FINAL PERFORMANCE REPORT

Federal Aid Grant No. F17AF01257 (W-191-R-1)

Status and Trends in Bobcat Populations

Oklahoma Department of Wildlife Conservation

Grant Period: January 1, 2018 – March 31, 2023

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Graduate Student Investigator: Nathan Proudman

EXECUTIVE SUMMARY:
Bobcats (*Lynx rufus*) are an important resource in Oklahoma as one of the most harvested furbearer species in the state and due to the ecological and environmental services they provide as predators of small mammals. However, their secretive nature makes them difficult to monitor with, for example, roadside surveys, and data from fur harvest may be biased with respect to where bobcats are harvested and factors such as weather during trapping season and fur prices. We conducted a study testing the use of hair snares and occupancy modeling as a method to assess bobcat population trends across the state, and the use of hair snares with genetic capture-recapture methods to estimate bobcat population sizes in three Wildlife Management Areas (WMAs) representing different ecoregions in Oklahoma. We used camera traps on the WMAs to assess the efficacy of the hair snares in detecting bobcats on the WMAs. In addition, we used small mammal trapping to ascertain the relationship between prey abundance and bobcat density among the WMAs.

While the hair snare cubbies were successful in sampling mammal hair, with 25-28% identified as bobcat using genetic analyses, overall accuracy of morphological identification of hairs was low when compared against genetic analysis of the same samples. For this reason, hair snares as a stand-alone method for monitoring bobcat populations without genetic backup may not be useful without highly trained technicians (forensic or museum professionals). Camera trap methods involving a platform for images from trail cameras submitted by the public, as well as targeted camera-trap placements by agency personnel, combined with occupancy modeling might be a better method for monitoring bobcats independent of harvest data. However, based on conservative identifications of snared bobcat hair and occupancy modeling we were able to develop a probability of occupancy map for most of the state. Because the standard error was high, particularly in two regions of the state, likely due to insufficient resampling and bobcat detections, occupancy predictions should be used with caution. The lack of cubby placements within certain regions of the state means those parts of the map are particularly less supported in terms of predictions of bobcat occupancy.

Due to small sample sizes and low success rate of genetic analyses of hair and scat samples from the WMAs, genetic capture-recapture was not a viable method for estimating bobcat population sizes in these intensive study areas. However, we were able to estimate bobcat population densities on the WMAs with capture-recapture analysis using the trail cameras set up to assess efficacy of hair snares for capturing bobcat hair. We identified individual bobcats based on unique natural pelage patterns. Using this method, we estimated bobcat density at James Collins WMA to be 0.33 bobcats/km^2, at Packsaddle 0.14 bobcats/km^2, and 0.02

bobcats/km^2 at Sandy Sanders. These densities fall within the range of previously estimated bobcat densities in different parts of Texas.

While small mammal abundance was highest at Sandy Sanders WMA and lowest at James Collins WMA, differences in small mammal abundance were not statistically significant. Both species richness and Shannon diversity were significantly higher at Packsaddle WMA. Activity patterns of bobcats and coyotes, based on camera trap data, indicate that bobcats and coyotes are not avoiding potential competition by being active at different times, especially on James Collins WMA in eastern Oklahoma where overlap in activity periods was >90%. Overlap in activity periods between bobcats and coyotes was higher than between bobcats and lagomorphs (64% - 77%) on all three WMAs. Overlap of coyote and lagomorph activity patterns on Packsaddle and Sandy Sanders WMAs was higher than overlap of bobcat and lagomorph activity patterns, suggesting that coyotes may be focusing more on lagomorphs as prey than are bobcats.

BACKGROUND:

Bobcats (*Lynx rufus*) are one of most-harvested furbearers during the trapping season in Oklahoma, and when fur prices are good, can represent a significant source of additional income for trappers. In addition, bobcats can be important predators of small mammals, assisting in control of rodent and rabbit populations. Despite being a valued resource, the secretive nature of bobcats and their widespread distribution across the state make it difficult to monitor populations. Between 1977 and 1981, the Oklahoma Department of Wildlife Conservation (ODWC) conducted scent station surveys in 59-77 counties in Oklahoma for 2 nights in August of each year (Rolley 1985). These surveys suggested declining population trends for bobcats statewide. Currently, bobcat populations are monitored in 2 ways in Oklahoma – with roadside surveys and fur sales. Roadside surveys in 2008-2017 suggested declining bobcat numbers in the state (Fig. 1), but roadside surveys may not be ideal for monitoring population trends in a secretive species like bobcats. Fur sales, on the other hand, may reflect fur prices more than bobcat population status because hunters vary their effort based on potential for economic gain.

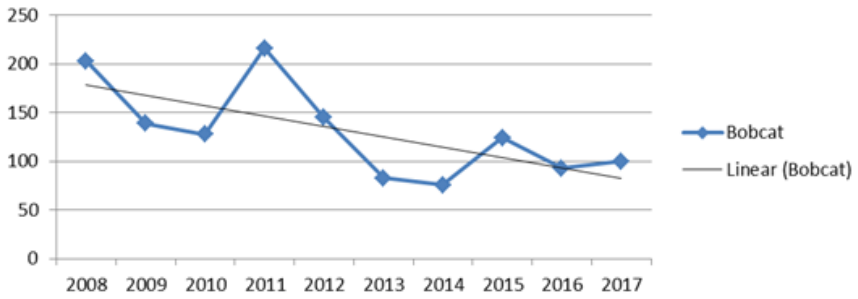

Figure 1. Number of bobcats recorded during Annual Roadside Survey in Oklahoma, 2018-2017 (J.Davis, ODWC).

A more accurate method of monitoring bobcats on a large spatial scale, independent of fur prices, would assist in management of bobcats in Oklahoma. Occupancy modeling is an approach that allows relatively rapid collection of presence/absence data of a species over a large area (MacKenzie et al. 2002, Long et al. 2011). The method provides information on distribution and trends in species occurrence in different parts of the state, while taking into

account differences in detectability. The latter is particularly important for a species such as bobcats, which occur in a wide variety of habitats. Inexpensive, non-invasive methods such as hair snares can be used to determine presence/absence of bobcats over a large spatial scale. An advantage to using hair snares rather than camera traps is that, if individuals can be genetically identified with DNA extracted from hair follicles, the data can be used in genetic capture-recapture analyses to estimate population size. While species with variable markings, such as bobcats, can be identified visually, pictures from camera traps may not have the correct angle or be clear enough for individual identification, especially in low light conditions.

In this study, we used hair snare cubbies and occupancy modeling at a large scale to assess trends in bobcat populations state-wide. This involved undergraduate volunteers from Oklahoma State University and other Oklahoma colleges and universities setting up hair snare cubbies in their home counties for 3 weeks over the winter break and morphological identification of mammal hairs to species at OSU. On James Collins, Sandy Sanders, and Packsaddle WMAs, more intensive studies were conducted to estimate population sizes using hair snare cubbies and genetic capture-recapture methods. We also trapped small mammals on the three WMAs and measured other potential habitat features as covariates to explain differences in bobcat populations across the WMAs. Genetic results and camera trapping were used to assess the efficacy of the hair snares as a noninvasive method to assess bobcat population trends and population ecology of bobcats across the state and among three ecoregions. Management of bobcats will be supported by knowledge generated from this study concerning the status and population trends, as well as monitoring methods, in the species in Oklahoma.

OBJECTIVES:

Objective 1: To assess trends in bobcat populations across Oklahoma using non-invasive detection methods and occupancy modeling. Presence/absence by location will be provided to the ODWC in performance reports.

Objective 2: To estimate bobcat density, using intensive sampling with hair snares and genetic mark-recapture methods, in 3 areas of particular interest in different ecoregions of Oklahoma.

METHODS:

Hair Snare Design

The project built upon the original hair-snare cubby design from West Virginia (Rounsville 2018), with some minor modifications, including decreased cubby length. Cubbies were made from corrugated plastic sheeting, folded and anchored to the ground with four tent stakes (Fig. 2). Two pairs of 30-caliber rifle bore brush pairs were fixed at each entrance of the cubby, at alternating heights and angled slightly inwards, to snag hair from investigating bobcats. Bobcats were lured to cubbies by a combination of scent lure ("Dixie cat" food-gland mix [Okie Cable and Trap Supply, Crowder, OK]) applied to a carpet square in the cubby interior, and curiosity lures (such as feathers, Christmas baubles, and tinsel), which were applied to overhanging branches or the cubby exterior. Scent lure was re-applied and gun brushes removed, frozen, and replaced, weekly.

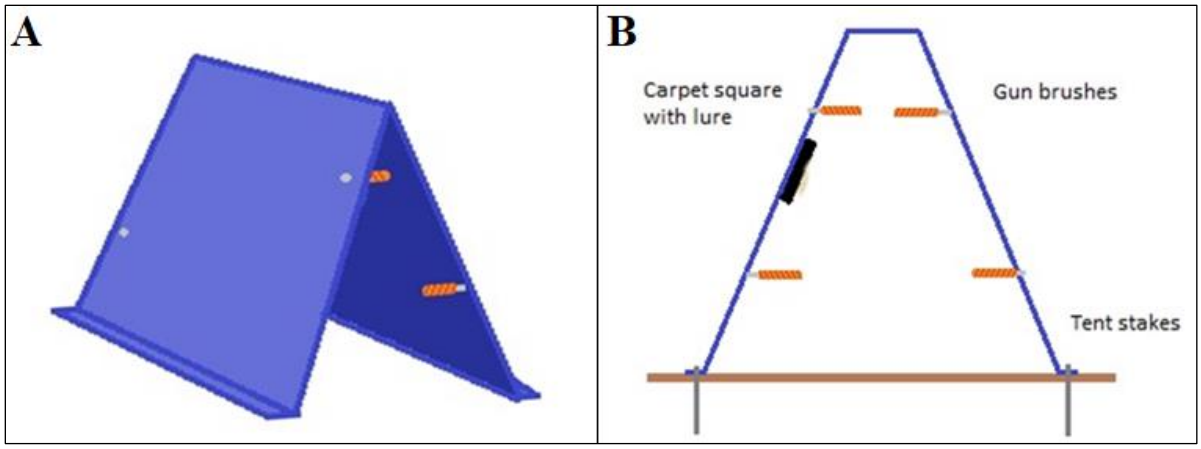

Figure 2. Hair-snare cubby design exterior (A) and interior (B). Cubbies are constructed from corrugated plastic with gun brushes fixed to entrances at alternate heights, carpet squares fixed to cubby interior for scent lure application, and tent stakes to anchor the cubby in place (see Rounsville 2018). Diagrams courtesy of K. Branham (former OSU and UCO student).

Objective 1 Methods

To maximize the number of Oklahoma counties sampled with hair snares, we recruited >60 volunteers from learning institutions across Oklahoma, including Oklahoma State University, University of Central Oklahoma, Southeastern Oklahoma State University, Southwestern Oklahoma State University, Cameron University, Northeastern State University and Medicine Park Aquarium & Natural Sciences Center. Volunteers were provided with the equipment and trained in how to attract bobcats by an experienced trapper and/or video tutorial. Each student volunteer deployed two or more cubbies, with a minimum distance of 500 m between each cubby, for three weeks over the winter holidays of 2018/19, 2019/20, and 2020/21.

Volunteers were instructed to replace gun brushes and re-apply scent lure weekly, freezing gun brushes until they were returned to OSU. Students were also required to provide GPS and basic habitat data for each cubby location for use as covariates during analyses. However, some volunteers did not provide GPS locations for their cubbies, and not all gun brush sets received were correctly labelled by volunteers. Any data received from these cubbies were not used in subsequent analyses.

All mammalian hairs were extracted from gun brushes in the lab at OSU. For the 2018/19 and 2019/20 field seasons, follicles were clipped, if present, and frozen for later genetic analysis. Casts and permanent slides were made for all mammalian hairs for morphological identification to species. In the 2020/21 field season, all mammalian hairs were stored in plastic tubes for genetic analysis, though when hairs formed clumps on brushes, single hairs were extracted for morphological identification as a control sub-sample and comparison. All genetic hair samples were frozen until DNA extraction and analysis to assess the accuracy of morphological identification of hair (see *Genetic analyses of hair samples* below).

Morphological identification of hairs

For each mammalian hair extracted from gun brushes, 1) a cast of the scale pattern (Fig. 3A) was created by pressing the hair into clear nail polish, and 2) a permanent slide of the hair was made to examine the medulla structure (Fig. 3B). Hairs were identified to genus or species level based on external scale pattern, medulla, color, and width, facilitated by photomicrographs, dichotomous keys and guides, by two to five lab technicians (median = 3).

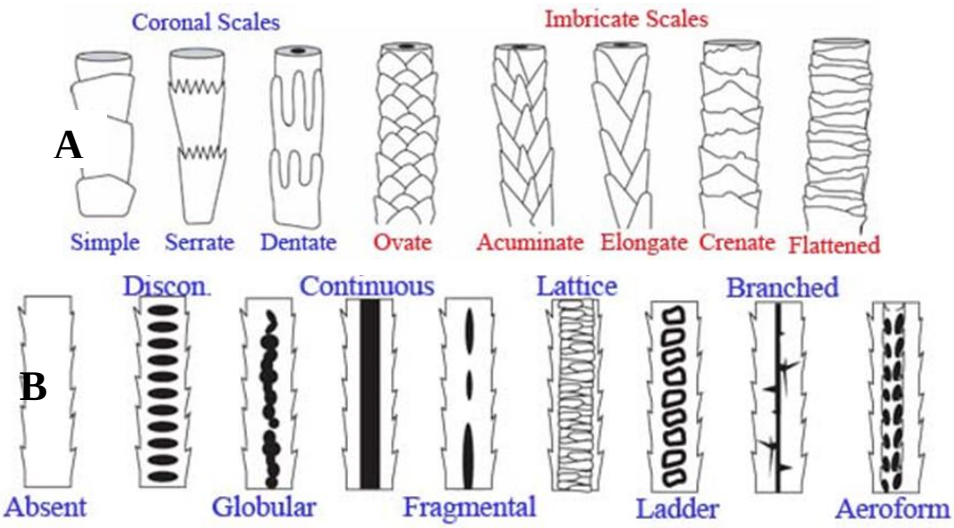

Figure 3. Unique characteristics of mammalian hair used for identification of hair samples obtained from occupancy study volunteers across Oklahoma. A) External scale patterns and B) medulla structures of mammal hairs are shown (from MicrolabNW Photomicrograph Gallery website).

Statistical Analyses

To determine the probability of occupancy for bobcats across Oklahoma, we employed dynamic (multi-season) occupancy models using the unmarked package in R. After eliminating hair-snare cubbies unsuitable for analysis (e.g., due to absence of GPS coordinates), we used presence/absence data from 166 cubby sites, of which 10 cubby sites from 2019/20 were resampled in 2020/21. For presence/absence scores, we used all sources of bobcat presence (morphological ID, genetic ID, or both genetic and morphological ID). In some cases, in which cubbies had both genetic and morphological IDs for a given week, species identifications differed between identification methods. However, this may be due to different hairs being used for each identification method, because often several sub-samples were extracted from cubbies on a given week, and follicles clipped from very few hairs. Therefore, we used any morphological IDs that achieved bobcat identification consensus (majority of technician IDs) as bobcat presence per cubby site on a given week.

