provide a more detailed perspective on the habitat use and extent of distribution of the otter population, but large-scale measures of range size and affinity to latrine sites would be relevant to both techniques. If the distribution of otter was restricted by frequent visitation to latrines or habitats near latrines, then both telemetry and noninvasive DNA sampling should provide similar power to reveal the spatial extent of the monitored population.

We used two analyses to assess the efficacy of hair samples for monitoring the spatial extent of the otter population. First, we used a two-sample t test to compare the average distance of telemetered animals from known latrine sites with sets of random locations. For this analysis, we included only latrine sites and otter locations on Tezzeron Lake or within an area 500 m upland from the shore and the two major tributaries of the lake. The same spatial constraint applied to the calculation of comparison random locations. Because of the small sample size of otter locations relative to the study area, we conducted 500 replicates of the t test including unique sets of random locations for each replicate. As a second analysis, we compared the total area and overlap of the minimum convex polygons of seasonal ranges for individual otter monitored using radio-telemetry and hair sampled at latrine sites. We stratified data by sex and hair collection season and used only those telemetry locations from an equivalent time frame for which we collected hair.

Cost-effectiveness of data collection

We calculated the cost (US dollars) per datum of using radio-telemetry or DNA to monitor otters. Recognizing that expenses would vary depending on objectives, field logistics, and jurisdiction, we identified expenses that would remain consistent across projects. Our calculations provided itemized expenses of major supplies and services, type and volume of data collected for each method, unit cost per datum for each expense, and total cost for each datum.

Sampling intensity and effort was based on our stated methods for trapping and implanting otters with radio transmitters and monitoring a fixed number of latrine sites for hair during five sessions of 9–11 days of continuous monitoring. In the case of fecal sampling, we adopted a monthly sampling interval of all known latrine sites for a total of four collections. Because the otters would continue to defecate, regardless of our sampling schedule, we saw no benefit in matching the sampling periods for the hair and fecal matter. We assumed that the laboratory cost of genotyping a fecal sample was similar to a hair sample, although this was likely conservative. The success rate of identifying a 9-locus genotype for fecal samples was taken from the most recent literature (Mowry et al. 2011). Documenting the location of latrine sites was a fixed and required expense for all three sampling methods, thus we did not include that cost in our calculations. This cost included labor, boat expenses, and field accommodation during the survey of lake shorelines (see "Animal capture, marking and relocation"). Expenses that were variable among techniques included labor, accommodation, field supplies, boat, and sample analysis.

Results

Efficacy: hair sampling

During the summer of 2009 (June 12–August 23), we deployed 35 noninvasive knaplock snag traps and 37 releasable body snares at 15 latrine sites used consis-

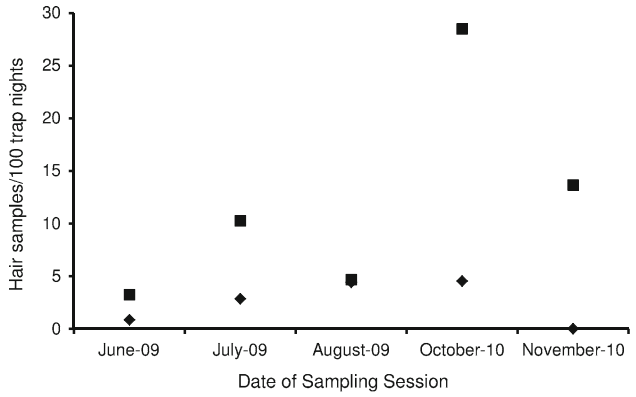

Fig. 2 Total number of guard hair samples (standardized to 100 trap nights) collected from river otters in central British Columbia, Canada, over five trapping sessions using noninvasive knaplock snag traps (*filled diamond*) and body snares (*filled square*)

