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RINGTAIL (*BASSARISCUS ASTUTUS*) NONINVASIVE SURVEY METHODS, DENSITY, AND OCCUPANCY IN CENTRAL NEW MEXICO, USA

Robert L. Harrison[1]

ABSTRACT.—I tested 3 hair-collection devices used for population surveys of ringtails (*Bassariscus astutus*): PVC pipes, modified cage traps, and triangular Coroplast® tunnels. Coroplast tunnels were the most efficient, with hair obtained by 92% of tunnels tested on radio-collared ringtails. I used the tunnels to survey ringtails on the Sevilleta National Wildlife Refuge in central New Mexico. Probability of detection in areas where ringtails were present was 74% (95% CI 0.56–0.86). Population density was estimated to be 0.17–0.33 ringtails · km^{-2} and occupancy (Ψ) by ringtails was 0.56 (95% CI 0.35–0.75).

RESUMEN.—Examiné tres dispositivos para colectar muestras de pelo que se utilizan en los monitoreos de poblaciones del Cacomixtle Norteño (*Bassariscus astutus*): tubos de PVC, trampas-jaula modificadas y túneles triangulares de *Coroplast*® para roedores. Los túneles de coroplast fueron los más eficientes, ya que se obtuvo pelo en el 92% de los túneles que se examinaron donde se registró la presencia de Cacomixtle Norteño con radio-collares. Utilicé los túneles para monitorear a los cacomixtles norteños en *Sevilleta National Wildlife Refuge*, en la parte central de Nuevo México. La probabilidad de detección en las áreas donde se registró la presencia de Cacomixtle Norteño fue 74% (95% I.C. 0.56 a 0.86). La densidad de la población se estimó entre 0.17 y 0.33 Cacomixtle Norteño/km^2 y la ocupación (Ψ) fue del 0.56 (95% I.C. 0.35 a 0.75).

The distribution and abundance of species are central and fundamental aspects of species' ecological relationships (Krebs 1985). Estimation of population size and density and study of population survey methods are thus often primary objectives of field research and wildlife management (Lancia et al. 2005, Long et al. 2008). Density estimates are particularly valuable because they may be used to estimate population sizes in areas beyond the original study area. However, density estimates are vulnerable to difficulties of accurate estimation of the area surveyed (Foster and Harmsen 2012). Occupancy (the percentage of area occupied by a species) may often be estimated more easily and less expensively than density and offers the advantage of explicitly including detection probability (MacKenzie et al. 2002, 2006). Essential to estimation of both density and occupancy are methods which produce high probability of detections.

A variety of field methods have been developed to detect animals and conduct population surveys. Invasive methods, which require physical contact with the animal, include trapping, reports of hunting and commercial trapping, and road mortality counts. Noninvasive methods, which do not require physical contact, are favored for investigations where the welfare of individual animals is of concern, such as for endangered species, and include track and scat surveys, aerial sightings, hair collection, and automatic cameras (Long et al. 2008). Noninvasive survey methods may be less expensive than invasive methods (Barea-Azcón et al. 2007). Although more than one species may be surveyed simultaneously with a single survey technique (such as scat surveys), a given technique is not likely to be optimal for all species detected (Gompper et al. 2006). Also, the probability of detection, which is explicit in the calculation of abundance and occupancy, varies with species and survey method. Thus, to obtain the best possible estimation of population size, density, and occupancy for a given species, an investigation of survey techniques designed for that specific species may be required.

Ringtails (*Bassariscus astutus*) are small (1 kg) members of the family Procyonidae that occur from southern Mexico through the southwestern United States and Texas northward to southern Kansas, Colorado, and Oregon (Hall 1981). Previous estimates of population sizes