Volunteers were instructed to place cubbies > 500m apart from another cubby, but despite this, almost half of all cubby sites were within this proximity. To avoid losing this data, we accounted for this by including a detection covariate which quantified the number of cubbies within a 1km radius of the cubby site. To account for further heterogeneity among detection probabilities for bobcats, we created three more detection parameters for our occupancy models; average weekly temperature, average weekly wind speed, and number of days the cubby was active per week. For the former two, we used climate data from a weather station in Oklahoma City as a coarse estimate of weather conditions throughout the state. We understand that this estimate is coarse, due to local climatic variation. However, because cubbies were deployed on different days, these data account for temporal differences in deployment dates and factor in extreme weather events (cold snaps and wind storms) that may impact bobcat detection, which would affect all cubbies during that period. We also included the number of days cubbies were active per week (range = 0 – 16, median = 7) as a measure of trapping effort.

For occupancy covariates, we leveraged data from the National Land Cover Database (Dewitz, 2021) using a buffer radius of 2 km around cubby sites and calculating the proportional area of each landcover class within the buffer. The 2km radius buffer was chosen because it mostly closely matched the scale in which Oklahoma was stratified into 11.73km^2 hexes for predictive purposes. The landcover classes chosen as occupancy covariates were those we thought most relevant to bobcats, and included the proportion of cropland, decidf, dev_high, dev_med, wetland, conif, pasture, herb, mixedf, water, and shrub_scrub (see Table 1 for covariate definitions) within a 2km radius of cubby sites. To identify the best models for predicting bobcat occupancy, we created an R loop, in which every combination of variables was used sequentially, with a maximum of three variables per model (to reduce computational time and ensure better model convergence). The best models were then ranked according to AIC and model-averaged to determine best predictors of bobcat occupancy. We used the model averages to predict bobcat occupancy for a state-wide stratification of 15,019 hexes. We kept colonization and extinction constant in our occupancy models to increase the probability of model fit, considering our small number of detections. Also, considering our small number of resampled cubby sites (n = 10), colonization and extinction probabilities are not informative parameters.

Table 1. Occupancy and detection variables used in occupancy modelling and GLM analysis (For more detailed description of variables see https://www.mrlc.gov/ and https://www.ncei.noaa.gov/).

Variable	Type	Description
conif	occupancy	Proportion of evergreen forest within a 2km radius of cubby
cropland	occupancy	Proportion of cropland within a 2km radius of cubby
decidf	occupancy	Proportion of deciduous forest within a 2km radius of cubby
dev_high	occupancy	Proportion of high development area within a 2km radius of cubby
dev_med	occupancy	Proportion of medium development area within a 2km radius of cubby
herb	occupancy	Proportion of herbaceous within a 2km radius of cubby
mixedf	occupancy	Proportion of mixed forest within a 2km radius of cubby
pasture	occupancy	Proportion of pasture within a 2km radius of cubby
shrub_scrub	occupancy	Proportion of shrubland/scrubland within a 2km radius of cubby
water	occupancy	Proportion of open water within a 2km radius of cubby
wetland	occupancy	Proportion of wetland within a 2km radius of cubby
active	detection	Number of days cubby was active on a given week
cubs1km	detection	Number of cubbies within a 1km radius of cubby
AWND	detection	Average wind speed over duration of study week (NOAA)
TAVG	detection	Average temperature over duration of study week (NOAA)

In addition, due to poor performance of occupancy models due to small sample size, general linear models (GLMs) were constructed for cubby data in R. Mixed models (binomial/poisson GLMMs) that used detection parameters (ncubs1km, AWND, TAVG, active) as random effects, were initially used, but resulted in low model convergence. It was likely these models were too complicated for our small sample sizes. Therefore, GLMs were used. A binomial GLM was performed for bobcat presence/absence over all three study weeks and a Poisson GLM was used for bobcat counts (sum of bobcat presence/absences over three weeks [0 - 3]), with NLCD variables as predictor variables for both models.

Backwards stepwise selection was used to find the most parsimonious model, according to AIC.

Objective 2 Methods

Intensive studies of bobcat populations were conducted on three WMAs representing three different ecoregions in Oklahoma. In addition, western and eastern study sites sit on either side of the partition between the two accepted (IUCN) identified subspecies of bobcat in North America; *Lynx rufus rufus* and *L. r. fasciatus* (Kitchener et al. 2017). James Collins WMA occurs in the Arkansas Valley ecoregion and spans 86.41 km² of southeastern Oklahoma in Pittsburg and Latimer counties. It is dominated by oak-hickory-pine (*Quercus spp.-Carya spp.-Pinus spp.*) woodlands and major mammalian predators include bobcats, coyotes and gray foxes (*Urocyon cinereoargenteus*). Common prey items include abundant Eastern cottontail rabbits, gray (*Sciurus carolinensis*) and fox squirrels (*Sciurus niger*), bobwhite quail (*Colinus virginianus*), white-tailed deer (*Odocoileus virginianus*), and lesser abundances of eastern wild turkey (*Meleagris gallopavo silvestris*) (Oklahoma Department of Wildlife Conservation [ODWC], 2019). Sandy Sanders WMA, located in in the Southwestern Tablelands ecoregion of southwestern Oklahoma, spans 120.46 km² of Greer, Beckham, and Harmon counties. It consists of rugged terrain, dominated by mesquite-juniper (*Prosopis spp.-Juniperus spp.*) and harbors bobcats and coyotes, as well as bobwhite quail, white-tailed deer, black-tailed jackrabbits (*Lepus californicus*), Eastern cottontail rabbits, and desert cottontail rabbits (*Sylvilagus audubonii*) with relatively low frequencies of Rio Grande wild turkey (*Meleagris gallopavo intermedia*) (ODWC 2019). There is considerable cattle-grazing in certain areas of the WMA, but human presence is comparatively low. Packsaddle WMA occurs in the Central Great Plains ecoregion and spans 79.56 km² of Ellis County in western Oklahoma. Bounded by the Canadian River to its south, it is composed primarily of mixed grass prairie and shinnery oak (*Quercus havardii*). It harbors bobcats and coyotes, as well as good numbers of bobwhite quail, white-tailed deer, Rio Grande wild turkey, and Eastern cottontail rabbits, with occasional black-tailed jackrabbits and desert cottontail rabbits (ODWC 2019). Extensive oil drilling occurs across much of Packsaddle WMA, with related vehicular traffic considerably high.

Hair snare and camera placement on intensive study areas

Forty hair-snare cubbies were deployed at each study site (total n = 120) with a minimum distance of 500 m between each cubby for a total of six weeks, January-March 2019, 2020 and 2021 (Fig. 4-6). This period of the year is during the height of bobcat mating season (Larivière and Walton 1997), which should increase the effectiveness of lures and result in greater movement rates (Chamberlain et al. 2003). Cubby deployment locations prioritized areas in which bobcat encounters were thought most likely, including trails used by wildlife and/or livestock, dirt roads, and river crossings. The average distance between cubby sites was 660 m, though this differed between study sites, with our smallest site James Collins averaging 544m in a denser array, and our largest site Sandy Sanders averaging 752 m between cubbies.

We included camera traps at a number of our hair-snare cubbies to assess efficacy of hair snares (Figs. 4-6). For 2019 and 2020 field seasons, we deployed 60 Stealth Cam G42NG motion-sensor camera traps at half of all hair-snare cubby locations (about 20 cameras per site). For the 2021 field season, we deployed 53 Stealth Cam G42NG, 21 Reconyx Hyperfire HC500, 16 Campark T30, and 2 WildCam camera traps (about 32 cameras per site). The average distance between camera sites was 960.78 m; camera density differed among study sites and study seasons (due to increased number of cameras deployed; in 2021; Table 2).

Reconyx and Stealth Cam cameras were set to 5 photo bursts with an interval period of 30 seconds, whilst Campark cameras were set to 2 photo bursts followed by a 30 second video and an interval period of 1 min. All media were time stamped and GPS recorded.

Gun brush collection and replacement, scent lure re-application, and swapping of camera-trap SD cards, occurred weekly. All gun brushes remained frozen until hair extraction. Mammalian hairs collected from the cubbies were identified morphologically according to the methods above. Follicles or whole hairs were frozen for genetic analyses.

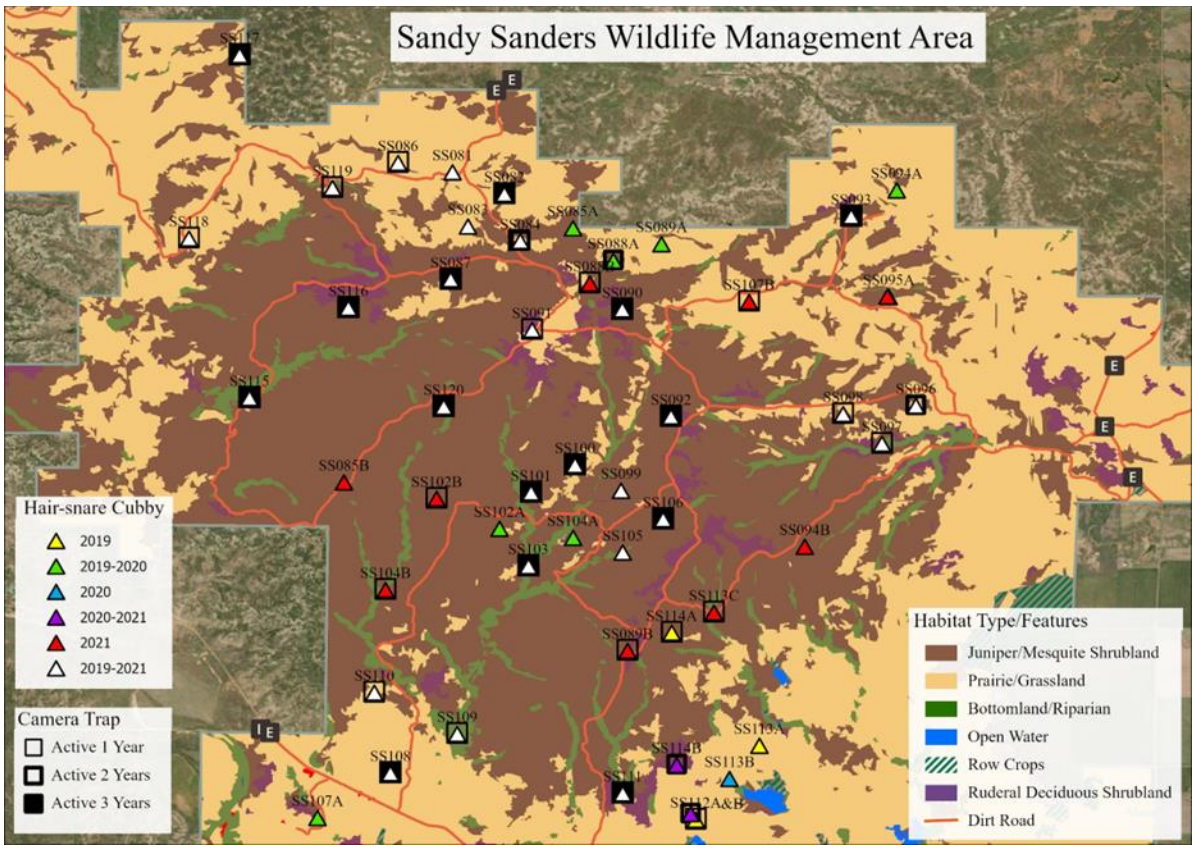

Figure 4. Sandy Sanders Wildlife Management Area, Greer, Beckham, and Harmon counties in southwestern Oklahoma. Map shows landcover types reclassified from Diamond and Elliot (2015; Appendix I), WMA features, and hair-snare cubby and camera trap locations 2019 – 2021. Hair-snare cubby locations and camera-trap locations are color-coded and symbol-coded, respectively, to signify the number of study seasons deployed at a given location. Map was created using ArcGIS Pro.

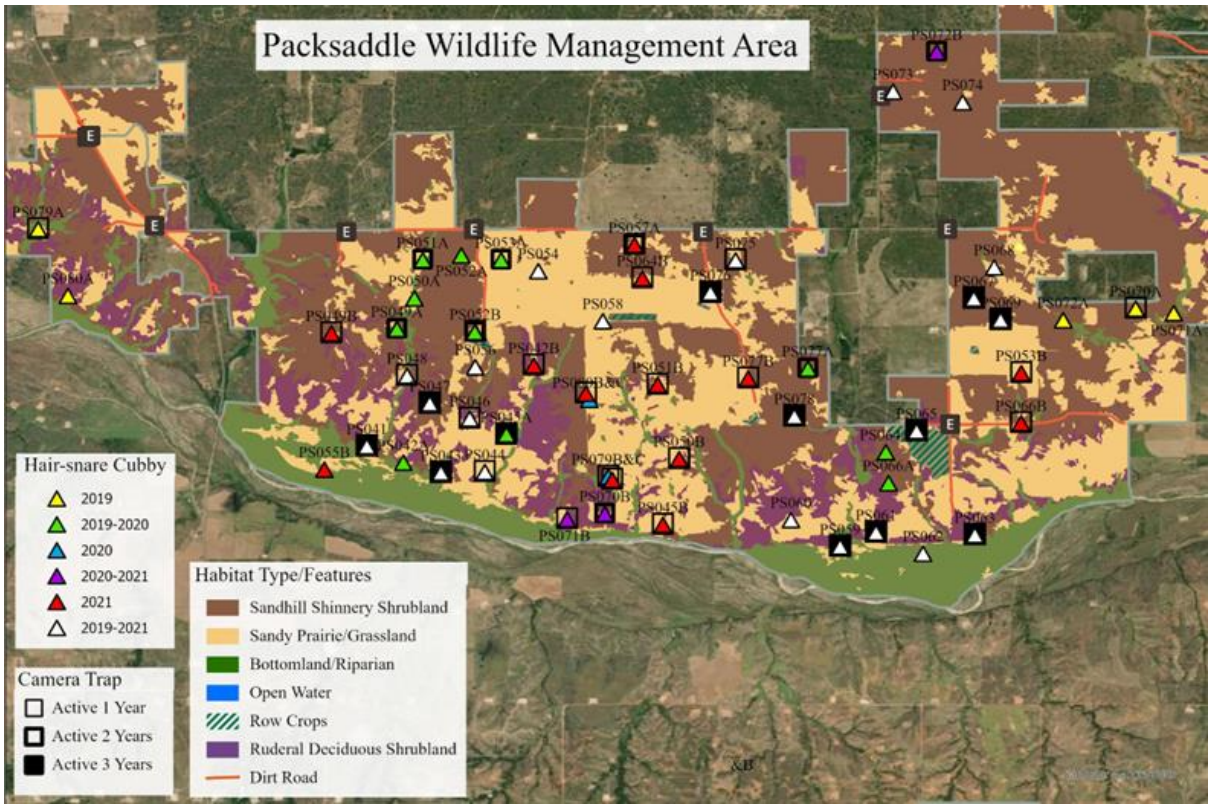

Figure 5. Packsaddle Wildlife Management Area, Ellis County in western Oklahoma. Map shows landcover types reclassified from Diamond and Elliot (2015; Appendix I), WMA features, and hair-snare cubby and camera trap locations 2019 – 2021. Hair-snare cubby locations and camera-trap locations are color-coded and symbol-coded, respectively, to signify the number of study seasons deployed at a given location. Map was created using ArcGIS Pro.

