Original Article

Use of Modified Snares to Estimate Bobcat Abundance

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ABSTRACT Although genetic and analytical methods for estimating wildlife abundance have improved rapidly over the last decade, effective methods for collecting hair samples from terrestrial carnivores in a mark–recapture framework have lagged. Hair samples are generally collected using methods that permit sampling of multiple individuals during a single sampling period that can cause genotyping errors due to cross-contamination. We evaluated a modified body snare as a single-sample method to obtain bobcat hair samples suitable for individual identification using DNA analyses to estimate population size. We used a systematic grid (2.5 × 2.5 km) overlaid on a 278.5 km^2 study area in Michigan's Upper Peninsula to distribute sampling effort. In each of 44 grid cells, we placed 2–6 snares at established sampling stations and collected hair samples weekly for 8 weeks during January–March 2010. We collected 230 hair samples overall, with 91% of sampling stations obtaining at least 1 hair sample. Fifty-seven percent of samples had sufficient DNA for species identification, which included bobcat (*Lynx rufus*, *n* = 17); raccoon (*Procyon lotor*, *n* = 62); coyote, dog, or wolf (*Canis* spp., *n* = 29); fox (*Vulpes vulpes* or *Urocyon cinereoargenteus*, *n* = 4); and fisher (*Martes pennanti*, *n* = 1). We identified 8 individual bobcats and using Huggins closed capture population models with a one-half mean maximum distance moved buffer, estimated 10 individuals within the trapping area (95% confidence interval = 8–28) with a density of 3.0 bobcats/100 km^2. Our method provides an effective, single-sample technique for detecting bobcats and estimating abundance. © 2012 The Wildlife Society.

KEY WORDS bobcat, carnivore, DNA, hair snare, *Lynx rufus*, Michigan, mark–recapture, population estimation, semi-passive sampling.

The integration of DNA-based identification of individuals from tissue samples (e.g., hair) and non-invasive detection or capture techniques has markedly improved our ability to monitor wildlife populations. DNA can be extracted from hair follicles and through application of mark–recapture techniques, has been used to estimate population densities for numerous carnivore species including bears (*Ursus* spp.; Mowat and Strobeck 2000, Kendall et al. 2009), river otters (*Lontra canadensis*; DePue and Ben-David 2007), American marten (*Martes americana*; Mowat and Paetkau 2002) and fishers (*M. pennanti*; Williams et al. 2009).

Hair-capturing devices for felids include posts with barbed wire, glue, or brushes (Kendall and McKelvey 2008); which were scented with an olfactory lure such as catnip oil for Canada lynx (*Lynx canadensis*; McDaniel et al. 2000) and fatty-acid discs for bobcats (*L. rufus*; Diefenbach et al. 1994);

intended to elicit a rubbing behavior required for target species to leave hair samples. While these methods have successfully collected hair from target species in areas of high densities (e.g., McDaniel et al. 2000), semi-passive techniques that require elicitation of unique behavioral responses by bobcats (such as rub response; e.g., Harrison 2006, Downey et al. 2007) may decrease detection or capture rates (Comer et al. 2011), which can be problematic in areas of low density.

Another concern with many hair collection techniques is cross-contamination of samples from multiple individuals (Mowat and Strobeck 2000, Mowat and Paetkau 2002). Genotyping samples from traps that do not prevent cross-contamination due to visits by multiple individuals can require use of only one hair in DNA analysis because multiple hairs may be from more than one animal (DePue and Ben-David 2007). However, single hairs may yield insufficient DNA and lead to high genotyping error due to allelic dropout (Roon et al. 2005). To minimize sample cross-contamination, single-sample trap devices have been

designed for mesocarnivores (Belant 2003, Pauli et al. 2008), bears (Beier et al. 2005), and foxes (*Vulpes velox*; Bremner-Harrison et al. 2006), however these devices are generally designed to accommodate only single species or require animals to enter enclosed traps which may exclude some species.

Our goal was to develop and test a semi-passive (i.e., does not require specific behavioral response), inexpensive, single-sample method for collecting hair from bobcats that could be integrated in an experimental framework suitable for population estimation. We selected a modified body-snare design adapted from DePue and Ben-David (2007), originally developed for river otters, as a semi-passive device for collecting hair from bobcats. We evaluated use of this body snare in a capture–recapture framework to obtain hair samples for individual identification and population estimation.