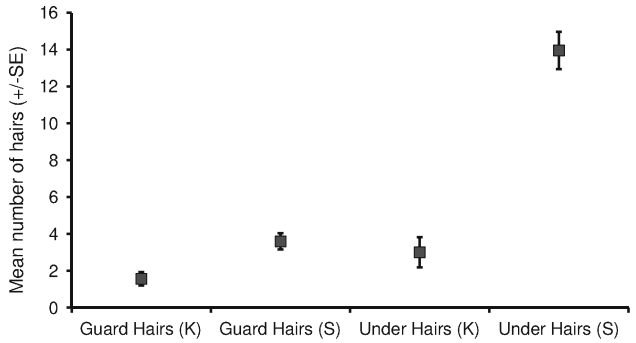

Fig. 3 Mean (±1 SE) number of guard hair and under hair sampled from river otter in central British Columbia, Canada, using body snares (*S*) or knaplock (*K*) snag traps. Statistics represent successful catch events only; estimation of total numbers of under hair per sample was approximate

tently by the river otter. We reduced trapping effort and duration during the autumn of 2010 (September 30–November 3), deploying and monitoring 22 knaplock snag traps and 30 body snares at nine latrine sites. For the 2 years of sampling, traps were monitored for a total of 50 days across five trapping sessions ($n = 3$, summer 2009; $n = 2$ autumn 2010). Body snares were a more efficient instrument for noninvasive sampling of hair from river otter when compared with knaplock traps. For each session, we collected an average of 12.1 (4.52 SE) individual guard hairs per 100 trap nights for body snares (n hairs = 187) and 2.5 (0.92 SE) guard hairs for the knaplock traps (n hairs = 39; Fig. 2). The number of hairs collected at each successful trap was significantly greater for the body snare when considering both guard hair ($t = -3.6$, $df = 72.59$, $p < 0.001$) and under hair ($t = -8.4$, $df = 72.68$, $p < 0.001$; Fig. 3).

During year 2 of the study, we achieved a genotyping success rate of 81.8 %. This was greater than for year 1 of the study (52.5 %) where we submitted trace amounts of hair. We fit six logistic regression models to determine the relationship between genotype success and sample

Table 2 Results of information-theoretic model comparison to identify the most parsimonious logistic regression relating number of hairs and trap type to the probability of identifying a 9-locus high-confidence genotype for individual river otter from a population in central British Columbia, Canada

Model	k	AIC_c	$AIC_c w_i$	AUC
Guard hair + under hair	6	118.1	0.62	0.81 (0.72, 0.90)
Guard hair + under hair + year	7	120.3	0.20	0.81 (0.71, 0.9)
Guard hair	2	149.7	0.00	0.45 (0.34, 0.56)
Under hair	5	139.9	0.00	0.65 (0.54, 0.76)
Trap type	2	121.3	0.12	0.49 (0.35, 0.63)
Trap type + year	3	123.2	0.05	0.61 (0.489, 0.73)

Area under the curve (AUC) and 95 % confidence intervals (brackets) for the receiver operating characteristic (ROC) represents the model's predictive power

type or technique. Although we observed some model selection uncertainty, the most parsimonious model included covariates for number of guard hairs and under hairs (Table 2). Using withheld data to test the model's predictive accuracy, the ROC score (AUC = 0.81) suggested that the combined number of guard and under hairs was a good predictor of developing a 9-locus high confidence genotype for river otter. Predictions from this model suggested that even one guard hair accompanied by >5 under hairs had a >59.0 % probability of producing a genotype. A model that included the number of hairs and year was slightly less parsimonious, but indicated a season effect relative to our trapping efforts. The model with trap type had poor predictive accuracy and was far less parsimonious than the top-ranked model, but a positive coefficient for body snares highlighted the relatively greater effectiveness of this collection instrument.