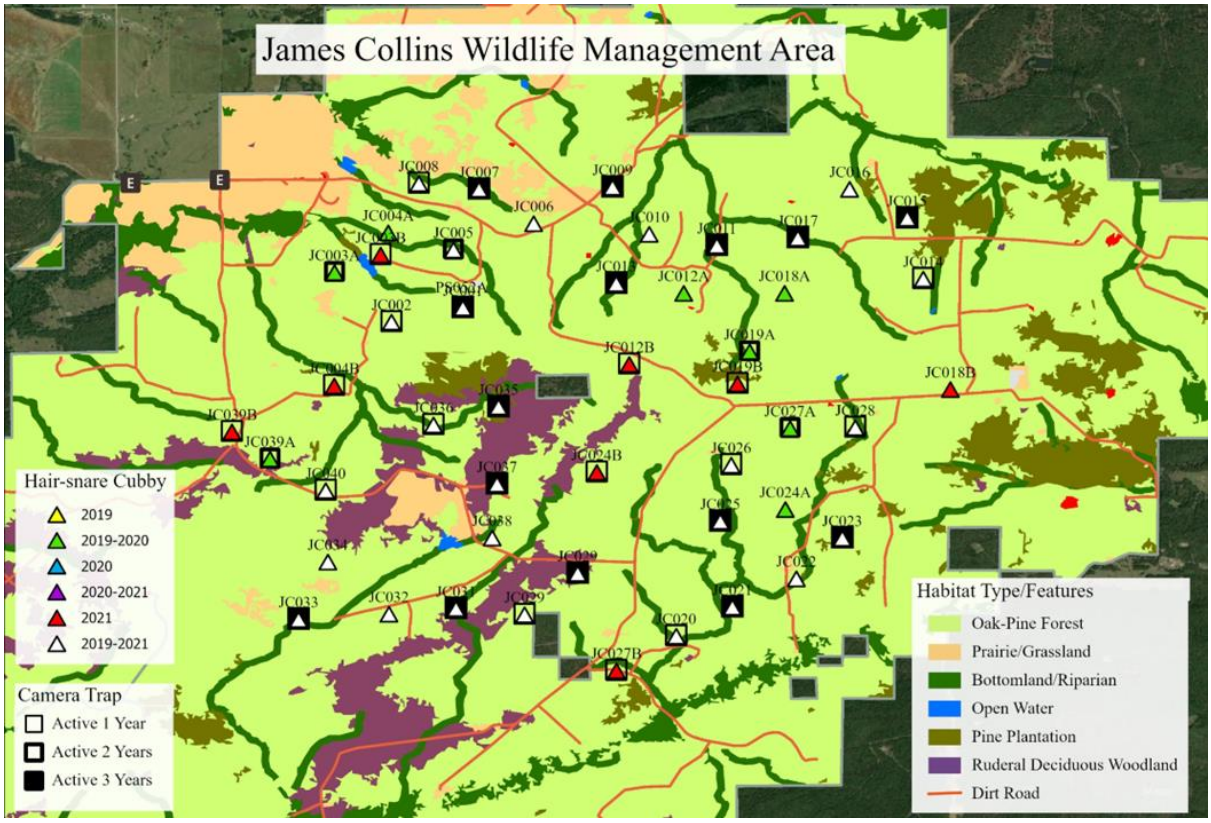

Figure 6. James Collins Wildlife Management Area, Pittsburg and Latimer counties in southeastern Oklahoma. Map shows landcover types reclassified from Diamond and Elliot (2015; Appendix I), WMA features, and hair-snare cubby and camera trap locations 2019 – 2021. Hair-snare cubby locations and camera-trap locations are color-coded and symbol-coded, respectively, to signify the number of study seasons deployed at a given location. Map was created using ArcGIS Pro.

Table 2. Average distance (m) between camera traps at each study site for each study season. JC = James Collins WMA; PS = Packsaddle WMA; SS = Sandy Sanders WMA.

Study Site	2019	2020	2021
JC	838.51 m	840.63 m	672.52 m
PS	1155.23 m	1099.18 m	843.84 m
SS	1184.56 m	1133.35 m	868.48 m

Genetic analyses of hair samples

Due to success rates being low, we used a series of different DNA extraction, DNA amplification via the polymerase chain reaction (PCR), and sample purification methods throughout the duration of the study in attempt to find an optimal method of species identification. In the end, samples were only extracted successfully with two different extraction methods: modified Qiagen DNeasy protocol and Promega DNA IQ protocol (3.B. Purification From Hair Follicles and Hair Shafts), whilst our organic extraction protocol was used for control samples only (hairs from bobcat roadkill), with limited success. For hair samples from our GCR study sites for the 2019 field season and for the 2018/19 - 2019/20 occupancy surveys, hair follicles were removed from hair samples and transferred directly to

a 1.5ml microcentrifuge tube. For GCR 2020 and 2021 field seasons and 2020/21 occupancy survey, whole hairs were collected (or collated from smaller tubes) into 1.5ml microcentrifuge tubes. The Qiagen protocol was modified as follows: 180µl of tissue lysis buffer ATL and 20µl of proteinase K was added to each sample (30µl of DTT [Dithiothreitol] was also added in later DNA extractions) and samples were incubated overnight at 56ºC or 60ºC. After the initial incubation step, DNA extraction followed the Qiagen DNeasy Blood and Tissue Kit protocol with the following modifications to the elution phase: decreasing buffer AE from 200µl to 150µl to increase DNA concentration and a double elution step (re-inserting elute into microcentrifuge tube and spinning for an additional 1min at 8000rpm).

We first determined the species represented by hair samples before genotyping samples identified as bobcat. Amplification via PCR followed the protocol suggested by Janečka et al. (2006) with the addition of BSA and an increase in cycle time as suggested by Schwartz et al. (2004). We tried numerous genetic primer sets for amplification; 16S, FurND1 (Garofalo *et al.* 2018), or MVZ05/400R, which target the 16S rRNA, ND1, and cytochrome b regions of the mitochondrial genome, respectively. The thermal profiles used in PCRs are shown in Appendix II. PCR products were subsequently electrophoresed on agarose gel and visually inspected under UV-light. Samples successfully amplified were cycle sequenced in both directions using 16S/FurND1/cyt b forward and reverse primers, following the recommended protocol for Big Dye version 3.1 (Applied Biosystems). The thermal profile was: 25 cycles of 96.0°C for 10 min, 50.0°C for 10 min, 60.0°C for 2 min. Samples were sequenced on an ABI3500 Genetic Analyzer. Resulting sequences were aligned when possible and submitted to the NCBI database BLAST (www.ncbi.nlm.nih.gov/BLAST/) for species identification.

Bobcat DNA samples were genotyped using a suite of 6 microsatellite loci developed for bobcats (Faircloth et al. 2005), domestic cats (*Felis catus*; Menotti-Raymond and O'Brien 1995; Menotti-Raymond et al. 1997; 1999; 2005), or Canada lynx (*L. canadensis*; Carmichael et al. 2000). After initial genotyping, we found insufficient variation in locus LC110, and subsequently only used loci BCE5T, BC1AT, FCA077, FCA090, and FCA096. Samples were genotyped on an ABI3500 Genetic Analyzer and alleles were scored using GeneMapper 5 software (Applied Biosystems). However, because hair and scat samples contained low quantity and low-quality DNA due to a number of factors, including environmental exposure and contaminants associated with collection protocols (e.g., presence of other species in scat samples), both species identification and individual identification proved difficult. Although microsatellite markers are designed to amplify small fragments (which might result during DNA degradation due to environmental exposure), they are subject to low copy number artefacts (e.g., allelic drop-in, allelic dropout). Additionally, the presence of multiple species or multiple individuals within a single collected sample (as might occur when two individuals rub against the same brush or prey species DNA is collected with predator species DNA in a scat sample). Due to insufficient genetic data to determine individual bobcats, and thus estimate densities, we used camera trap data for estimation.

Scat Analyses

To supplement our genetic identification of bobcats, we collected scat samples opportunistically during the field season (Jan- May) on the three study sites, and recorded GPS location. Most samples were obtained from dirt roads running throughout the study areas, used often by bobcats and coyotes, and where they are known to frequently defecate (Macdonald 1980). Due to inaccuracies that often arise when attempting to differentiate scats morphologically by species (Davison et al. 2002, Morin et al. 2016), our identifications were

more conservative, and we only differentiated coyote scat from unidentified meso-carnivores (bobcat, coyote) by large (> 30%) proportions of juniper (*Juniperus spp.*) berries within the scat. Coyotes are known to consume juniper berries in the western study areas, and as obligate carnivores, bobcats are not likely to consume large quantities of vegetation. By using the > 30% juniper presence criterion, we could conservatively rule-out presence of juniper due to contamination. Scats containing 0-30% juniper (i.e. not identified in the field), were identified genetically to determine depositing species.

We separated each scat sample into two sub-samples, one for diet analysis and one for genetic analysis to determine the depositing species. In the 2020 field season we collected genetic sub-samples in paper bags and placed in a freezer. Later, scrapings were taken in the lab from frozen samples by extracting the outer layer of the scat using a scalpel. In the 2021 field season, we took scrapings in the field using a scalpel sterilised in bleach solution and preserved the material in molecular-grade ethanol (EtOH). We froze the remainder of the sub-sample as backup material for genetic analysis. In the lab, we removed any ethanol before DNA extraction by air-drying for approximately 20 min. We extracted DNA using the QIAamp DNA Stool Mini Kit (Qiagen, Inc.) modified to include a heated suspension step after the addition of InhibitEX buffer for 5 min at 70ºC, extended incubation after the addition of proteinase K and buffer AL from 10 min at 70ºC to 1 hr at 70ºC, and a double elution step (performed the same as for hair samples). Amplification via the polymerase chain reaction (PCR) used 16S, FurND1 (Garofalo *et al.* 2018), or MVZ05/400R genetic primers, which target the 16S, ND1, and cytochrome b rRNA regions of the mitochondrial genome, respectively. The thermal profiles used in PCRs are shown in Appendix II. PCR products were subsequently electrophoresed on agarose gel and visually inspected under UV-light. Samples successfully amplified were cycle sequenced in both directions using 16S or FurND1 forward and reverse primers, following the Big Dye version 3.1 (Applied Biosystems) recommended protocol. The thermal profile was 96.0°C for 10 min, 50.0°C for 10 min, 60.0°C for 2 h. After linear amplification, corresponding samples were aligned when possible and implemented into the NCBI database BLAST (www.ncbi.nlm.nih.gov/BLAST/) for species identification.

The diet sub-sample was collected in a labelled paper bag in the field and stored at room temperature for later diet analysis. After initial air-drying, scat sub-samples were placed into doubled and knotted nylon stockings and placed in hot soapy water for >1 hr to soften and rinsed to remove matrix material. This process was repeated as needed. Scats were dried under a fume hood for >12 hr and oven-dried at 50°C for >48 h and stored for potential future analysis of mesocarnivore diets.

Density estimates and meso-carnivore/prey activity patterns from camera trap data
We estimated bobcat density on Sandy Sanders, Packsaddle, and James Collins WMAs by first identifying individual bobcats in camera-trap images by their unique pelage patterns. Observations in which pelage patterns were not visible (e.g. infrared flare, subject obscured by foliage) were not counted. We estimated bobcat density at each study area by first calculating the ½ mean maximum distance moved (½MMDM) by individual bobcats captured by multiple cameras, pooling data over the three field seasons. By pooling data, we assume no home range shifts occurred during the 3-year period. However, due to low number of recaptures within single field seasons, we believe pooling data resulted in more accurate estimates. We obtained MMDM calculations via the MMDM function in R package *secr* (Efford 2022) and halved the values. We then created a geodesic buffer of this radius length around camera site locations in ArcGIS, dissolving buffers into a single feature, and

calculated the resultant area in square kilometers. Density was estimated as the number of bobcat individuals divided by the estimated sampling area.

The activity patterns of bobcats and their sympatric meso-carnivores: coyotes, opossums, raccoons, and striped skunks, and their potential prey species: rabbits (*Sylivagus spp.*), were compared using the compareCkern function using the *activity* package (Rowcliffe 2021) in R (R Core Team 2017). This function uses fitted kernel densities of radian time-of-day data to calculate the overlap index $\hat{\Delta}_4$ for the fitted distributions, which ranges from 0 (no overlap) to 1 (complete overlap), then completes a randomisation test for the probability that the two sets of circular observations come from the same distribution (Rowcliffe 2021). We compared activity patterns of bobcats among study areas, coyotes among study areas, and among bobcats, coyotes and rabbits across all areas, with data pooled from all three study seasons. Number of bootstrap iterations was 999. We then compared coefficients of overlap $\hat{\Delta}_4$ for all species pairs (meso-carnivores and rabbits) among study areas, with data also pooled over all three field seasons. Samples sizes of < 20 at a given site for a given species were omitted from comparisons.

Small mammal live-trapping

Due to the effect small mammal communities can have in influencing bobcat abundance, space-use, and population dynamics (Bailey 1974, Knick 1990, Hansen 2012), estimating the small mammal and lagomorph densities at each intensive study site can improve our understanding of the bobcat-prey dynamics exhibited in ecologically distinct regions of Oklahoma. Small mammal (primarily rodent) abundance data was collected via live trapping between March – May 2020 and 2021. A total of 120 large (7.62 cm x 9.53 cm x 30.48 cm) and 240 small (5.08 cm x 6.35cm x 16.51 cm) Sherman live-traps were deployed on each of the three intensive study areas. Landcover within the study areas was reclassified from landcover maps by Diamond and Elliott (2015), into three or four distinct landcover types (Fig. 7-9; Appendix I). Four 300m linear transects were deployed on each landcover type per study area, with each transect composed of 30 trapping stations. Due to damage sustained to traps leading to trap shortages, we reduced trapping stations to 28 (280m transects) at Sandy Sanders during the 2020 season only. Transects were used instead of trapping grids because they are more effective at assessing small mammal abundances, especially when abundances are low (Pearson and Ruggiero 2003). Although transects were mostly linear, environmental obstacles and fine-scale deviations in landcover resulted in non-linear transects. This was especially true for riparian transects, which followed the course of streams or rivers. Trapping stations, placed at 10m intervals along each transect, were composed of two small traps and one large trap placed within a 1.5 m radius. Traps were baited with rolled oats and peanut butter and active for three consecutive nights. Rodents and shrews were identified to genus or species level and sexed in the field. Individuals were marked to avoid pseudo-replication during recaptures, using a permanent (non-toxic) marker on the base of the skull and inside the ears, which we found to be the most difficult areas for rodents to remove during the relevant time period. In 2021, markers were colour-coded to provide coarse capture histories. In addition, we obtained weights of individual rodents during the 2020 field season.