STUDY AREA

We conducted this study in Delta and Menominee counties in Michigan's Upper Peninsula. The area represents diverse forest types with variable topography ranging from upland hardwood forests dominated by maple (*Acer* spp.), birch (*Betula* spp.), and aspen (*Populus* spp.), to low-lying swampy areas containing spruce (*Picea* spp.), eastern hemlock (*Tsuga canadensis*), and other species consistent with boreal coniferous wetlands. The study area was mostly forested (77%) but included intermittent agricultural areas (10%), wetlands (4%), and sparse urban areas (3%). Winter temperatures average about $-7°$ C in December, $-10.9°$ C in January–February and $-2°$ C in March. Mean annual total snowfall ranges from 127 to 254 cm. We obtained climate data from the National Oceanic and Atmospheric Administration (NOAA 2010).

METHODS

Snare Design

We constructed body-snares using 152 cm-long pieces of 2.4-mm diameter 7×7 galvanized aircraft cable following DePue and Ben-David (2007). We cut microstrands about 4 mm in length and pulled the strands outward from the main cable in groups of 2–4, every 2.5 cm along the body-gripping portion of the cable (about 50 cm), forming barbs to snag hair. These snares work similar to conventional snares (Etter and Belant 2011) except, we replaced locks with a size 1 aluminum paper clip, which was able to pass over the barbs and allowed the snare to break away rather than capture the animal (see DePue and Ben-David 2007). This breakaway method prevented multiple visits by different individuals, which eliminated cross-contamination. Cost of material for each snare was about US $1.

Field Methods

We established 44 sampling stations within a 2.5×2.5 km^2 grid cell system overlaid on a portion of the study area, with one station per cell. Sampling stations consisted of several snares surrounding a single bait (described below). Grid cells truncated >50% due to Lake Michigan were combined with adjacent complete cells. This sampling station density meets

Otis et al. (1978) recommendations for ≥4 traps/individual home range for mark–recapture studies, using average bobcat home ranges at this latitude (Fuller et al. 1985, Lovallo and Anderson 1996).

We deployed snares from 12 January–13 March 2010. Although samples collected during summer typically yield more hair due to shedding (Kendall and McKelvey 2008), plucked (i.e., non-shed) hairs collected during winter more frequently have follicles attached and contain greater amounts of DNA (Goossens et al. 1998). Additionally, fewer prey are generally available during winter, which can increase carnivore attraction to bait. To maximize encounters, we placed sampling stations in suitable bobcat winter habitat (Fuller et al. 1985, Lovallo and Anderson 1996, Preuss and Gehring 2007).

We baited stations with vehicle- or hunter-killed white-tailed deer (*Odocoileus virginianus*) or skinned beaver (*Castor canadensis*) carcasses provided by local trappers. We also used commercial lures and animal blood as attractants. We wired baits to a tree to prevent removal by animals. We mixed lures with mineral oil to prevent freezing and applied this mixture to cotton balls to maintain scent (McDaniel et al. 2000). We placed the lure-saturated cotton balls in plastic photo film canisters and hung the canisters about 2.5 m above ground near the bait. We perforated film canisters with 6–7 small holes about 5 mm in diameter to allow transfer of scent. We kept lures consistent within sampling intervals.

Before deploying snares, a 3-week pre-baiting period allowed animals to locate baits and establish trails through the snow to sampling stations. Snares ($n = 161$) were set on these and other traversable trails, using vegetation and woody debris to create barriers to eliminate alternate entry points. We set 2–6 snares within 2–3 m of baits at each site, dependent on the number of trails, with carcass baits generally at the circular center of snares deployed. We set snares with about 20-cm loop diameters with the bottom of the snare 20–25 cm above ground (or packed snow) level (Virchow and Hogeland 1994: Fig. 1). Snares were attached to 45-cm-long
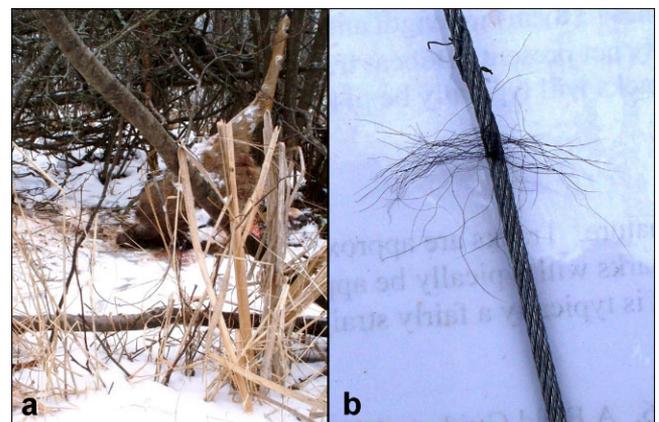