Analysis of abundance

Noninvasive body snares and knaplock snag traps were an effective method for marking and recapturing river otter at latrine sites. Genotypes identified using hair samples collected in the summer of 2009 and autumn of 2010 resulted in 59 independent captures of 13 male and eight female river otters [2.81 (0.501 SE) recaptures per individual] across both years. A larger number of unique individuals were captured in 2009 (n = 11M:4F) compared to 2010 (n = 2M:4F), although sampling effort was greater during the summer of 2009. Across both years, one individual was recaptured eight times, two individuals seven times, and 61.9 % of the marked otters were recaptured greater than once. Recapture success was slightly higher in the summer [2.53 (0.515 SE) recaptures per individual] relative to the autumn [1.75 (0.218 SE) recaptures per individual] sampling periods. Ten additional captures (total capture n = 69) were the result of multiple hair samples from individuals at a single latrine site within a capture session.

We used CAPWIRE to produce a population estimate with independent and the full set of capture samples (i.e., unique to latrine site and sampling interval, $n = 59$ and $n = 69$). For both data sets, we produced population estimates for the summer (2009) and autumn (2010) sampling periods. For three of the four population estimates, a likelihood ratio test suggested that the two innate rates model was the most appropriate. Given the higher proportion of males in our sample, we assumed that the choice of the multi-strata model was specific to this source of capture heterogeneity. Based on the independent capture data, we generated a population estimate of 19 otters in 2009 (95 % CI = 15–29) and 16 in 2010 (95 % CI = 12–23). The 2010 estimate was generated using the equal catchability model. We generated similar population estimates using the non-independent data with a corresponding larger sample of captures: 18 otters in 2009 (95 % CI = 15–27) and 17 in 2010 (95 % CI = 12–26).

Efficacy: analysis of distribution

We identified 21 individual otters and 69 relocations at latrine sites using genotypes extracted from the DNA in hair. For that same sampling period, we collected 95 relocations from five telemetered otters located on or near Tezzeron Lake. Both data sets suggested that otter have a strong spatial association with latrine sites. On average, we located otters 622.0 m (2.66 SE) from a latrine whereas the median mean distance for the 500 sets of random locations was 10,713 m (61.96 SE). Of the 500 sets of random locations we tested relative to the observed distribution of otter, all produced t tests with statistically significant differences in distances from latrine sites ($t > 2.8$, $p < 0.003$). However, few locations from telemetered otters were collected in the immediate vicinity of latrine sites. Five of 95 locations were found within 100 m of the nearest latrine and 44 of 95 locations occurred within 500 m of a latrine. This is in obvious contrast to genotyped otter with locations collected exclusively at latrine sites.

The spatial extent of otter, as represented by the global and seasonal minimum convex polygon home ranges, was described incompletely when using noninvasive hair samples collected at latrines. The pooled home range of all telemetered otter was 3.3 times the size of the home range inferred using hair samples (Table 3). For three of the four season-by-sex combinations of data, the home range size was larger for male and female otters relocated with radio-telemetry. In the case of female otters during autumn and male otters during summer, home ranges overlapped completely, but this was the result of telemetry-based ranges completely eclipsing the smaller ranges generated using genotypes identified at latrine sites. For male otters during autumn, there was no spatial overlap between the home ranges generated using the two data samples.

Table 3 Area (km^2), number of locations (n), and percentage overlap of composite minimum convex polygons generated for river otter of central British Columbia, Canada, using radiotelemetry locations for five individuals and DNA captures of 21 individuals at latrine sites

Season/sex	Telemetry		Hair snags		% Area overlap
	km^2	N	km^2	n	
Pooled data	221	95	68	69	100
Female					
Summer	70	34	66	16	48
Autumn	62	11	3	11	100
Male					
Summer	196	36	15	26	100
Autumn	5	14	18	16	0

Percentage overlap represents total area of home range generated from hair snag data within the home range of telemetered otter

Cost-effectiveness

Genotyped hair samples were the most cost-effective technique for monitoring otters (Table 4). Based on our sampling effort and associated expenses, we incurred a cost of $168.50 per genotyped hair sample. The cost of genotyping fecal samples was greater by nearly $100.00 per datum. This cost difference was a result of the relatively low success rate (24 %) of amplifying DNA from fecal material. With the exception of the laboratory analysis, fecal sampling was the most affordable technique on a per datum basis when comparing the other cost categories. Capturing and relocating river otter using radio-telemetry was by far the most expensive technique. We calculated an average cost of $2,273.00 to capture and implant a radio transmitter in one otter. Although the cost per relocation ($59.20) was relatively low, when including capture and handling expenses the total cost of conducting radio-telemetry was $264.50 per relocation.