Small Mammal Statistical Analyses. Because the aim of this project was to quantify and reliably predict rodent abundances at the different landcover types represented within our study sites, generalised linear mixed models (GLMM) were constructed using the lme4 package v1.29 (Bates et al. 2015) in RStudio v4.2.2 (RStudio Team, 2020), with the number of unique rodent individuals captured at each trapping station as the response variable and landcover type kept as a fixed effect. As stations were nested within transects, transect ID

was included as a random effect. Separate analyses were performed for each study area due to the unique environments and rodent species present at each site. To control for heterogeneity among capture probabilities as a result of weather conditions preceding each day of capture, we included total precipitation, average maximum temperature, and average minimum temperature for the day prior to each of the three capture days as a random effect. Because the response variable was count data in nature, a Poisson link was specified. Overdispersion analyses were performed for all models. If a set of models was overdispersed, a negative binomial link was specified. Candidate models included transect ID plus all combinations of the environmental variables, including no variable, as random effects (e.g. n_indiv ~ landcover + (1 | transect) + (1 | AVGTMIN)), and were weighted by AIC. Models with ΔAIC <2 were considered as showing equal support, and in all cases models ΔAIC >2 were shown to have singular fits. Therefore, the best model was selected from this shortlist of models (ΔAIC <2) based upon biological relevance, and was used to predict the expected number of individual rodents captured on a given transect within each landcover type, using the predict() function. To assess differences in ecological metrics among landcover types at each site, we compared species richness and Shannon diversity using the vegan package v2.7 (Oksanen et al. 2020). We used linear models to determine significant differences in metric values between landcover types of each study area. To contextualize bobcat densities, between-site analysis was performed for all study areas, using the same methodology as above to compare abundances and ecological metrics.

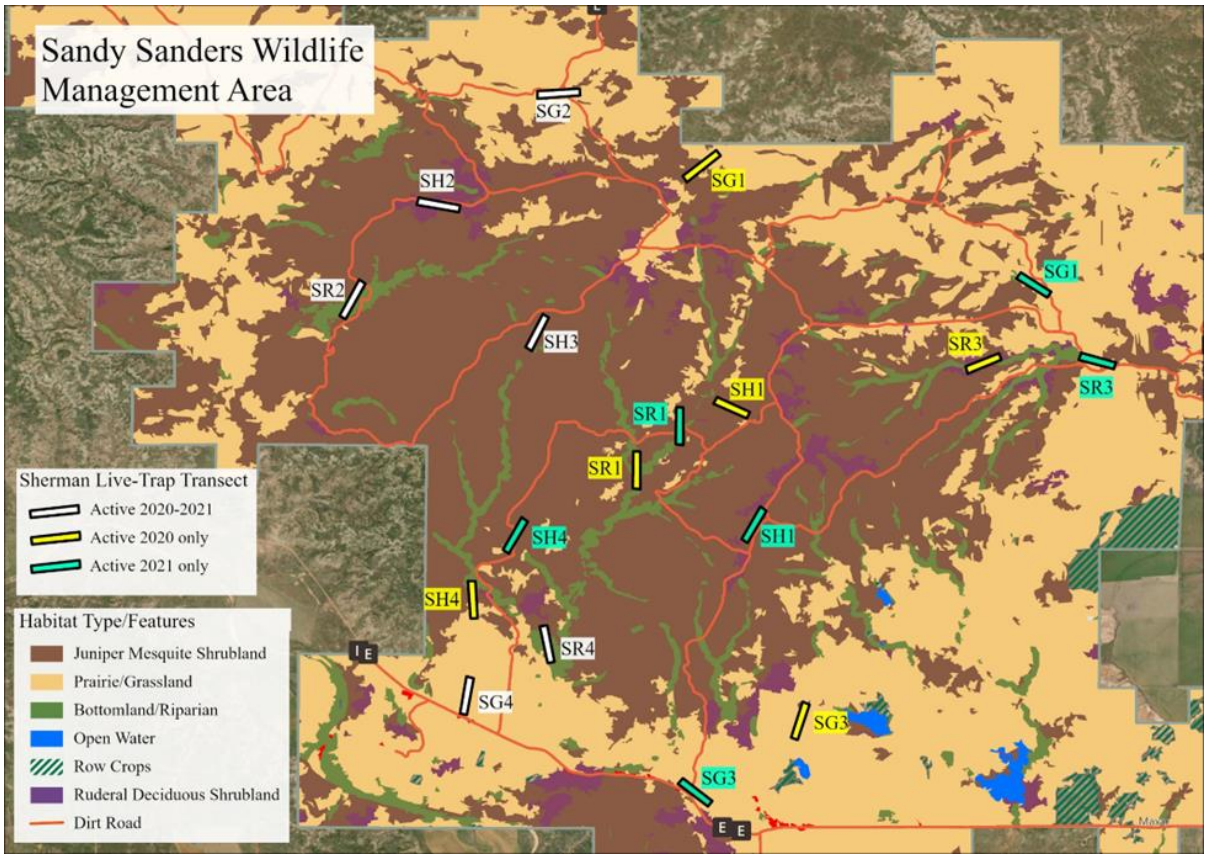

Figure 7. Sandy Sanders Wildlife Management Area, Greer, Beckham, and Harmon counties in southwestern Oklahoma. Map shows landcover types reclassified from Diamond and Elliot (2015; Appendix I), WMA features, and small mammal transect locations 2020 – 2021. Transects are labelled and color-coded to signify the number of study seasons deployed at a given location. Map was created using ArcGIS Pro.

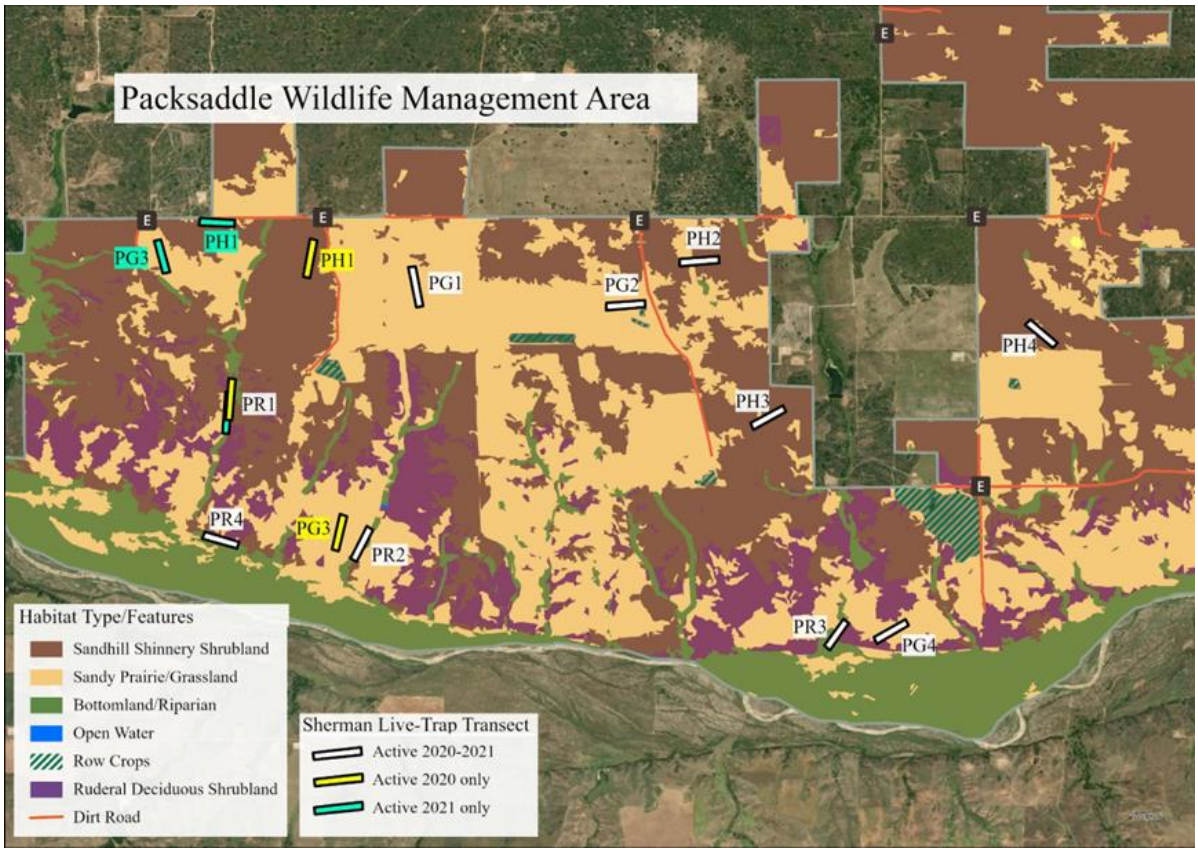

Figure 8. Packsaddle Wildlife Management Area, Ellis County in western Oklahoma. Map shows landcover types reclassified from Diamond and Elliot (2015; Appendix I), WMA features, and small mammal transect locations 2020 – 2021. Transects are labelled and color-coded to signify the number of study seasons deployed at a given location. Map was created using ArcGIS Pro.

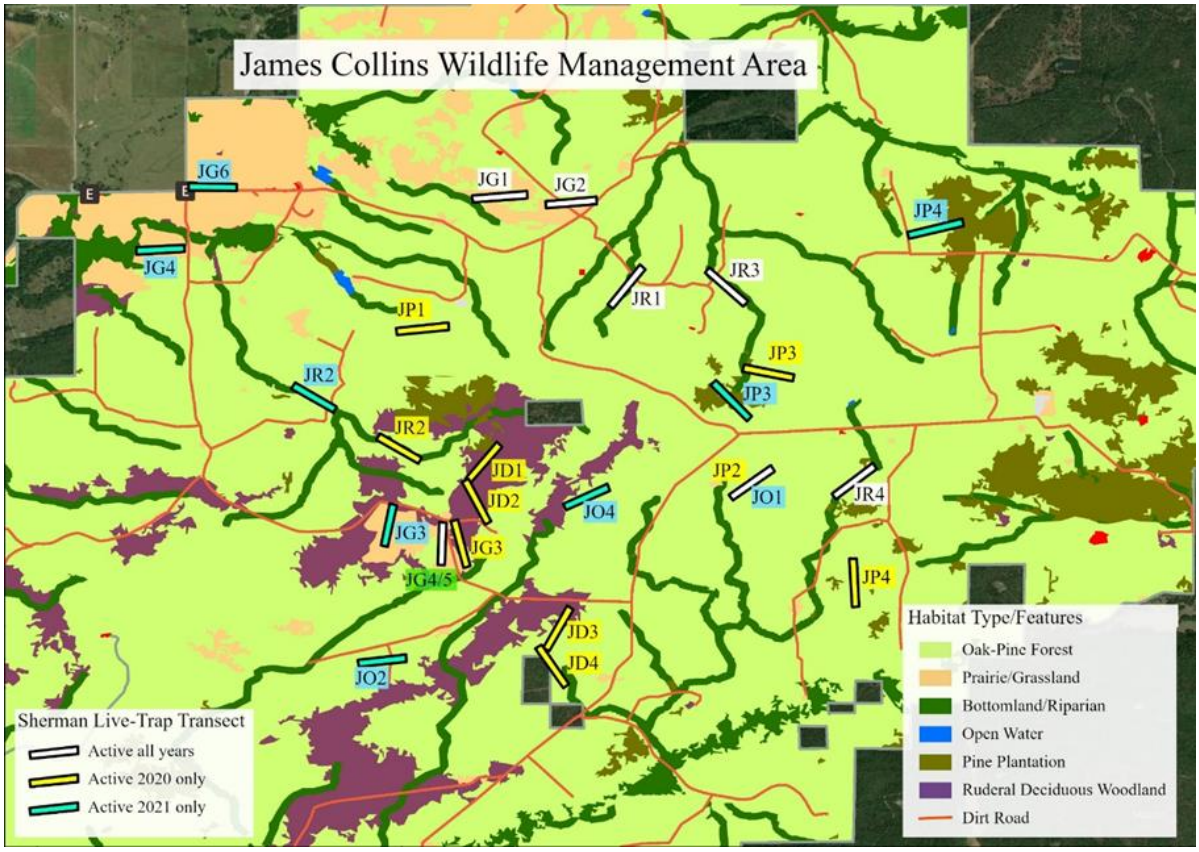

Figure 9. James Collins Wildlife Management Area, Pittsburg and Latimer counties in southeastern Oklahoma. Map shows landcover types reclassified from Diamond and Elliot (2015; Appendix I), WMA features, and small mammal transect locations 2020 – 2021. Transects are labelled and color-coded to signify the number of study seasons deployed at a given location. Map was created using ArcGIS Pro.

RESULTS

Accuracy of morphological identification of hairs to species

We considered a 'sample' as hair obtained from all brushes from a cubby on a given week, which may consist of many sub-samples. In the state-wide occupancy study we obtained 201 hair samples from cubbies and morphologically identified 56 hair samples to species in 2018/19, 52 in 2019/20, and 28 in 2020/21. Of these, at least one hair was identified as bobcat in 72 (53%) samples by at least 50% of technicians, which we considered identification consensus. Of the 263 hair samples collected on the WMA study areas, we identified 43 to species in 2019 and 18 in 2021, of which 3 and 6 were identified as bobcat by consensus, respectively.

To evaluate the accuracy of morphological identification of hair, we compared 77 sub-samples for which we had both morphological and genetic identification. We considered genetic identification to species to be accurate. Due to our protocol shifts (from clipping follicles to collecting whole hairs) and the presence of multiple sub-samples, we present our accuracy ratings as a range of values, representing the highest and lowest possible accuracy and highest and lowest possible number of false positives. Accuracy, defined as the number of correct identifications made per hair-lab technician assigned to a sub-sample, was calculated as between 20.20% and 25.93%. However, we had a significant number of hair

sub-samples identified as domestic cattle (*Bos taurus*, n = 26), which were likely not included in our dichotomous keys and hair guides intended for wild animals. We did, however, classify morphological identifications of bison (*Bison bison*) as correctly identifying cow hair. When removing cow hair sub-samples from the comparisons, accuracy increased to between 29.85% and 35.82%. Species-specific accuracy, calculated as the percentage of morphological IDs that matched genetic IDs, was highest for bobcat hairs (54.81 - 56.73%) and opossum hairs (36.17 - 51.06%), and lowest for coyote hairs (0%). There were between 32 and 58 false positives for bobcats, indicating an identification bias for bobcats, as well as 21 false negatives. However, identification consensuses (species ID made by the majority of technicians) may be a more useful statistic to measure accuracy.