Figure 1. Body snare deployed at bait site (a) modified to snag carnivore hair (b), Upper Peninsula of Michigan, USA, January–March 2010. Galvanized aircraft cable was modified with 4 mm microstrands protruding to form barbs, which snag hair. The original locking mechanism was replaced with a size-1 paper clip to prevent animal capture; snare cable diameter in panel (b) is 2.4 mm.

rebar staked into the ground or attached to a tree, and supported with 9-gauge wire.

We checked hair snares on 7-day intervals for 8 consecutive weeks. We chose this check frequency to reduce DNA degradation due to ultraviolet light and moisture from snow (Kendall and McKelvey 2008). We put individual hair samples (i.e., all hair on a single snare) in paper coin envelopes, which we placed in sealed shipping envelopes with desiccant and sent weekly to the Wildlife Disease Laboratory of the Michigan Department of Natural Resources (MDNR; Lansing, Michigan) for DNA extraction. Before reuse, snares with hair were flame-sterilized in the field using a butane torch. We replaced damaged snares as needed; snares and baits were not moved between sessions although occasionally additional snares were added to sites as new trails developed.

Laboratory Methods

DNA extraction.—We extracted DNA from samples 6–19 days after field collection. We subjected tissue samples (i.e., hair follicles) to overnight proteinase K digestion, followed by manufacturer recommended protocol of the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) with the following modifications to steps 1, 2, and 7: Step 1) For each sample, we clipped all possible hair follicles into the tube using tweezers and scissors; Step 2) The incubation was set at 55° C; Step 7) 65 µl Buffer AE was used and the incubation was 60° C for 5 minutes and elution was not repeated. We quantified purified DNA using a Nanodrop ND-1000 Spectrophotometer (Thermo Scientific, Waltham, MA) and diluted to a concentration of 20 ng/µl for use in polymerase chain reactions (PCR), but did not quantify DNA purified from hair follicles to conserve the small amount of DNA obtained.

Species identification.—We chose the mitochondrial 16S ribosomal RNA (rRNA) gene for species distinction as it is commonly used for carnivores (Hoelzel and Green 1992, Mills et al. 2000, Schwartz and Monfort 2008) and gene sequences for species of interest are available in GenBank. We chose a small but taxonomically informative region of the 16S rRNA gene over other regions of the mitochondrial genome (e.g., the cytochrome oxidate I gene) that is commonly used in DNA Barcoding (e.g., Borisenko et al. 2008) because of expectations of low DNA yield and potentially low DNA quality characteristic of non-invasively sampled DNA sources such as hair (Paetkau 2003). A primer pair (Mam_16SF: 5′-tgaaattgaccttcccgtgaa-3′ and Mam_16SR: 5′-tagggtaacttgttccgttga-3′) was designed for this study based on comparison of carnivore 16S rRNA gene sequences obtained from GenBank, and produces an approximately 250 bp product. We identified differences in sequence among species that allowed target species/groups to be distinguished based on this region of the gene.

We tested primers for amplification using carnivore DNA extracted from tissue samples. We conducted reactions in 25 µl volumes including 2.5 µl of 2 mM dNTP mix, 1.5 µl of 50 mM $MgCl_2$, 1.5 µl of both forward and reverse primers (each at a concentration of 10 pmol/µl), 0.5 unit of ChromaTaq™ DNA polymerase, 5 µl of ChromaTaq™ $5\times$ PCR buffer (Denville Scientific, Metuchen, NJ) and 5 µl of template DNA at 20 ng/µl. Thermocycling conditions were: 2 minutes at 95° C, 35 cycles of 1 minute at 95° C, 1 minute at 57° C, 1 minute at 72° C, and a final extension of 5 minutes at 72° C. We visualized products on a 1% agarose gel using ethidium bromide staining.

We determined gene sequences from both strands by automated fluorescent DNA cycle sequencing using the forward and reverse amplification primers and the Big Dye Terminator kit v3.1 (Applied Biosystems, Foster City, CA), and visualized on an ABI 3730xl DNA Sequencer (Applied Biosystems). We conducted sequencing at the Michigan State University Research Technology Support Facility in East Lansing, Michigan. We inspected chromatograms, aligned sequences to the extent possible by eye, then trimmed sequences using the computer program MEGA4 (Tamura et al. 2007). We obtained about 200 nucleotides of reliable sequence from each sample, and queried sequences against the GenBank nucleotide database using the BLAST algorithm (Altschul et al. 1990) to determine the closest match.