Discussion

In our system, river otters are actively trapped and recognized as a furbearer sensitive to harvest. Historical declines of populations in the United States and Europe (*Lutra lutra*) suggests that otters are sensitive to anthropogenic disturbances and over harvest (Raesly 2001). Fundamental information requirements for population recovery and sound management include a better understanding of: (1) the habitat requirements and foraging ecology of otter; (2) sensitivity to human-caused disturbances and mortality resulting from industrial (e.g., mining, forestry), recreation, and trapping activities; and (3) responses in population distribution and abundance to long-term changes in the composition and functioning of aquatic and terrestrial communities (i.e., habitats). Given the large range of the species, these information gaps can be addressed only through the development of efficient methods that allow the

Table 4 Comparative costs per datum of capturing and relocating (telemetry) river otters and collecting and genotyping hair or fecal material sampled at latrine sites

	Animal capture	Telemetry	Genotype-hair	Genotype-fecal
Expense item				
Veterinarian/day	390.00	NA	NA	NA
Biologist/day[a]	200.00	200.00	200.00	200.00
Field accommodation	50.00	50.00	50.00	50.00
Field supplies[b]	2,597.00	NA	784.00	200.00
Boat expenses/day	50.00	25.00	25.00	25.00
Lab cost/sample	NA	NA	58.00	58.00
Collection effort and data				
No. of field days	19	17	12	25[c]
No. of samples submitted	NA	NA	101	1,210[d]
No. of animals or relocations	12	95	59	290[e]
No. of individuals monitored	9	4	21	>21[f]
Unit cost per datum				
% Data success	26.9/35.9[g]	100.0	81.8	24.0
Veterinary rate	823.30	NA	NA	NA
Biologist rate	844.40	35.80	40.70	17.20
Field accommodation	211.10	8.90	10.20	4.30
Field supplies	288.60	NA	13.30	0.70
Boat expenses	105.60	4.50	5.10	2.20
Lab cost/sample	NA	NA	99.30	241.70[h]
Total $ cost per datum	2,273.00	59.20 (264.50)[i]	168.50	266.00

Costs are approximate, but represent realized expenses of monitoring a population of river otter from central British Columbia, Canada, from 2007 to 2010

Costs per datum refers to the field and lab costs associated with the collection of each animal location

[a]Two biologists required per day for trapping, one biologist per day for all other activities

[b]Field supplies for animal capture included leg-hold traps and radio transmitters; supplies for hair snags included wire, knaplock, and time to construct snags

[c]Assumed collection time of 2 min per sample including travel time

[d]Observed defecation rate recorded for monitored latrines over 2 years

[e]Assumed 24 % amplification rate as reported by Mowry et al. (2011)

[f]Assumed larger number of samples would reveal a greater number of otter relative to hair sampling

[g]Number of trap nights per captured otter was calculated for total number of individuals and total number of individuals implanted with a transmitter and was based on trapping success in this study

[h]Consistent with hair, failed samples (~76 %) would be charged full lab fee

[i]Cost in bracket is cost for each location after including costs of capturing animals

identification and relocation of individuals and the assessment of trends in population size.

Efficacy of sample collection

The method for using body snares to sample hair was previously published (Depue and Ben-David 2007), but we were uncertain about the ability of the method to collect a sufficient number of hairs to amplify DNA. As was reported for other furbearer species (e.g., Boulanger et al. 2004; Sawaya et al. 2011; Stricker et al. 2012), our data suggested that noninvasive hair collection was an efficient and cost-effective technique for monitoring the abundance of river otter with known latrine sites. Much of our success, however, was due to laboratory protocols that allowed for high-confidence genotyping (i.e., 9 loci) of individuals from few hairs per sample. We achieved a reasonable probability of a genotype (>59.0 % probability) with as little as one guard hair and >5 under hairs (Fig. 4).