Across our 77 sub-samples, 58 reached morphological identification consensus (Table 3). Nineteen (32.76%) of these morphological identifications matched with our genetic identifications. Again, when removing hairs genetically identified as domestic cattle, accuracy increased (to 45.24%), but remained below 50% accuracy. Among our 58 comparative sub-samples with consensus, 16 identified the hair as bobcat, however only 50% matched the genetic identification, most commonly raccoon or domestic dog. On the other hand, most sub-samples genetically identified as bobcat matched morphological identifications (66.66%), and mismatches were misattributed morphologically to a range of species. This suggests that hair-lab technicians had an identification bias for bobcats. Mismatches between morphological and genetic identification may be explained by mislabelling, contamination, or other source of human error, by morphological and genetic identifications being based on hairs from different species if hair from multiple species was collected on a hair snare, or the difficulty of technicians to identify hairs to species accurately.

Table3. Morphological identification consensuses reached by technicians for 58 comparative sub-samples and the number and percentage that matched the corresponding genetic identification.

Identification consensus	n	match	% correct
Lynx rufus	16	8	50
Didelphis virginiana	9	6	67
Canis latrans	8	0	0
Odocoileus virginianus	4	1	25
Mouse or shrew	4	0	0
Mephitidae	3	0	0
Homo sapiens	3	0	0
Ondatra zibethicus	3	0	0
Procyon lotor	2	2	100
Neogale vison	2	0	0
Ovis spp.	1	1	100
Canis lupus familiaris	1	1	100
Syvilagus spp.	1	0	0

Objective 1

In the state-wide occupancy study, we obtained 201 mammalian hair samples over about 586 hair-snare trapping weeks for an overall snag rate of 0.34 samples/week. Bobcat detections at each cubby varied between 0 and 3 for the 3 weeks each cubby was deployed. We identified hairs morphologically for 2018/19 and 2019/20, and clipped 38 and 39 follicle samples, respectively. We were able to identify 18 (47.37%) and 6 (15.38%) follicle samples, respectively, to species (Table 4). In the 2020/21 field season, we only collected samples for genetic identification, collecting a total of 88 samples and genetically identifying 26 (29.55%) to species. For all 50 hair samples identified genetically (Fig.10), bobcats were the most numerous species (25%), followed closely by opossums (23%), domestic cattle (22%), raccoons (12%), and domestic dogs (8%). Domestic animals represented 38% of all bycatches.

Table 4. Number of genetic samples (total = 165) identified for each field season

Species Identified	2018/19	2019/20	2020/21	Total
No	20	33	62	115
Yes	18	6	26	50
% Identified	47.37	15.38	29.55	30.30

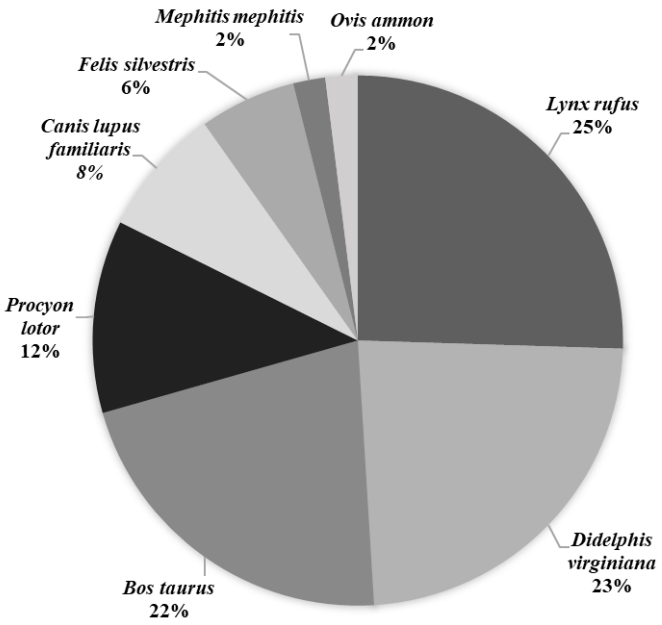

Figure 10. Percentage of species identifications of 50 genetic samples obtained from hair-snare cubbies in the state-wide occupancy study, 2018/19 – 2020/21.

Occupancy models selected with $\Delta AIC \leq 2$ included the covariates conif, decidf, herb, mixedf, pasture, and shrub_scrub, suggesting these land cover classes were important determinants of bobcat occupancy. Model averaged coefficient values indicated that average weekly temperature (TAVG) significantly increased detection probability, though the effect

size (coefficient) was small (Table 5). Model-averaged coefficient estimates suggested conif, decidf, mixedf and shrub_scrub all decreased the probability of bobcat occupancy, whilst herb and pasture increased occupancy probability. However, all estimates had considerable standard error. In the state-wide occupancy map (Fig. 11), bobcat occupancy appears lowest in the Ouachita Mountains region in the southeast, and in the western portion of the state. However, the standard errors for these estimates are also highest in these areas (Fig.12). These low estimates and high standard errors are likely due to low sample sizes, which, in land cover types with particularly few deployments, resulted in the highest inaccuracy in occupancy predictions.

Table 5. Model-averaged coefficient values from # occupancy models with $\Delta AIC \leq 2$. Variable definitions in Table 1. col(Int) = intercept for colonization; ext(Int) = intercept for extinction. * denotes significance at $p < 0.05$.

Coefficient	Estimate	Std. Error	z value	Pr(>|z|)
p(Int)	-1.43728	2.1435	0.671	0.5025
p(active)	-0.1937	0.16671	1.162	0.2453
p(AWND)	-0.1581	0.11159	1.417	0.1565
p(cubs1km)	-0.28902	0.1881	1.537	0.1244
p(TAVG)	0.08467	0.04146	2.042	0.0411 *
col(Int)	-34.43711	400.62683	0.086	0.9315
ext(Int)	-1.11824	0.5627	1.987	0.0469 *
psi(Int)	1.8857	1.98342	0.951	0.3417
psi(conif)	-15.65078	26.199	0.597	0.5503
psi(decidf)	-2.80536	6.31147	0.444	0.6567
psi(herb)	0.36433	1.50537	0.242	0.8088
psi(mixedf)	-114.27543	113.0184	1.011	0.312
psi(pasture)	100.25757	91.27749	1.098	0.272
psi(shrub_scrub)	-2.13071	6.90701	0.308	0.7577

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

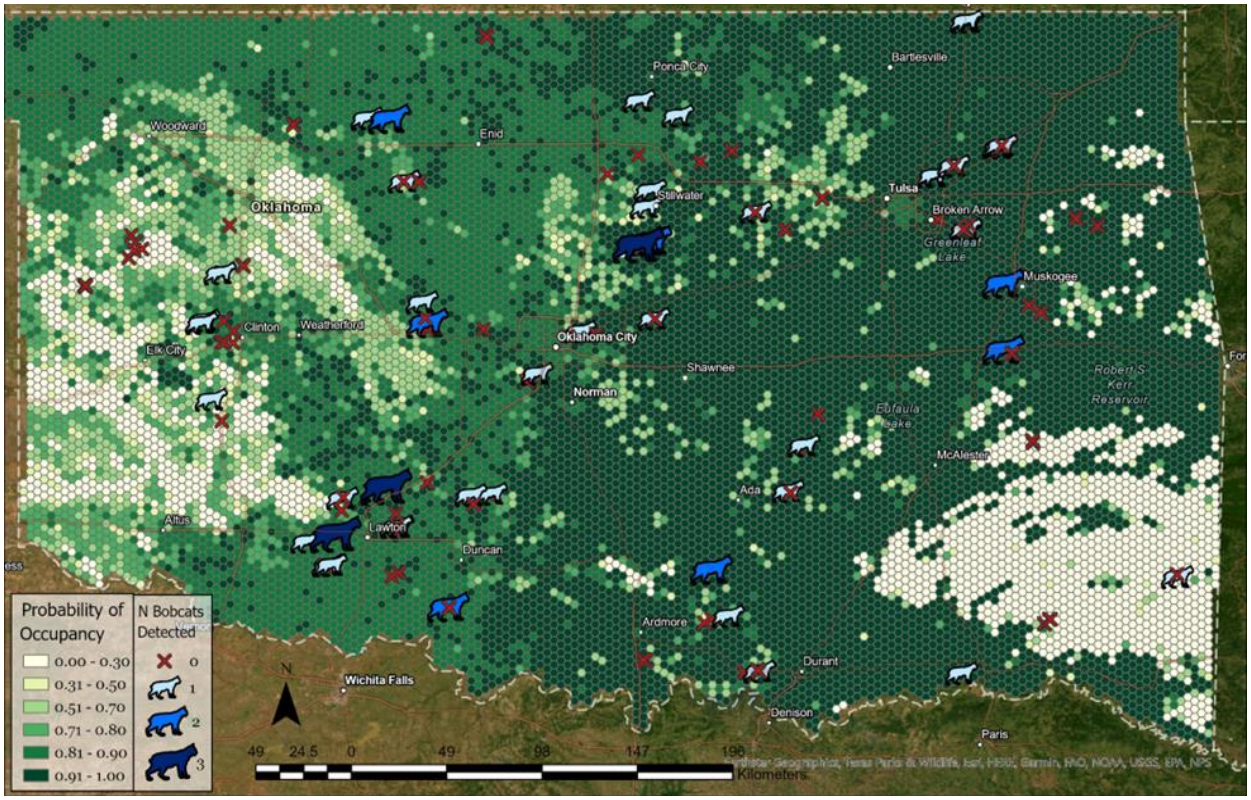

Figure 11. Predicted probability of bobcat occupancy for 11.73-km^2 hexes of Oklahoma based on model-averages of best occupancy models (ΔAIC $\leq$ 2). Also shown is the number of bobcat detections over the three weeks that each hair-snare cubby was deployed in the state-wide occupancy study, 2018/19 – 2020/21.

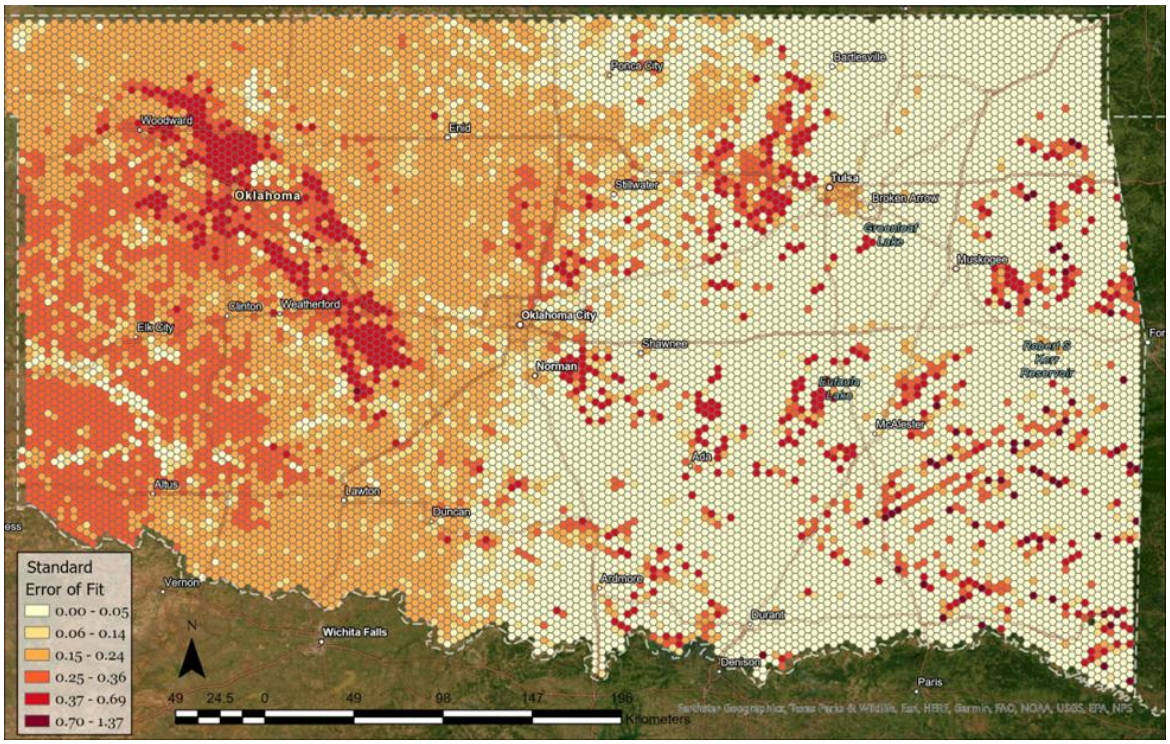

Figure 12. Standard error of bobcat occupancy predictions for 11.73-km^2 hexes of Oklahoma, based on model-averages of best occupancy models (ΔAIC $\leq$ 2).

Stepwise selection resulted in the best binomial GLM model as the one that included conif, dev_med and dev_high. Medium human development (dev_med) was significant at the α=0.1 level and indicated increasing bobcat presence with increased are of medium development, whilst conif and dev_high predicted large, but non-significant decreases in bobcat presence (Table 6). For the poisson model, stepwise selection also selected conif, dev_med, and dev_high in the best model, but also shrub_scrub (Table 7). dev_med significantly increased bobcat presence/absence counts, but dev_high significantly decreased it. These models have considerable error and fit the data quite poorly, likely due to insufficient detections of bobcats. Therefore, these results should be taken with caution, but may be useful to show potential relationships between land cover types and bobcat presence/absence that occupancy models may be missing, particularly the relationship between bobcat occupancy and medium and high development areas. Bobcats are likely to benefit from medium development (such as suburban areas) due to increases in prey abundances, but will likely find high development areas inhospitable.

Table 6. Best binomial GLM model for bobcat presence/absence at cubby sites, resulting from stepwise selection.

Coefficient	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.7511	0.2083	-3.606	0.00031 ***
conif	-13.022	8.51	-1.53	0.125964
dev_high	-83.6414	52.5298	-1.592	0.111325
dev_med	28.9522	17.2718	1.676	0.093684 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 7. Best Poisson GLM model for counts of bobcat counts at cubby sites, resulting from stepwise selection.

Coefficient	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.7519	0.1601	-4.697	2.64E-06 ***
conif	-8.3624	6.5619	-1.274	0.2025
shrub_scrub	-5.0137	3.2089	-1.562	0.1182
dev_high	-61.0123	32.9657	-1.851	0.0642 .
dev_med	19.5118	9.4216	2.071	0.0384 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Objective 2

Results from hair-snare and scat data from three WMAs

Cubby width (entrance width) was adjusted throughout the three field seasons, beginning with an approximate width of 75 cm in 2019. Based on low return of hair samples, even when bobcats were observed entering cubbies on camera trap photographs, we decreased the width to approximately 35 cm in the 2020 season, in hopes to increase snag rates from snares. However, after reviewing camera photographs of the 2020 season, this new width appeared to dissuade some individuals from entering the cubby, so we increased the cubby width to approximately 60 cm in our final field season. Our success rate of obtaining mammalian hair samples increased every field season (Table 8), however it is unclear what difference the cubby width made to our success rate of obtaining specifically bobcat hair.