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and densities of ringtails have been obtained from trapping either with or without marking and recapture (Grinnell et al. 1937, Toweill and Teer 1980, Belluomini 1983, Lacy 1983) or from subjective estimates by trappers (Grinnell et al. 1937, Taylor 1954). Home-range sizes obtained from radiotelemetry have been used as relative indices of population density (Lacy 1983). Noninvasive methods have not been used to survey ringtail populations, but ringtails deposit scats in conspicuous locations, such as outcrops, and often create latrines (Trapp 1973, Barja and List 2006), suggesting that scat surveys may be useful in determining ringtail presence or in generating an index of relative density (Heinemeyer et al. 2008). However, to generate an abundance estimate from scats, DNA analysis is required, and it is necessary that scats be collected when very fresh (Murphy et al. 2007). In the arid climate of New Mexico, scats dehydrate quickly. Discriminating between fresh and older scats in the field can be a problem. To obtain fresh scats often requires 2 visits to the study site, the first to remove or mark older scats and the second to collect fresh scats. Thus, the cost of surveying by scat collection is increased. Additional problems of using scats for abundance analyses include damage by mold, confusion of DNA of the target species with DNA of prey within scats, misidentification of scats in the field, and chemical inhibitors within scats (Heinemeyer et al. 2008, Schwartz and Monfort 2008).

DNA extracted from hair follicles often yields better analysis success rates than DNA from scats (Schwartz and Monfort 2008), suggesting that hair snares may be useful survey devices for ringtails when individual identification is required. Zielinski et al. (2006) observed that ringtails entered enclosures with track plates and hair snares intended for fishers (*Martes pennanti*) and martens (*Martes americana*). Ringtails are similar in size to martens, suggesting that noninvasive survey devices developed to collect hair from martens may be useful for ringtails (Foran et al. 1997, Belant 2003, Pauli et al. 2008).

Foran et al. (1997) surveyed martens using hair snares that consisted of 2 boards attached to a tree to create a small triangular tunnel open at each end. Bait was attached in the center of the tunnel. Glue patches placed between the entrances and the bait collected hair. Belant (2003) designed a survey device for martens that consisted of a cage-type trap with the locking mechanism of the trap disabled. He attached currycombs to the lower edge of the door so that when an animal entered, sprang the trap, and left, hair was collected on the combs. Pauli et al. (2008) surveyed martens using PVC pipes that were open on one end and that contained metal brushes on the inside to collect hair. When a marten entered a tunnel and seized the bait, a door closed. The marten was able to exit through the door, but no additional animals could enter.

In order to develop a population survey method for ringtails, I tested 3 hair-collecting survey devices based on the designs of Foran et al. (1997), Belant (2003), and Pauli et al. (2008) in an area with free-ranging, radio-collared ringtails. To test and demonstrate the utility of the most efficient device, I surveyed the ringtail population on the Sevilleta National Wildlife Refuge in central New Mexico, generating estimates of population density and occupancy (percentage of area occupied). I also conducted a scat survey for comparison.

STUDY AREA

The study was conducted in the Los Pinos Range of the Sevilleta National Wildlife Refuge, Socorro County, in central New Mexico (Fig. 1). Habitat was primarily juniper (*Juniperus monosperma*) savanna and piñon-juniper (*Pinus edulis–J. monosperma*) woodland (Dick-Peddie 1993; see also http://sev.lternet.edu [research sites Goat Draw and Cerro Montoso]). Dominant grasses were grama grasses *Bouteloua eriopoda, Bouteloua gracilis, Bouteloua curtipendula,* and *Bouteloua hirsuta.* Dominant shrubs were mountain mahogany (*Cercocarpus montanus*), scrub live oak (*Quercus turbinella*), gray oak (*Quercus grisea*), and Apache plume (*Fallugia paradoxa*). Two cactus species, purple-fruited prickly pear (*Opuntia phaeacantha*) and candelabra cholla (*Cylindropuntia imbricata*), were common. Elevations were 1900–2300 m. Topography was hilly and generally very rocky, with many cliffs, outcroppings, talus slopes, and gravel fields. From 2001 to 2010, mean monthly maximum and minimum temperatures were 19.8 °C and 6.1 °C, respectively, and mean annual

Fig. 1. Location of Sevilleta National Wildlife Refuge, New Mexico.

precipitation was 318 mm (D. Moore, Sevilleta Long Term Ecological Research, personal communication).