We amplified and sequenced unknown hair DNA samples as described above for the test tissue sample DNAs, with slight modification. We did not quantify the DNA concentration of hair-derived samples; thus, we used 5 µl of a sample containing an unknown quantity of DNA as the template in all PCR amplifications. Also, we increased the number of cycles during PCR amplification of the 16S rRNA gene from 35 to 40 to obtain sufficient product for sequencing. If a clean, readable DNA sequence was obtained, we again compared its sequence against GenBank to ascertain species identity.

Individual identification and population estimation.—We used seven microsatellite primers based on level of polymorphism and quality of product for bobcat: 6HDZ056, 6HDZ057, 6HDZ064, 6HDZ463, 6HDZ610, 6HDZ635, and 6HDZ700 (Williamson et al. 2002). We PCR-amplified bobcat DNA with each primer pair in 10 µl reactions with the following conditions: 2 pmol reverse primer, 2 pmol forward primer, dNTPs at 200 µM each, 200 µM $10\times$ PCR2 buffer (1 mM Tris–HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton X-100), variable amounts of 25 mM $MgCl_2$, and 0.3 units of Taq DNA polymerase. The thermal profile for PCR amplification was 95° C for 3 minutes, followed by 35 cycles of 95° C for 45 seconds, primer specific annealing temperature for 1 minute, and 72° C for 1 minute. We separated amplification products for bobcats on a 6.5% polyacrylamide gel using a LiCor IR^2 DNA Sequencer (NEN™, Lincoln, NE). Fragments were viewed using Saga Generation 2 software (LiCor, Lincoln, NE).

We used a series of quality control protocols to minimize genotyping errors. All genotypes were scored independently by two experienced laboratory personnel. Samples that could not be genotyped at 5 of 7 loci were not used in analysis due to poor quality DNA. For samples where a sufficient number of loci were resolved, we performed an additional PCR

reaction to attempt to resolve missing genotypes. We used program GENECAP (Wilberg and Dreher 2004) to match sample multi-locus profiles and identify sample pairs characterized by 1 and 2 allele mismatches (as per Paetkau 2003, Dreher et al. 2007). Using a modified multi-tube approach (Taberlet et al. 1996), we performed an additional PCR reaction for the locus or loci characterized by a mismatch. Genotypes were scored again by two experienced laboratory personnel. If the mismatch remained, we considered samples to be from different individuals.

We used genetic capture–recapture data to assemble capture histories and estimate population size of bobcats in the study area. Because of the short duration of our study, we considered our study area geographically and demographically closed. Therefore, we used Huggins closed mark–recapture models available in program MARK (White and Burnham 1999) to estimate bobcat population size, including time and behavior as model covariates. We used Akaike Information Criteria adjusted for small samples to select the most parsimonious model (Burnham and Anderson 2002). For the density estimate, we computed effective trapping area using one-half of the mean maximum distance moved (MMDM; Wilson and Anderson 1985), estimated from bobcats captured $\geq$2 times. The MMDM estimation was added to the outer trap polygon as a buffer width and used to calculate bobcat density in the trapping area, expressed as number of bobcats/100 km^2.

RESULTS

Snares were activated on 322 occasions and we collected 230 hair samples from 91% (40 of 44) of stations (Fig. 2). The number of snares visited by animals per sample period increased slightly across weeks, although additional snares were added through week 4 (Fig. 3). Of the 230 samples collected, 76.5% contained follicles for DNA extraction. Of these samples, 91% had sufficient DNA to produce a PCR product and 73% (56% of total samples) produced a sequence that could be identified to species or species group. Hair samples, which produced enough amplified DNA for identification contained 1–30 follicles (mean = 4.5). We identified hair samples as bobcat ($n = 17$); raccoon (*Procyon lotor*, $n = 62$); coyote, dog, or wolf (*Canis* spp., $n = 29$); fox (*Vulpes vulpes* or *Urocyon cinereoargenteus*, $n = 4$); and fisher ($n = 1$). Seven samples were unknown and included possible striped skunk (*Mephitis mephitis*) and rodent samples. Due to cost constraints and depletion of genetic material, we did not analyze canid samples to species or individuals.