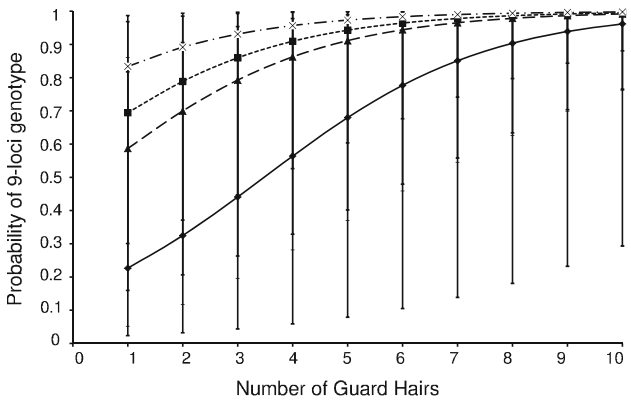

Fig. 4 Probability of successfully identifying a 9-locus genotype for river otter in central British Columbia, Canada, relative to total number of sampled guard hair and under hair. Number of guard hair and under hair (5 = *filled diamond*, 10 = *filled square*, 15 = *filled triangle*, ≥20 = *multiple sign*) were additive as reported for the most parsimonious logistic regression model (Table 2). Median and 95th percentile coefficients and resulting predictions were generated from 2,000 bootstrap replicates

Guard hair is thought to regenerate from August to November (Ben-David et al. 2005), but the timing is unclear for the population that we studied. Thus, seasonal variation in hair growth (i.e., molt) may influence the amount of hair collected and genotype success, including sample contamination. This was not a

confounding factor for this study as the sampling period for each technique was consistent. However, we did find that the body snare provided more samples and a greater number of hairs per sample compared to knaplock snags (Figs. 2, 3, 4). Additionally, the body snare was a single-capture instrument with a low probability of DNA contamination (see Stricker et al. 2012). In comparison, the knaplock snags had the potential to collect hairs from multiple individuals within one trapping session. We did not observe a large number of hairs at these snags, reducing the probability of cross-contamination; the genotyping protocol and associated error testing diagnosed few such samples.

Although the body snare was a more efficient approach, we did increase our capture rate and total sample of marked individuals with the knaplock snags. This instrument was easy to construct, deploy, and monitor. Thus, where sufficient shoreline substrate is available for attachment, we recommend using both techniques in tandem. However, there is a cost to using the less efficient hair collection method. Failed samples provide no data for estimating the abundance or distribution of otter, but they still incur a lab fee.

During year 2 of the study, we achieved a greater genotyping success rate: 81.8 versus 52.5 %. Following the pilot analysis of year 1, we adopted a collection threshold of a minimum of 1 guard hair or >15 under hairs. These amplification results compare favorably to other studies employing similar noninvasive sampling methods. For example, Settlage et al. (2008) reported a success rate of 82.0 % for hair collected from black bear (*Ursus americanus*), Clevenger and Sawaya (2010) successfully genotyped 70.0 % of hair samples from bears (*U. americanus*, *U. arctos*), and Stricker et al. (2012) found that 57.0 % of hair samples collected using a body snare had sufficient DNA to identify species. Contrasting otter hair with the more commonly sampled fecal material, Arrendal et al. (2007), Mowry et al. (2011), and Guertin et al. (2010) achieved a consensus genotype for 31.0, 24.0, and 12.0 % of samples, respectively. When using fecal material, genotype success often was dependent on sample type—fresh, old, or anal jelly—and time of year, with drier or colder periods providing greater preservation of DNA. Our design and analysis was not confounded by this wide range of uncertainty in sampling protocol.