METHODS

I tested 3 survey devices: Coroplast® tunnels, modified cage traps, and PVC tubes. Coroplast tunnels were based on the design of Foran et al. (1997) and consisted of a tunnel that in cross section was an equilateral triangle. Tunnels were made of Coroplast plastic (Coroplast, Dallas, TX) and were 12 or 14.5 cm wide by 60 cm long and open on both ends. In the center of the tunnel, I attached a 9 × 12.5-cm plastic pastry dish to the floor of the tunnel with a 2 × 2-cm velcro patch. On each side of the ceiling of each end of the Coroplast tunnels, I used thumbtacks to attach one 2 × 10-cm strip of glue paper (Tomcat® glue board, Motomco, Clearwater, FL) one-third of the distance between the entrance and the pastry dish, for a total of 4 strips. I placed raisins in the dish and at the entrances to the tunnels. Each tunnel was cut from a single piece of Coroplast plastic and folded to create a tunnel. Sides of the tunnel were held in place with two 2 × 8-cm strips of velcro tape. Coroplast tunnels did not prevent more than one animal from entering.

Modified cage traps were based on Belant (2003) and consisted of collapsible 17 × 17 × 49-cm traps (Tomahawk Live Traps, Hazelhurst, WI), with the locking mechanism of the door rendered inoperative via cable ties. At the lower edge of the door, I attached metal brushes (#1610, Papa John's Toolbox, Brooklyn, NY) or a 1.7 × 1.7 × 14-cm wooden bar to which 1.5 × 14-cm strips of glue paper were attached. Traps were baited with chicken wings, sardines, or raisins. In a previous study that used these baits (Harrison 2012), I found that type of bait did not affect success of trapping ringtails. Therefore I did not investigate type of bait as a factor affecting the efficiency of survey devices.

PVC tubes followed the design of Pauli et al. (2008) and consisted of PVC sewer pipe tubes that were 10 cm in diameter and 35 or 60 cm in length. Two cylindrical metal brushes were inserted diagonally in each tube. One end of the tube had a door that closed after an animal entered and attempted to remove the bait (a chicken wing). The door-release mechanism required that the bait be tied to fishing line; thus, it was not possible to use soft baits such as sardines. The other end of the tube was covered with either hardware cloth or a cap. The latter allowed an animal to see the bait but not reach it. I secured PVC tubes and Coroplast tunnels to the ground with stakes and wire, or with rocks. I found sufficiently high success rates with Coroplast tunnels (see results) and so did not test other possible configurations of the 3 devices, such as using glue strips in PVC tunnels.

I tested the devices by placing them within 0.5 m of occupied dens of radio-collared ringtails and inspecting the devices for hair the following day. I assumed that all collected hair was from ringtails, and I did not otherwise identify the species of origin during tests of survey devices at ringtail dens. I evaluated the efficacy of each device by the percentage of trials in which hair was collected.

After selecting 12-cm Coroplast tunnels as the most efficient survey device, I surveyed the Los Pinos Range of the Sevilleta National Wildlife Refuge for ringtails by placing tunnels in 22 transects of 4 tunnels each. I located transects along cliffs and rocky hillsides where I expected ringtail activity to be greatest. Average transect length was 780 m (range 432–1416 m). Average distance between tunnels was 260 m (range 88–959 m). I attempted to place tunnels near ringtail latrines or on outcrops. I baited the tunnels with raisins and placed 2 cm^3 of an olfactory lure (Big Stinky, A.M. Grawe, Wahpeton, ND) near the tunnel. I returned to each transect once per week for 3 weeks to collect hair and replace glue strips, bait, and lure. I also searched for ringtail scats while visiting transects. I identified ringtail scat by size (4–14 mm maximum diameter) and location (on ledges or prominent rocks). In a previous study, I confirmed this method of ringtail scat identification with mitochondrial DNA analysis (Harrison 2012). Fieldwork was conducted between November 2010 and April 2011.