We identified 8 individual bobcats from the 17 samples collected, with 6 individuals captured once, 1 individual captured 4 times at three different locations, and 1 individual captured 7 times at the same location. The most parsimonious model included temporal variation in capture rates (M_t) and estimated 10 individuals (95% CI, 8–24) within the trapping area. The calculated one-half MMDM buffer provided an effective trapping area of 330.3 km^2, resulting in an estimated density of 3.0 bobcats/100 km^2.

Initial set-up of each snare site (e.g., arrangement of vegetation, placement and securing bait, placement of snares) required 30–40 minutes. Subsequent checking of snare sites including collecting hair samples, replacing snares, and maintaining bait and lure required about 10 minutes each.
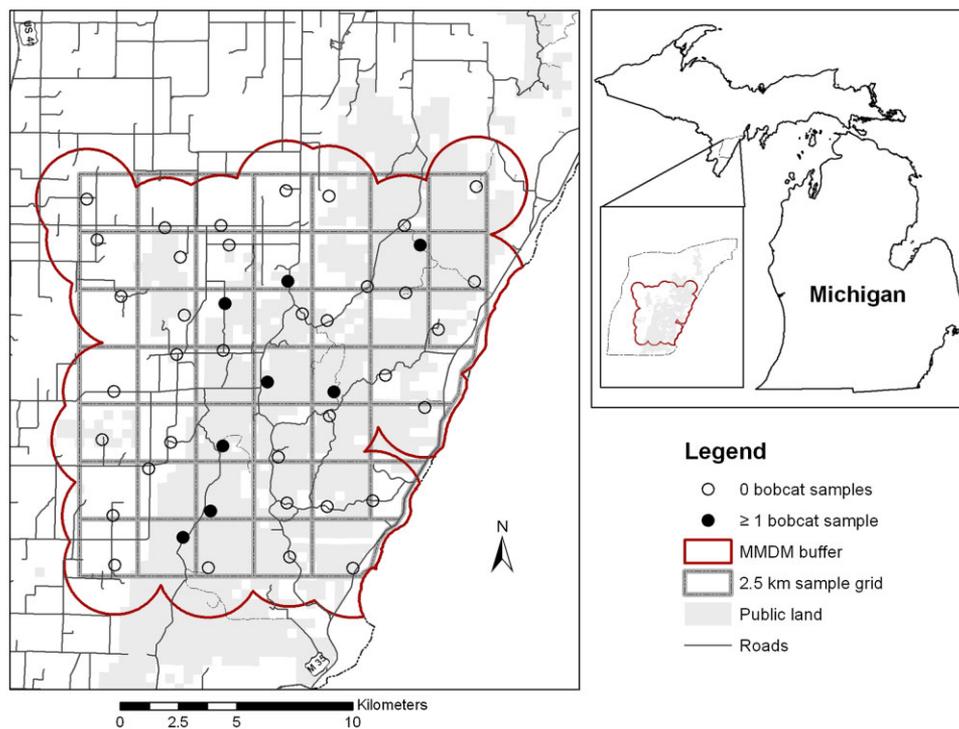

Figure 2. Distribution of 44-hair snare sampling sites within mean maximum distance moved (MMDM) buffer (330.3 km^2 effective sampling area) to estimate bobcat abundance, Upper Peninsula of Michigan, USA, January–March 2010. Solid black circles represent snare sites with $\geq$1 bobcat detection; open circles are sampling sites with no bobcat detections.
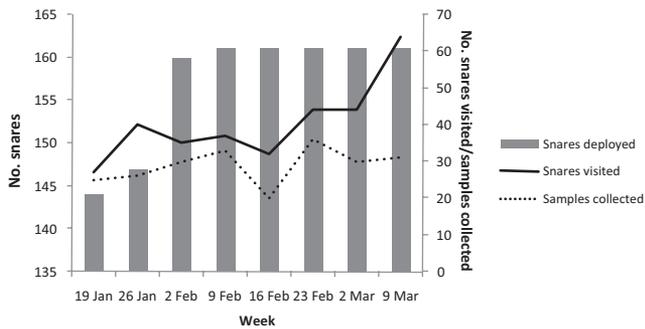
Figure 3. Number of carnivore hair snares deployed, activated (i.e., hair sample collected or disturbed only), and hair samples collected, Upper Peninsula of Michigan, USA, January–March 2010.