Accounting for assumptions in population estimation

For unbiased population estimates, White et al. (1982) recommended capture probabilities of >0.30 and total captures of >20 when populations are small ($n < 100$). Our capture data suggested that we met those criteria. Using a combination of body snares and knaplock hair snags, we achieved a total rate of independent recaptures of 66.7 % (capture $n = 38$) during the summer of 2009 and 41.7 % (capture $n = 21$) during the shorter trapping season the following year. Applying the program CAPWIRE and the full set of pooled capture data, we generated a population estimate of 18 otters in 2009 (95 % CI = 15–27) and 17 in 2010 (95 % CI = 12–26). Our mark–recapture estimate suggested a density of 1 otter/4.6 km of shoreline. This is similar to otter populations found in northern Idaho (1 otter/3.9 km; Melquist and Hornocker 1983), northeastern Alberta (1 otter/5.7 km; Reid et al. 1987), and south-central Missouri (1 otter/4.2 km; Mowry et al. 2011). Although, density likely varies with habitat quality including broad differences in topography and hydrology such as lake (our study), river (Mowry et al. 2011), valley (Melquist and Hornocker 1983), marshes and wetlands (Helon 2006) or marine systems (Bowyer et al. 1995).

CAPWIRE is designed for small populations, using ad hoc sampling protocols that allow for multiple recaptures of an individual within a single location. This method has been employed with apparent success for unstructured latrine and snow surveys of fecal material from otter (*L. lutra*, *L. canadensis*) (Arrendal et al. 2007; Mowry et al. 2011) and tested using known population numbers for other species (Miller et al. 2005). For our data, CAPWIRE was best parameterized using a model that represented two components of the population with different capture rates. We marked and captured an uneven number of female and male otters likely explaining the rejection of the even capture model. This sex bias could be a result of smaller seasonal ranges, as we observed for radio-telemetered females, or naturally occurring differences in the number of females and males (Mowry et al. 2011). Although CAPWIRE allowed us to use the full set of capture data (i.e., multiple relocations at a latrine within a sampling location), it did not take advantage of our multi-session design across discrete sampling locations. We could, for example, apply the "robust" mark–recapture model to the five sessions of data and calculate a population estimate in addition to other parameters (Kendall et al. 1995).

A lack of population closure is one possible source of bias in our estimates. Otters have large annual and seasonal ranges, especially males, and sizeable linear movements are not uncommon (Reid et al. 1994). Over the course of 13 months of continuous monitoring of telemetered animals in both Tezzeron and Pinchi Lakes, we observed only three large-scale inter-lake movements. Recognizing a possibility of violating the closure assumption across years, we stratified our data into two seasons with relatively short intervals.

A second source of possible bias in our mark–recapture estimates was insufficient spatial coverage of sampling locations. Where animals are not provided with an opportunity to be marked and recaptured, the estimate will be consistently low. This form of capture heterogeneity can be difficult to address for large study areas where sampling is difficult (Settlage et al. 2008). The average minimum distance among our trapped latrines was only 1,843.3 m (SD = 896.6) and telemetry data suggested that otters easily moved among adjacent sites on a daily basis. Finally, attractants, as deployed

for other mustelid species and bears, can increase capture heterogeneity and bias population estimates (Beier et al. 2005; Pauli et al. 2008). Applying body snares and knaplock hair snags at latrines, sites with known and consistent visitation, eliminated the need for bait or lure.

Monitoring distribution

Although the genetic data collected from hair samples was effective at identifying individual otters and generating a mark–recapture population estimate, this source of information had relatively little power to identify the spatial extent and movements of the study population. Telemetry data revealed that otter were spatially associated with latrine sites; however, they frequently moved among latrines and used other areas of their range for activities such as hunting. Furthermore, there was a poor match between the home ranges generated with the locations collected from telemetered otter and hair at latrine sites. Some of this variation may be attributed to the independence of data sets and differences in sampling intensity. A better match between otter distribution and latrine use might be achieved if additional latrines were sampled for hair over a longer period of time. This would not account for long inter-lake movements or forays into upland terrestrial habitats, as we observed (Fig. 1).