Glue strips containing hair samples collected during the survey portion of this research were covered with Rite-in-the-Rain® paper and placed in paper envelopes. DNA was extracted using QIAGEN DNeasy Tissue kits (Qiagen, Valencia, CA) following the manufacturer's instructions. Guard hair roots (#10) were used when available. When underfur hairs were used, entire clumps of whole underfur were extracted rather than individual roots. Solvent (Goo Gone®, Magic American Products, Bedford Heights, OH) was applied to 32 of 148 samples to release the hairs from the adhesive. Success rates are not compromised when solvent is required. Sample quality was excellent, with an average of 7.5 guard hairs per extracted sample (treating each underfur clump as equivalent to 0.2 guard hairs). A mtDNA species test was performed on 148 DNA extracts. The species test was a sequence-based analysis of the mitochondrial 16S rRNA gene. Selection of primers and analysis conditions followed Johnson and O'Brien (1997). Six microsatellite markers that amplified well in ringtails were identified: 2 mustelid markers (*MP114, Mvis72*), 3 ursid markers (*G10C*, *G1D*, *G10U*), and 1 canid marker (*CXX20*). Error-checking followed the protocol of Paetkau (2003). Data for mismatching markers were confirmed to rule out genotyping error. Hair samples that contained 3 or more alleles at an individual marker were designated as originating from a mixed sample of more than one ringtail. A mixed sample was assigned to a previously identified individual if an identified ringtail had been observed at that site during the weeks before and after the sample in question. This procedure potentially produces a conservative estimate of the number of ringtails present.

I estimated the population size of ringtails represented by the survey sample with program CAPWIRE (Miller et al. 2005). CAPWIRE was chosen because of its superior performance at small sample sizes (Miller et al. 2005). To determine density, the effective survey area must be estimated. For geographically open populations, such as the ringtail population in this study, effective survey area is typically determined by buffering transects with a distance based on observed animal movements. Buffer distances are either 100% or 50% of the average distance between observations of the same individual at multiple sites (e.g., mean maximum distance moved) or 100% or 50% of the diameter of an assumed circular home range of average size as observed by radiotelemetry (Foster and Harmsen 2012). At present, the most appropriate method has not been determined (Foster and Harmsen 2012). Because the number of tunnels available to an individual ringtail was small (4 per transect), I estimated the size of the area sampled by buffering the transect survey routes with buffers of 2000 m and 1000 m. These buffers equal approximately 100% and 50%, respectively, of the diameter of an assumed circular home range equal in size to the average home-range of 300 ha. This average size was determined by radiotelemetry in the same area (Harrison 2012). I deleted areas of unsuitable ringtail habitat, such as open grassland or barren ground, from the area estimate. I used program PRESENCE (MacKenzie et al. 2003) to estimate occupancy (proportion of total area surveyed that was occupied by ringtails, Ψ) and probability of detection (p) of ringtails in the area surveyed, with individual transects as the sample units.

RESULTS

During initial tests of survey devices, hair was obtained by 6% of PVC tubes ($n = 8$ tubes with caps, $n = 9$ tubes with hardware cloth), 23% of modified traps with brushes ($n = 13$), 41% of modified traps with glue paper ($n = 17$), 89% of 14.5-cm Coroplast tunnels ($n = 9$), and 100% of 12-cm Coroplast tunnels ($n = 3$). Because gray foxes (*Urocyon cinereoargenteus*) are common on the study area and small enough to enter the larger tunnels but not the smaller tunnels, I selected 12-cm Coroplast tunnels for the study area survey.

A total of 148 hair samples were obtained from the survey, including 66 ringtails, 2 mixed ringtails and other species, 44 rock squirrels (*Spermophilus variegatus*), 28 woodrats (*Neotoma* spp.), 1 gray fox, 1 brush mouse (*Peromyscus boylii*), 1 piñon mouse (*Peromyscus truei*), and 5 unknown. High-confidence data scores (Paetkau 2003) were obtained for all 6 markers for 61 ringtail samples. Nineteen individual ringtails were identified. Mean observed heterozygosity across 6 markers and 19 individuals was 0.78, which is sufficient to ensure a very low match probability (Paetkau 2003). Five ringtail hair samples were assigned to previously identified individuals on the basis of location and known visits to tunnels at the same sites before and after the hair samples were obtained. Under these circumstances, it is unlikely that the number of individuals identified was inflated through undetected genotyping error (Kendall et al. 2009).