DISCUSSION

We obtained sufficient samples of bobcat hair within a short sampling period to estimate abundance and density (3/100 km^2) using genetic-based mark–recapture analysis. We are unaware of other recent estimates of bobcat abundance or density in the western Great Lakes region, which makes direct comparisons difficult, although this region appears to have among the lowest reported bobcat densities in the United States (Anderson 1987). However, our subjective assessment of bobcat abundance based on sign (e.g., tracks, scats) observed in the study area also suggests the population was low. The few studies that successfully used non-invasive or semi-passive techniques to estimate bobcat population size incorporated scat collections in California (Ruell et al. 2009) and camera traps in Texas and Washington (Heilbrun et al. 2006, Larrucea et al. 2007), with reported densities from 25–42 bobcats/100 km^2. Scent stations have been used to document bobcat relative abundance but low visitation rates (e.g., Conner et al. 1983) typically precludes estimates of population size (Diefenbach et al. 1994). Statistical population reconstruction (e.g., Skalski et al. 2011) can be applied to bobcats, but only for harvested populations. Our technique offers an additional tool for estimating bobcat abundance that can be applied to non-harvested populations.

We are uncertain why overall recapture rates were low yet 2 individuals were recaptured frequently. Heterogeneity in captures among individuals may be a consequence of numerous factors including differences in individual behavior (e.g., "trap happy" animals; Phillips and Winchell 2011), or variation in movements among individuals, sexes, or age classes (Bellemain et al. 2005). Also, our low capture and recapture rates may have conservatively biased our abundance estimate and inflated estimates of precision (White et al. 1982). Future studies using this technique could incorporate several measures to increase captures and recaptures including extending the length of study, increasing the density of snares or sampling stations, increasing the frequency of snare checks, and including data from harvested animals if available to provide an additional capture–recapture episode (Dreher et al. 2007).

Efficacy of this technique could be improved by reducing genetic analyses of hair samples from non-target species and the number of activated snares without hair samples. Genetic analysis of non-target species could be reduced by using gross morphology of hairs in conjunction with hair keys or reference collections. Similarly, combining use of hair snares with camera traps or track identification (when conditions permit) to identify non-target species could reduce costs (Zielinski et al. 2006). Snare disturbance by non-target species in our study (e.g., white-tailed deer, corvids) could be reduced by placement of sticks over snares, which would force deer to jump over snares, and setting snares further from baits to reduce activation by corvids.

The number of bobcat hair samples we obtained/unit effort appeared intermediate as compared with previous hair snare studies for *Lynx* spp. (e.g., McKelvey et al. 2006, Long et al. 2007). However, our success rate for identifying species and/or species groups was low relative to other studies. For example, Ruell et al. (2009) identified 77–89% of scats collected to species and 88–90% of bobcat scats were identified to individual. McKelvey et al. (2006) obtained DNA amplification rates of 84% for lynx hair samples from hair snares during summer and 70–86% for lynx hair samples and 94–100% for lynx scat samples collected along lynx trails during winter. Although McKelvey et al. (2006) did not quantify number of hairs collected/sample, increasing the number of hairs collected per sample would undoubtedly increase DNA amplification rates (Goossens et al. 1998). For snares used in this study, increasing the number or length of microstrands on each snare may increase numbers of hairs collected/sample.

Our technique has several advantages over other techniques for collecting hair samples including not requiring elicited behaviors such as rubbing a scent post (e.g., Zielinski et al. 2006) which can limit the number of samples collected (Comer et al. 2011). Also, snare placement does not require animals to enter enclosed spaces as do some enclosed hair collection traps (e.g., Belant 2003, Bremner-Harrison et al. 2006, Pauli et al. 2008), which some individuals or species may be wary of. Finally, our design effectively removed potential for cross-contamination by multiple individuals or species (DePue and Ben-David 2007).

That we detected ≥5 carnivore species suggests that our technique can be adapted to detect and estimate abundance of other carnivore species. For example, snare height above ground and loop diameters can be modified to increase capture rates for other species (e.g., Etter and Belant 2011). Sampling periods can be altered to coincide with periods of increased movements (e.g., breeding season) or vulnerability to detection (e.g., during periods of relative food scarcity). Snare site density (i.e., grid cell size) can also be modified based on the target species' home range size during the period of interest. Finally, baits and lures can be changed to increase site visitation. We have demonstrated the potential of modified snares to collect genetic information suitable for abundance estimation of bobcats. We suggest our approach is applicable to other species and encourage additional refinements to improve overall efficacy.

ACKNOWLEDGMENTS

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