For simplification, we counted a 'sample' as hair obtained from all brushes of a cubby on a given week, which may consist of many sub-samples. From hair-snare cubbies on WMAs, we obtained 263 mammalian hair samples over 2073 trapping weeks for an overall snag rate of 0.13, with no obvious pattern in snag rate across sampling weeks (Fig13). The majority of identifications for the 2019 field season were morphologically derived, but of the 26 follicles clipped from hairs we identified 9 (34.62%) to species. For the 2020-2021 field seasons, whole hairs were extracted for genetic identification. In 2021, sub-samples of hairs were also identified morphologically. Of the 91 hair samples collected in 2020, and 148 collected in 2021, we genetically identified to species 27 (29.67%) and 13 (8.78%), respectively.

Table 8. Hair-snare snag rate for cubbies on Sandy Sanders, Packsaddle, and James Collins WMAs measured as the percentage of hair-snare cubbies returning with at least one mammalian hair sample for each week during our three field seasons.

year	week 1	week 2	week 3	week 4	week 5	week 6
2019	10.38	4.17	3.33	18.33	4.17	2.50
2020	10.09	7.56	16.81	14.17	7.50	13.33
2021	33.33	20.00	22.50	11.67	22.50	24.00

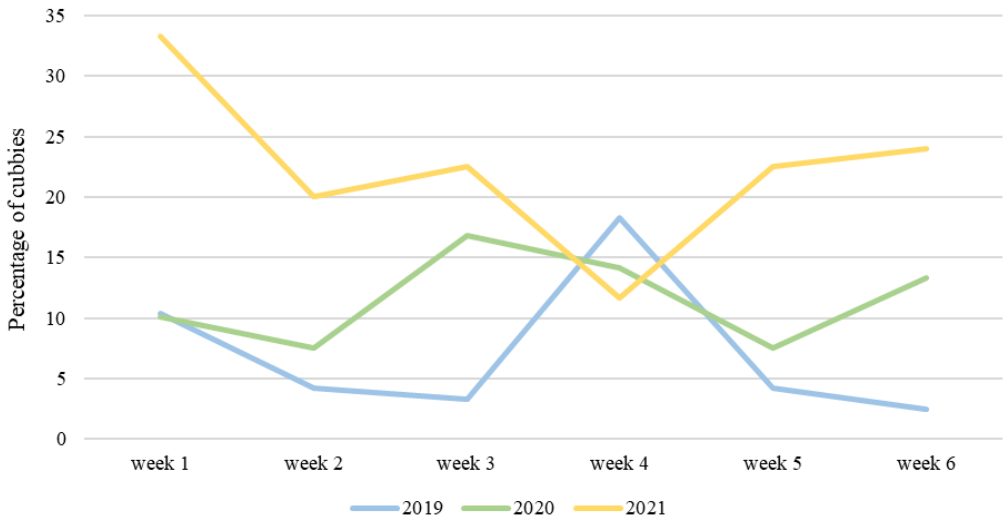

Fig.13: Hair-snare snag rate for cubbies on Sandy Sanders, Packsaddle, and James Collins WMAs measured as the percentage of hair-snare cubbies with at least one mammalian hair sample for each week during our three field seasons.

From hair-snare cubbies on WMAs, of the 39 genetic samples we were able to identify to species using mtDNA, 28% were identified as bobcat (Fig.14). Opossums (*Didelphis virginiana*) were the most numerous species (33%), with other common bycatch species including domestic dog (*Canis lupus familiaris* 10%), domestic cattle (*Bos taurus* 10%), and white-tailed deer (*Odocoileus virginianus* 5%). Domestic species represented 29% of all bycatches. After genotyping, we were able to identify a further 10 samples as likely bobcat. Therefore, for years in which we took primarily genetic samples (2020 and 2021), trapping rate for bobcats was 1.24/100 trapping weeks.

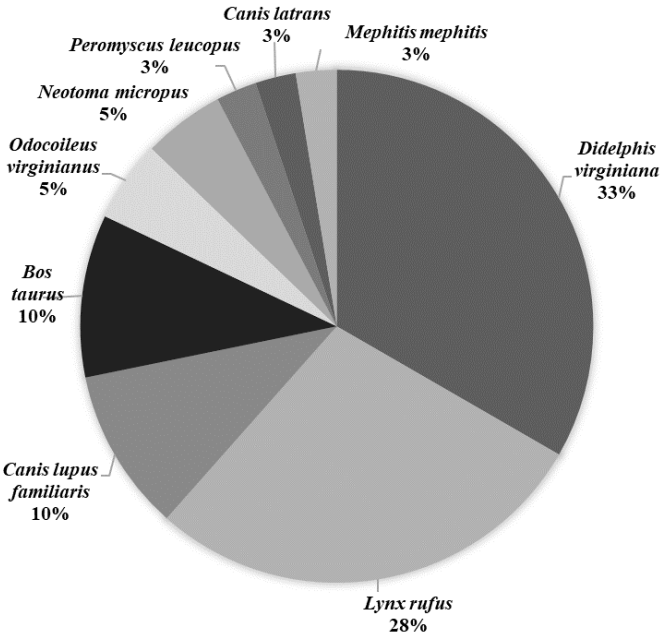

Figure 14. Percentage of species identifications of 39 genetic samples obtained from hair-snare cubbies on Sandy Sanders, Packsaddle, and James Collins WMAs, 2019-2021.

We collected 243 predator scats across all three study sites over the 2020 and 2021 hair-snare and small-mammal-trapping field seasons. Fifty-nine of the scats were identified to species, 34 via morphology (presence of juniper, SS only) and 25 via genetic analysis (Table 9). Fifty-two scat samples were from coyotes, 6 were from bobcats, and 1 was from grey fox.

Table 9. Identification of predator scats collected at James Collins WMA, Packsaddle WMA, and Sandy Sanders WMA, 2020 and 2021. (N = 243 scats)

study site	un-identified	identified	ID via DNA	ID via morph.	coyote	bobcat	grey fox
JC	72	18	18		13	4	1
PS	40	5	5		5		
SS	72	36	6	30	34	2	

Camera-trap results

Total camera-trapping nights over all study areas and study seasons was 8425 trapnights. Two cameras malfunctioned during the 2019 field season, five cameras in 2020 and four cameras in 2021. All data from these cameras were removed from analyses. We obtained 134 observations of bobcats over all three field seasons, resulting in a trapping rate of 1.59 bobcats/100 trapnights. The highest frequency of bobcat observations occurred at Packsaddle and lowest frequency at Sandy Sanders, though this differed between years (Fig. 15). Of all 109 unique camera-trap locations across all study sites and field seasons, 55.96% had at least one bobcat observation (James Collins = 61.11%, Packsaddle = 57.89%, Sandy Sanders = 55.96%), though this varied by study area and year (Fig. 16). There appeared to be no pattern

in observation frequency among study weeks (Fig 17). We also collected significant data on sympatric species (n = 1710, excluding white-tailed deer), including those relevant to bobcats, including 284 rabbit and 258 coyote observations (see Appendix IV).

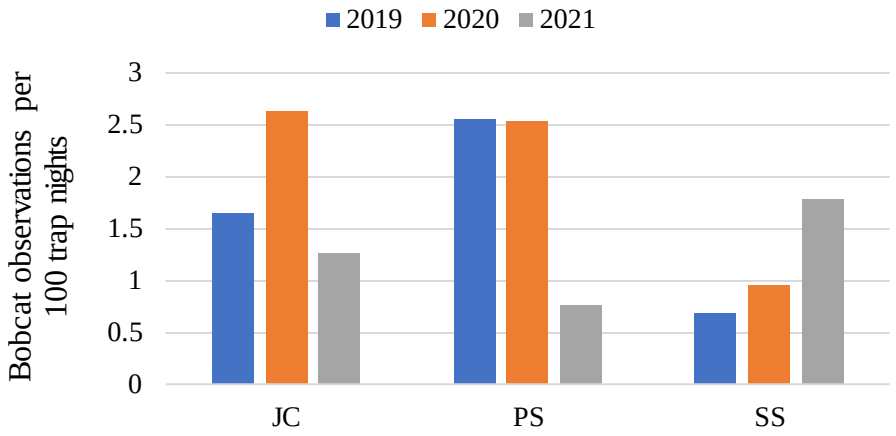

Figure 15. Camera-trapping rate for bobcats per 100 trapnights (total trapnights = 8425) for each study site and field season. JC = James Collins WMA; PS = Packsaddle WMA; SS = Sandy Sanders WMA.

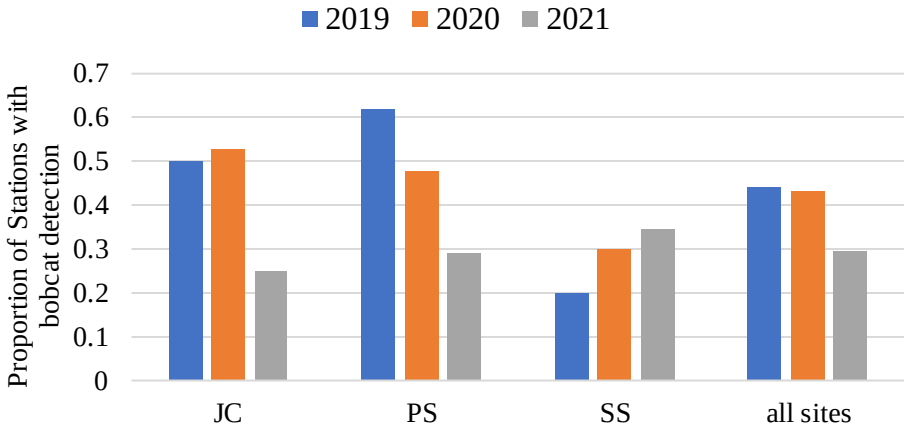

Figure 16. Proportion of camera-trap stations with at least one bobcat (*Lynx rufus*) observation for each study area and all sites combined, per study season. JC = James Collins WMA; PS = Packsaddle WMA; SS = Sandy Sanders WMA.

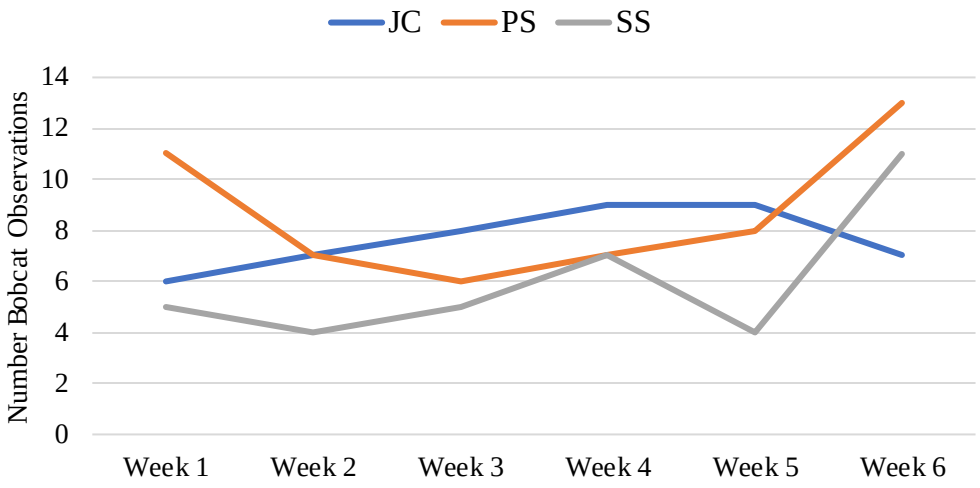

Figure 17. Number of bobcat observations by camera trap per week. All camera data pooled across all field seasons 2019-2021.

Bobcat density estimation on WMAs based on camera trap data. At James Collins WMA, we identified 12 distinct individual bobcats. The MMDM was calculated as 2038.34 m, resulting in a sampling area of 36 km^2 (Table 10). This resulted in a bobcat density estimate of 0.33 bobcats/km^2. For Sandy Sanders WMA, we identified 2 individuals and the MMDM calculated as 4883.97 m. Therefore, the sampling area was estimated at 115 km^2 and the bobcat density at 0.02 bobcats/km^2. At Packsaddle WMA we identified 14 individuals and MMDM calculated as 3143.40 m. The sampling area totalled 98 km^2, resulting in a bobcat density of 0.14 bobcats/km^2. However, at this study site there were several observations of just one flank of the animal, making it difficult to assign those images to a specific individual. Therefore, the estimated density at Packsaddle should be considered a rough estimate. To visualize potential minimum home ranges (HR) of bobcats, minimum convex polygons (MCPs) were constructed for each bobcat individual photographed at ≥3 different camera-trap locations (Fig.18-20).

Table 10. Bobcat density estimates for Wildlife Management Areas.

Study Site	Number of Bobcat Observations	Number of Individuals Identified	MMDM (km)	Sampling Area (km^2)	Bobcat Density (/km^2)
James Collins	46	12	2.04	36	0.33
Packsaddle	52	14*	3.14	98	0.14
Sandy Sanders	36	2	4.56	115	0.02

*Several images showed just one flank, so assignment to a known individual was less certain.