CAPWIRE chose the two innate rates model (TIRM), which assumes heterogeneity of trap response, and estimated the population to be 21 ringtails (95% CI 19–25). The area surveyed was estimated to be 124.6 km^2 with a 2000-m buffer and 63.5 km^2 with a 1000-m buffer. Population density within the surveyed area was estimated to be 0.17 ringtails · km^{-2} (95% CI 0.15–0.20 ringtails · km^{-2}) with a 2000-m buffer or 0.33 ringtails · km^{-2} (95% CI 0.30–0.39 ringtails · km^{-2}) with a 1000-m buffer.

PRESENCE did not reject the null hypothesis that detection rate was constant between transects ($\chi^2 = 0.86$, $v = 2$, $P = 0.662$). PRESENCE estimated occupancy (Ψ) of ringtails in the area surveyed to be 0.56 (95% CI 0.35–0.75) and probability of detection (p) to be 0.74 (95% CI 0.56–0.86).

Confirmed ringtail hair was obtained on 12 transects. I found ringtail scats on 15 transects, including all 12 transects where ringtail hair was obtained. Latrines of ≥2 scats were found on 13 transects, including 11 transects where ringtail hair was obtained.

DISCUSSION

Coroplast® tunnels were effective survey devices for ringtails. They collected ringtail hair more efficiently than modified cage traps or PVC tubes, produced a high probability of detection, and enabled estimates of population size, density, and occupancy. No previous study of survey methods designed specifically for ringtails is available for comparison, but comparison may be made with studies of survey methods for martens (Mowat and Paetkau 2002, Belant 2003, Zielinski et al. 2006, Pauli et al. 2008). Mowat and Paetkau (2002) used wooden triangular tunnels (Foran et al. 1997) to survey martens and reported that 92% of sites visited by martens collected hair. The detection rate determined by Foran et al. (1997) was similar to that found in this study with Coroplast tunnels. Belant (2003) reported that 100% of triggering events in cage traps with curry combs produced adequate hair. Pauli et al. (2008) reported that all 5 captive martens tested entered PVC tubes and left hair on brushes and that PVC tubes successfully collected hair in the field. Both Belant (2003) and Pauli et al. (2008) reported greater success for their devices than found in this study. The reasons for this difference are not obvious. Ringtails readily enter cage traps (Harrison 2012), but adequate hair was collected in <50% of tests. Martens, which generally occur at higher elevations and latitudes than ringtails, may have thicker fur, thus enabling them to leave more hair on brushes. The reluctance of ringtails to enter PVC tubes is peculiar, as they are carnivorous and often use small rock crevices for denning (Gehrt 2003). I speculate that martens may be more aggressive than ringtails and more willing to enter small or unfamiliar spaces to acquire food. In a study of devices designed for martens and fishers, but which also detected ringtails, gray foxes, and western spotted skunks (*Spilogale gracilis*), Zielinski et al. (2006) reported that 3 ringtails that entered the devices left hair on glue strips and 2 of 4 entering ringtails left hair on barbed wire. Only 1 of the 7 events resulted in sufficient hair for DNA analysis. Zielinski et al. (2006) concluded that, in general, glue strips were superior to barbed wire in their devices. This conclusion is similar to results here that glue strips collected ringtail hair more frequently in modified cage traps than brushes did.

The use of Coroplast tunnels to survey ringtails is a significant improvement over methods used previously for ringtails. Previous methods involved trapping (Grinnell et al.