Cost-effectiveness of sample protocol

With the exception of sampling efficacy (e.g., Settlage et al. 2008; Clevenger and Sawaya 2010; Ruibal et al. 2010; Sawaya et al. 2011; Stricker et al. 2012), there have been few formal studies documenting the cost-effectiveness of mark–recapture techniques for monitoring the distribution and abundance of medium-sized or large mammals (but see Harrison 2006). We studied multiple aspects of the ecology of river otter allowing a comparison of three methods. When considering the efficacy and cost-effectiveness of fecal samples, we applied the cost for genotyping hair samples and we assumed that amplification success would be similar to the most recent work (Mowry et al. 2011). Although we did not provide a complete three-way comparison among methods, our data strongly suggested that hair sampling was the most cost-effective approach for conducting mark–recapture estimates of otter and other furbearers with similar sampling opportunities. This includes species that are spatially associated with rendezvous sites or dens (e.g., Lucchini et al. 2002; Banks et al. 2003; Frantz et al. 2004; Meijer et al. 2007). Also, hair provides some description of otter distribution, but the efficacy is dependent on the number of latrines monitored and the required detail of range occupancy and movements. Genotyping DNA extracted from fecal samples is more cost-effective if a higher amplification rate is achieved. This is possible through better sample preservation (e.g., winter) or by collecting fecal material with higher quantities of DNA (i.e., anal jelly; Guertin et al. 2010; Mowry et al. 2011). We have some concern about the contamination of DNA in fecal matter, also reducing sample viability (e.g., Pauli et al. 2008). Using wildlife cameras, we observed otters rolling on and possibly mixing fecal samples, multiple individuals defecating in the same location, and other species foraging on fecal matter. This is not a concern when using body snares (Stricker et al. 2012).

The cost per radio-telemetry relocation would be reduced if we monitored otters more frequently or over a longer duration. For this study, we reported the collection of 95 locations over the same time frame that we collected hair. If otters survived for the duration of the battery life in a transmitter, we would expect a much lower cost per sample; including capture cost, three locations per day (as achieved) over 1,598 days (reported battery life) would result in a cost of $220.70 per location. Still, this estimate is greater than the cost of collecting and genotyping hair samples ($168.50/sample) and assumes a best-case scenario with no animal mortality and a frequent relocation interval that is unlikely throughout the year.

Animal and human welfare is an important consideration during sample design. Both fecal and hair sampling are noninvasive. We did not observe latrine abandonment during 2 years of frequent monitoring on Tezzeron and Pinchi lakes. Likewise, video monitoring of body snares suggested that otters experienced little discomfort or aversion to being caught in a snare (see http://www.youtube.com/watch?v=zT48AYW72HY). Alternatively, two captured and implanted otter died prematurely, likely as a result of surgery. Furthermore, otter are large, strong mustelids that are difficult to remove from leg-hold traps and potentially hazardous for field staff.

Conclusions

Genotyped hair samples can be a relatively precise and cost-effective method for monitoring the number of river otters over time and for calculating demographic parameters, contingent on proper sample design. Also, a high recapture rate, as observed for our study area, provides additional cost-free insights on distribution. Sampling DNA from fecal material provides similar data as hair snares and snags, but at a higher cost per sample and the possibility of additional bias related to sample quality and contamination. Also, fecal material is relatively rare at other predictable areas frequented by otter such as movement paths; we trapped such an area on Tezzeron Lake. Animal capture, surgery, and radiotelemetry have relatively high dollar costs and more complex logistics, but these relocations provide a greater resolution for observing fine-scale and infrequent large-scale movements by otter. Ultimately, the choice of method will depend on study objectives. However, our data suggest that sampling hair may be a more

cost-effective and efficient method for monitoring otters compared to the more commonly reported sampling of fecal material opportunistically or at latrines. Where hair sampling is the appropriate method, we recommend the body snare. These snares are easy to deploy in the field and provide a greater number of samples and total number of hairs when compared to the knaplock hair snag.

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