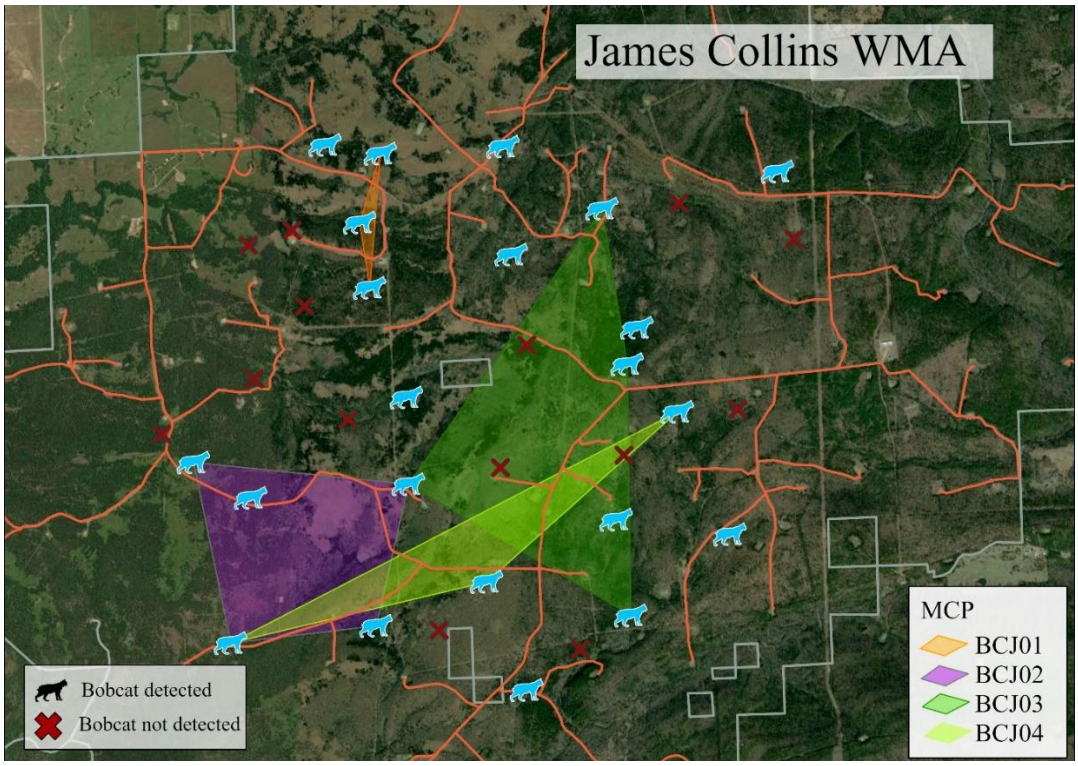

Figure18. Minimum convex polygons (MCPs) for bobcats captured ≥3 different camera-trap locations at James Collins WMA. MCPs are drawn around camera locations in which an individual was photographed. All other capture locations are denoted with a bobcat silhouette, whilst camera stations with no bobcat observations are denoted with an X.

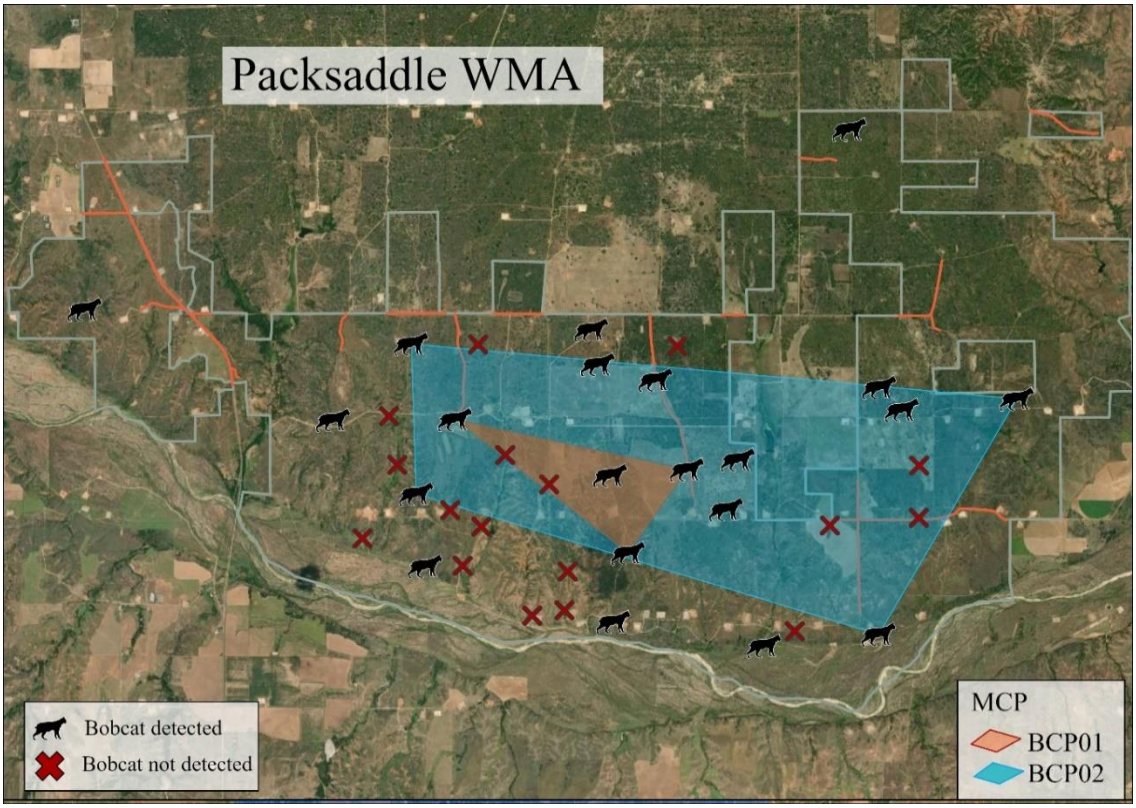

Figure19. Minimum convex polygons (MCPs) for bobcats captured ≥3 different camera-trap locations at Packsaddle WMA. MCPs are drawn around camera locations in which an individual was photographed. All other capture locations are denoted with a bobcat silhouette, whilst camera stations with no bobcat observations are denoted with an X.

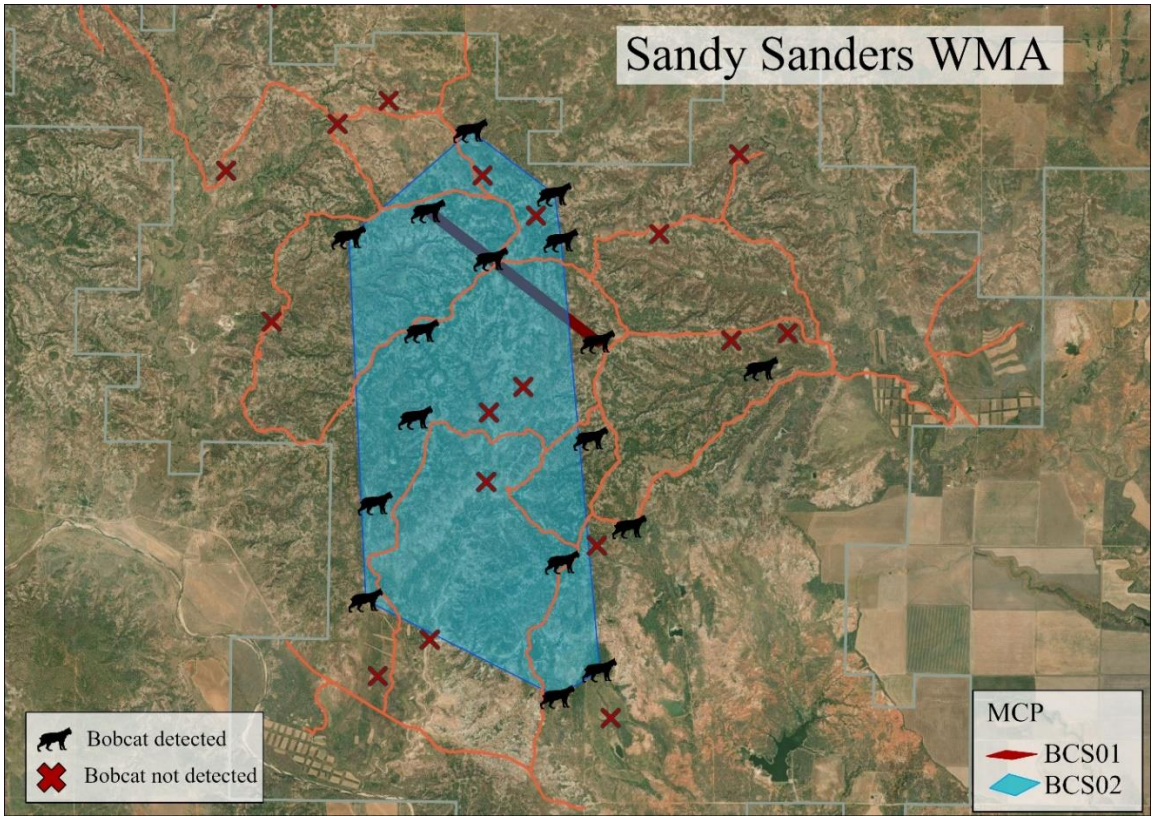

Figure 20. Minimum convex polygons (MCPs) for bobcats captured ≥3 different camera-trap locations at Sandy Sanders WMA. MCPs are drawn around camera locations in which an individual was photographed. All other capture locations are denoted with a bobcat silhouette, whilst camera stations with no bobcat observations are denoted with an X.

Activity Patterns

All meso-carnivores, as well as rabbits, were mostly nocturnal (> 60% observations). However, bobcats and coyotes had much more daytime observations than the other species, accounting for >16% of activity in both species (Table 11). These two species also had the most crepuscular activity, at 21% for bobcats and 15% for coyotes, with rabbits exhibiting 14% and raccoons 11%. Bobcats and coyotes overlapped significantly in activity patterns overall (Δ 0.88), whilst both species exhibited significantly different activities to rabbits ($p < 0.001$; Table 12), with bobcats overlapping the least with their assumed prey (Δ 0.72) compared to coyotes (Δ 0.82).

However, there were considerable differences in activity patterns between study sites. Both bobcats and coyotes showed variation in activity primarily on a west-east axis. Coyotes showed significant statistical differences in activity patterns between JC and PS (Δ 0.78, $p = 0.036$; Table 14), seeing a 21% reduction in nocturnal observations at JC compared to PS (52.83% and 74.17%, respectively), and 19% increase in daytime observations at JC compared to PS (30.19% and 10.83%, respectively). This was similar with bobcats, who showed a 15% reduction in nocturnal observations at JC compared to PS (54.35% and 69.44%, respectively), and 15% increase in daytime observations at JC compared to SS (26.09% and 11.11%, respectively), with statistically significant differences between JC and SS (Δ 0.72, $p = 0.047$; Table 13).

Of species pairs with samples sizes >20 at each study site, the greatest study-site differences in activity overlap were between coyotes and rabbits, from Δ 0.88 at PS to Δ 0.61 at JC, a difference of Δ 0.27 (Table 15). The second greatest study-site differences in activity overlap were between bobcats and coyotes, from Δ 0.92 at JC to Δ 0.78 at SS, though overlap remained high. Bobcats also showed large overlap coefficient shifts with rabbits, from Δ0.77 at PS to Δ0.64 at JC. These results show that activity pattern overlap is high among bobcats and coyotes, but highest at JC, where both species have increased daytime activity and asynchrony with their supposed prey. Both species overlapped to the most extent with rabbits at PS, though coyote-rabbit overlap was stronger than bobcat-rabbit, with coyote-rabbit being the highest overlap among species pairs at PS.

Table 11. Number of observations at each diel activity period, based on NOAA solar calculations.

Species	Study site	Day	Twilight	Night
Bobcat	JC	12	9	25
	PS	8	12	32
	SS	4	7	25
Coyote	JC	16	9	28
	PS	13	18	89
	SS	13	12	60
Rabbit	JC	1	11	76
	PS	2	6	71
	SS	4	24	89

Table 12. Overlap and statistical differences in activity periods between bobcats (*Lynx rufus*), coyotes (*Canis latrans*), and rabbits (*Sylvilagus* spp.), from data collected across all study sites 2019-2021, using coefficient of overlap (Δ) from 999 replications. Statistically significant ($\alpha = 0.05$) differences in activity from null are highlighted in grey.

Species Pair	**Observed Overlap**	**Null Overlap**	**Standard Error**	**P-value**
bobcats and coyotes	0.8797	0.8934	0.0260	0.2803
bobcats and rabbits	0.7234	0.9017	0.0253	0.0000
rabbits and coyotes	0.8152	0.9316	0.0210	0.0000

Table 13. Study site differences in bobcat (*Lynx rufus*) activity periods from data pooled from all field seasons, using coefficient of overlap (Δ) from 999 replications. Statistically significant differences in activity are highlighted in grey.

	Observed Overlap	**Mean Overlap**	**Standard Error**	**P-value**
JC vs PS	0.8374	0.8425	0.0500	0.4164
JC vs SS	0.7207	0.8284	0.0556	0.0413
PS vs SS	0.8094	0.8101	0.0538	0.4537
East vs West	0.7954	0.8612	0.0452	0.0815

Table 14. Study site differences in coyote (*Canis latrans*) activity periods from data pooled from all field seasons, using coefficient of overlap (Δ) from 999 replications. Statistically significant differences in activity are highlighted in grey.

	Observed Overlap	**Mean Overlap**	**Standard Error**	**P-value**
JC vs PS	0.7818	0.8770	0.0388	0.0180
JC vs SS	0.8043	0.8675	0.0429	0.0806
PS vs SS	0.8413	0.8800	0.0336	0.1241
East vs West	0.8007	0.8836	0.0380	0.0270

Table 15. Coefficient of overlap ($\hat{\Delta}_4$) for species pairs (data pooled 2019-2021) at each study site. Species with < 20 observations at a study site were excluded from comparisons (opossums at SS).

species pair	JC	PS	SS
bobcat-coyote	0.920	0.836	0.779
bobcat-rabbit	0.642	0.771	0.707
bobcat-skunk	0.636	0.679	0.725
bobcat-raccoon	0.692	0.740	0.717
bobcat-opossum	0.559	0.666	
coyote-rabbit	0.608	0.879	0.819
coyote-skunk	0.614	0.733	0.738
coyote-raccoon	0.648	0.781	0.768
coyote-opossum	0.544	0.625	
rabbit-skunk	0.847	0.783	0.805
rabbit-raccoon	0.760	0.836	0.850
rabbit-opossum	0.631	0.647	
skunk-raccoon	0.812	0.782	0.887
skunk-opossum	0.675	0.740	
raccoon-opossum	0.784	0.784	

Key
>0.90
>0.85
>0.80
>0.75
0.55-0.75
<0.55
<0.50

Results of Small Mammal Trapping

Over 21,087 trapping nights, we achieved 1212 captures of 832 unique individuals, with a trap success rate of 5.75%. Mortality rate was 5.08% of all captured individuals (5.53% of all capture events). The majority (>70%) of captures were of deer mice (*Peromyscus* spp.), which were found in abundance at all three study sites and were found in every landcover type (Tables 16 & Appendix V). *Reithrodontomys* spp. were also observed in large frequencies and were found at most habitat types (≥1 capture at 80% of landcover types), but were most abundant in grassland/prairie landcover (see Appendix V).

Table 16. Number of captures of novel individuals (does not include recaptures) of each small mammal classification over two field seasons, 2020 and 2021.