1937, Taylor 1954, Toweill and Teer 1980, Belluomini 1983, Lacy 1983). Because tunnels are noninvasive, there is no risk of harm to the animal, and unlike traps, tunnels do not need to be checked daily. They are lightweight, easy to transport, and may be assembled in the field, All 3 of these factors are especially significant where travel by foot is necessary to reach cliffs and other rocky, roadless habitats favored by ringtails (Poglayen-Neuwall and Toweill 1988). Tunnels used for previous marten surveys have been made of wood (Foran et al. 1997, Williams et al. 2009). Materials for Coroplast tunnels cost approximately $7 per tunnel, and tunnels may be reused. Cage traps of the size used in this study cost $38 (Tomahawk Live Traps, Hazelhurst, WI), and PVC tube assemblies cost $5 (Pauli et al. 2008). DNA extraction and species identification cost $24.25 per hair sample, plus hair extraction from glue at $3 per sample for 32 of 148 hair samples. Marker selection and microsatellite genotyping cost $4476 for 68 hair samples. Per sample costs decrease as the number of samples increases. Collected hair was adequate for species identification for 100% of hair samples and was adequate for individual identification for 89.7% of ringtail hair samples. Marten genotyping success reported by Pauli et al. (2008) and Williams et al. (2009) was 80% and 96%, respectively. Sample quality in this study was excellent, in part because the design of the tunnels prevented precipitation from reaching hair samples (D. Paetkau, personal communication). Probability of detection was good, but no comparisons with other hair collection survey devices for ringtails are available. One disadvantage of Coroplast tunnels is that hair samples may originate from more than one ringtail. In this study, 5 of 66 samples were mixed.

For a simple presence/nondetection survey, observation of scats in this study would have performed as well as or better than the hair-snare survey (Long and Zielinski 2008). Scats, both individually and in latrines, were found on all transects where hair was collected, plus 3 additional transects. Lynch et al. (2006) reported that scat surveys functioned as well as spring-loaded hair snares for surveying for the presence of European martens (*Martes martes*). However, DNA analysis is often required to verify species of scat origin (Davison et al. 2002). To confirm species identification from scat, Wildlife Genetics International charges $150 for project setup, plus $22.95 per scat. Because ringtails are readily attracted to lures and bait (Harrison 2012), automatic cameras may be useful presence/nondetection survey devices for ringtails (Kays and Slauson 2008). Cameras would not be useful for estimating population size because ringtails are small and lack obvious features to distinguish individuals. Cameras offer the advantage of recording many different species simultaneously. Other noninvasive survey methods, such as spotlighting, track searches, and track (scent) stations (Long et al. 2008), would be impractical in the steep, rocky habitats occupied by ringtails in this study.

Population density of ringtails calculated in this study should be regarded as conservative. Home ranges of ringtails in the study area are relatively large compared to other areas (Harrison 2012), which resulted in a large buffer around transects and thus a large estimate for the area surveyed. The density estimate reported here is among the lowest recorded. Other available density estimates range from 0.1 to 20.5 ringtails $\cdot$ km^{-2} (Grinnell et al. 1937, Toweill and Teer 1980, Belluomini 1983).

Differences of estimated density between this study and previous studies may be due to differences of habitat, methods, or both. Differences of habitat are likely significant factors. The Sevilleta National Wildlife Refuge study area is more arid than any of the previous study areas, and home ranges of other carnivore species in arid New Mexico are among the largest reported for their species (bobcats [*Lynx rufus*]—Harrison 2010; swift foxes [*Vulpes velox*]—Harrison 2003). In a previous study (Harrison 2012), I trapped ringtails in box traps on 3.4% of trap-nights, not including recaptures, and 8.4% of nights, including recaptures. In this study, I collected ringtail hair from 7.2% of tunnel-weeks, not including recaptures, and 25.0% of tunnel-weeks, including recaptures. A comparison of these rates is not entirely fair, as traps must be visited daily, in contrast to tunnels, which can be visited weekly or over other periods at the convenience of the investigator. A longer period between visits yields more opportunities for animals to be detected between visits. Shorter periods allow baits to be replaced more frequently, decreasing the time that survey devices sit inoperative after bait has been taken.

As measured over the intervals between visits in this study, tunnels were more efficient than traps at detecting ringtails. Tunnels also did not pose the risk of injury and stress to animals as does trapping.

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