Classification	Genus/Species	2020	2021	Total
deer mouse	*Peromyscus* spp.	313	256	569
harvest mouse	*Reithrodontomys* spp.	81	51	132
grasshopper mouse	*Onychomys leucogaster*	16	20	36
hispid cotton rat	*Sigmodon hispidus*	31	3	34
small pocket mouse	*Perognathus* spp.	1	16	17
wood rat	*Neotoma* spp.	6	6	12
ground squirrel	*Ictidomys spp. / Xerospermophilus spp.*	6	4	10
kangaroo rat	*Dipodomys ordii*	7	2	9
hispid pocket mouse	*Chaetopidus hispidus*	2	5	7
shrew	*Blarina* spp. / *Cryptotis parva*	4	0	4
marsh rice rat	*Oryzomys palustris*	0	1	1
meadow jumping mouse	*Zapus hudsonius*	1	0	1

Small mammal abundances and community composition. For James Collins WMA, three models were selected as showing equal support; one which included only transect ID as a random effect, one which also included average maximum temperature, and on which also included average minimum temperature. As we wanted to control for heterogeneity in capture probabilities created by incidental weather conditions, the selected best model included average maximum temperature as the random effect, as this also had higher variance than average minimum temperature. Subsequent rodent abundance prediction were based on this model. The model showed significantly more rodent individuals in grassland habitats compared all other habitats (all $p < 0.03$; Table 17) and predicted 13 individuals captured on a given grassland transect, whilst the other three habitat predictions ranged from 3 to 6 individuals (Table 18). For Packsaddle, two models were selected as showing equal support; one which included only transect ID as a random effect, and one which also included average minimum temperature. For the same reason as mentioned above, I the latter model was selected as the best model, which showed significantly fewer rodents within riparian habitat compared to shinnery shrubland and sandy prairie (Table 17). Model predictions showed 13 and 12 individuals for transects of the latter habitat types, respectively, whilst only 4 individuals for riparian (Table 18).

For Sandy Sanders WMA, four models selected as showing equal support; one which included only transect ID as a random effect, one which also included total precipitation, one which also included average maximum temperature, and one which also included average minimum temperature. We selected the one which included the average maximum temperature, as it was judged to be the most biologically meaningful. In all cases, across all sites, selection of any one of the shortlisted models resulted in only the most minor effects to coefficient, error and probability values, so we feel making judgement calls on the best model did not affect the outcome of the analysis. Shinnery shrubland exhibited significantly higher numbers of rodent individuals (Table 17), with a predicted 18 individuals per transect, compared to 7 for each of the other two habitat types (Table 18). No issues were found for any of the models during diagnostic testing.

Lastly, comparisons across all study sites saw three models showing equal support, all with transect as a fix effect, one with average minimum temperature and one with average maximum temperature. No significant differences were found among study sites (Table 19), and predicted abundances range from 0.23 at JC to 0.34 at SS.

Table 17. Best models fixed effects outputs for each of the study site analyses. Model intercepts are italicized.

Model	Coefficient (Habitat Type)	Estimate	Std. Error	z value	Pr(>|z|)
J3	*Grassland*	-0.7946	0.2754	-2.886	0.00391**
(JC site)	Dry Oak Woodland	-1.4358	0.631	-2.275	0.02289*
	Oak-Pine Forest	-1.1974	0.4117	-2.908	0.00363**
	Riparian	-0.7804	0.3593	-2.172	0.02986*
PNB4	*Riparian/ Bottomland*	-2.0728	0.2511	-8.255	< 2e-16***
(PS site)	Sandy Prairie	1.1382	0.2993	3.803	0.000143***
	Shinnery Shrubland	1.2465	0.3586	3.476	0.000509***
SNB3	*Grassland/ Prairie*	-1.5071	0.2613	-5.768	8.01E-09***
(SS site)	Riparian/ Bottomland	0.1057	0.3076	0.344	0.73104
	Juniper/ Mesquite Shrubland	1.0146	0.3157	3.214	0.00131***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

No significant differences in metric values among landcover types were found at James Collins or Sandy Sanders WMAs (Table 20; Fig. 1). However, both species richness and Shannon diversity were significantly different among landcover types at Packsaddle, with riparian/bottomland exhibiting significantly lower metric values (Table 20). Overall, Packsaddle WMA had more significantly higher species richness and Shannon diversity than did James Collins and Sandy Sanders (Table 21).

Table 18. Model predictions for each habitat type based on best respective GLMM.

Site	Habitat Type	N individuals per station	N individuals per transect (rounded to integer)
James Collins WMA	Grassland	0.452	13
	Dry Oak Woodland	0.107	3
	Oak-Pine Forest	0.136	4
	Riparian	0.207	6
Packsaddle WMA	Riparian/ Bottomland	0.126	4
	Sandy Prairie	0.393	12
	Shinnery Shrubland	0.438	13
Sandy Sanders WMA	Grassland/ Prairie	0.222	7
	Riparian/ Bottomland	0.246	7
	Juniper/ Mesquite Shrubland	0.611	18

Table 19: Negative binomial GLMM output for describing the effect of study site on small mammal abundances, with transect ID and average minimum temperature as a random effect (model intercept is shown parenthetically). Predicted abundances are also shown for each respective study site.

Coefficient	Estimate	Std. Error	z value	Pr(>|z|)	Predicted abundance
(Site: JC)	-1.38195	0.205539	-6.724	1.77E-11*	0.2275
Site: PS	0.002979	0.303983	0.01	0.992	0.2508
Site: SS	0.242843	0.295018	0.823	0.41	0.3384

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 21: Analysis of variance tables for linear models describing the relationship between habitat types within each study site and community metrics (species richness and Shannon diversity).

Site	Metric	DF	Sum Sq	Mean Sq	F value	Pr(>F)
JC	diversity	3	0.08527	0.028422	0.2652	0.8498
	richness	3	1.4185	0.47284	0.5502	0.6531
PS	diversity	2	3.5472	1.77361	17.935	<0.0001***
	richness	2	25	12.5000	11.413	<0.0004***
SS	diversity	2	0.46131	0.23065	1.7225	0.2029
	richness	2	2.3333	1.16667	1.3901	0.2711

Table 21. Outputs from linear models describing the relationship between study sites and community metrics (Shannon diversity, abundance, and species richness). Model intercepts are shown parenthetically.

Metric	Coefficient	Estimate	Std. Error	z value	Pr(>|z|)
Shannon	(Site: PS)	0.56567	0.08139	6.95	1.37E-09***
	Site: SS	-0.21944	0.11511	-1.906	0.0606.
	Site: JC	-0.22803	0.11186	-2.038	0.0452*
Abundance	(Site: PS)	10.4583	1.7629	5.933	9.55E-08***
	Site: SS	1.0417	2.4931	0.418	0.677
	Site: JC	-0.6435	2.4228	-0.266	0.791
Richness	(Site: PS)	2.5	0.2271	11.007	<2e-16***
	Site: SS	-0.7083	0.3212	-2.205	0.0306*
	Site: JC	-0.7593	0.3122	-2.432	0.0175*

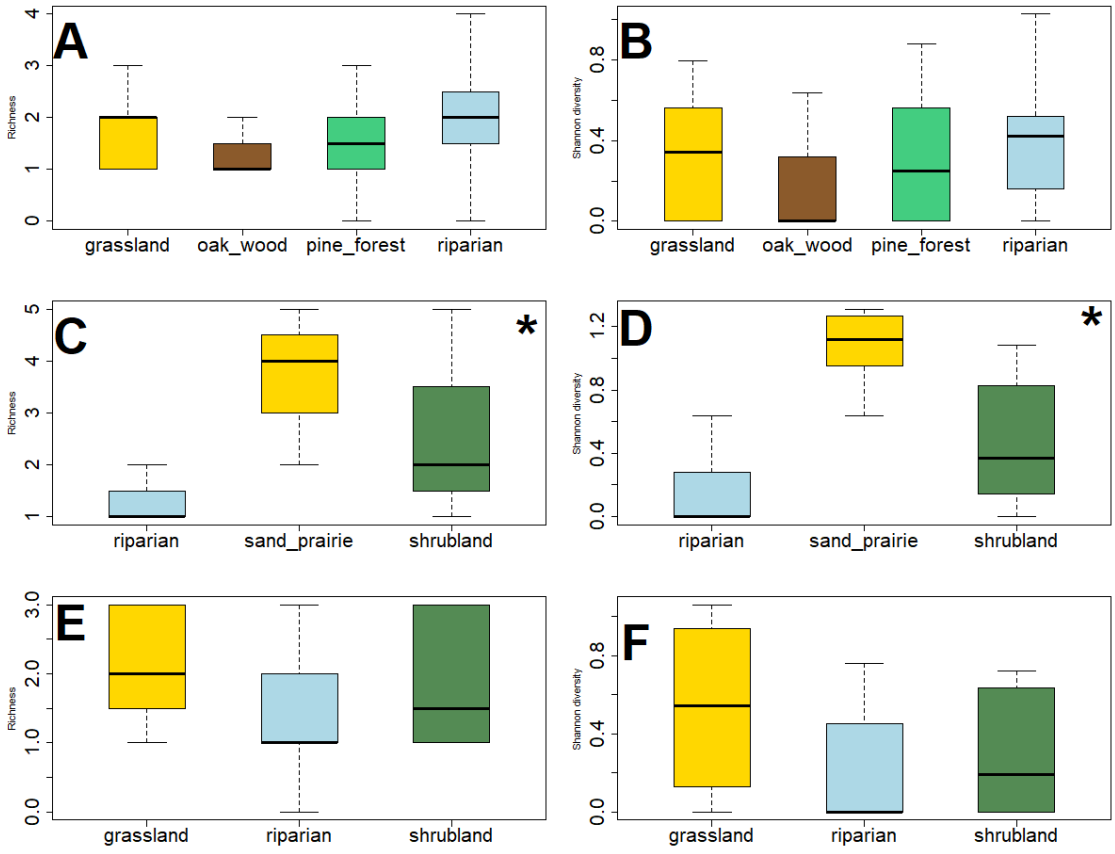

Figure 21. Model outputs from linear models, showing species richness (A, C, E) and Shannon diversity (B, D, F) values across respective habitat types for JC (A, B), PS (C, D), and SS (E, F). Significant differences in community metric values between study sites are denoted with asterisks.

DISCUSSION

Objective 1

The occupancy study had high volunteer participation, with an average of 21 volunteers per year. The advent of the pandemic in 2020 complicated recruitment for the 2020/21 field season, with fewer students participating. Because of this, we supplied more cubbies and equipment per volunteer for this season, which meant increased responsibility for those volunteers, but this did not seem to have affected results substantially. The interest generated in the project by visiting Oklahoma universities and recruiting volunteers was substantial, indicating the feasibility of using wildlife student volunteers as citizen-scientists for large-scale research projects, given the right tools.

In terms of hair-snare cubby performance, the snag rates for obtaining mammalian hair were high. However, the main issue was identifying these hairs to species. Morphologically, identification consensuses were comparatively rare, with many hair samples being identified as different species by different technicians. When compared to genetics results, we showed that the accuracy of these species identifications were low. It may be the case that morphological identification methods are not suitable for inexperienced identifiers, who rely on only dichotomous keys and reference guides, and may only be useful to professionals with years of experience working with hair samples (e.g. museum specimens). In addition, the fact that the study objectives were known by technicians (i.e. a bobcat study) may have biased results, resulting in far more bobcat identifications than would be expected, leading to many false positive bobcat detections.

In terms of the genetic analyses of occupancy-study hair samples, amplification rates were low (30%), with bobcats only accounting for 25% of genetic identifications. By-catches were very common, particularly opossums (23%), which may particularly be attracted to the food-gland scent lure we used for hair-snare cubbies. Other common by-catch species were domestic animals (38%), such as cattle and dogs. These high rates of domestic species may be a result of increased placement upon private lands, including ranches and suburban areas. Our hair-snare cubby results are contrasted by their use in West Virginia (Rounsville, 2018), which saw 62% of hair samples obtained suitable for genetic analysis, 61% of bobcat hairs successfully genotyped, and an overall bobcat trapping rate of 0.9/100 trapping nights. However, our results more closely matched the success rates of other bobcat hair-snare studies (Long et al. 2007, García-Alaníz et al. 2010, White 2010).

Occupancy modelling efforts were weakened by insufficient, high-quality data. For occupancy models to work best, there needs to be sufficient heterogeneity among bobcat presence/absence to detect patterns of occupancies. It is likely the case that we did not have enough presence/absence data, nor enough deployment locations and replications within representative habitats of Oklahoma, to accurately predict bobcat occupancy at the state-wide scale. It is also possible that we cannot accurately account for the large variation in cubby deployments made by volunteers, including inconsistent deployment durations and proximities, in order to account for variation in detection probability. These issues resulted in poor model fit to the data, both in the occupancy models and GLMs. However, theses analyses may provide a heavily caveated insight into the sort of relationship bobcats have with land cover types in Oklahoma, including an affinity for agricultural and suburban land, but an aversion to evergreen forests and high development areas. More (and higher quality) data is needed to explore this in more detail.

Objective 2

Similar to hair-snare cubbies used in the occupancy study, cubbies deployed on WMAs saw good snag rates for mammalian hair, but the number of hairs we could identify to species was small. We were able to identify only 39 hair samples to species using mtDNA, and a further 10 by genotyping using bobcat primers (total = 18.35% of hair samples). Bobcats accounted for <30% of mtDNA-identified hairs, whilst, similar to the occupancy study, opossums were the most common by-catch species. Despite their placement on WMAs, domestic animals still accounted for >20% of IDs, most likely from ranch cattle and hunting dogs. Due to the difficulties genetically analyzing bobcat hair, we used camera trap data to estimate bobcat densities on the WMAs. Camera traps outperformed hair-snare cubbies, not only with increased detection rate of bobcats, but also increasing the types of data we could obtain (density estimates, activity patterns, sympatric species data). For species with distinct pelage patterns, such as bobcats, camera traps may be far more efficient for any capture-recapture analyses in which individuals are identified. In addition, in contrast to hair-snare cubbies used in West Virginia (Rounsville et al. 2022), we had a high number of recaptures (Table 10).

Density estimates showed bobcats were more densely distributed at our eastern study site (James Collins WMA), which is characterized by oak-hickory-pine woodland. This density is higher than previous estimates in southeastern Oklahoma (Rolley 1983; 0.01/km^2) and western Arkansas (Rucker et al. 1989; 0.10/km^2), but similar to those found in eastern Texas (Symmank et al. 2008 = 0.29 - 0.58/km^2; Lombardi et al. 2017 = 0.48/km^2), and previous estimates from Illinois (Jacques et al. 2019 = 0.31/km^2). This is contrasted by estimated densities at Sandy Sanders WMA, with as few as 0.02 bobcat/km^2. This aligns with previous research within the Texas Panhandle (Thurmond 2014), with estimated home ranges sizes as large as 70 - 407km^2 for males and 55 – 204km^2 for females. Packsaddle WMA showed higher bobcat density estimates than Sandy Sanders WMA, aligning closely with more recent estimates from Illinois (Jacques et al. 2019 = 0.14/km^2) and Northern Texas (Thornton and Pekins 2015 = 0.13/km^2). In relation to the small mammal communities, we quantified at these study sites, increased bobcat density at Packsaddle WMA compared to Sandy Sanders WMA may be due to the higher small mammal diversity and species richness present at the study site. Whilst Sandy Sanders WMA had higher small mammal abundances, 95% were smaller-bodied rodents (*Peromyscus* spp., *Reithrodontomys* spp., and *Perognathus* spp.), whilst Packsaddle WMA had more medium and large rodents, including grasshopper mice, ground squirrels, and kangaroo rats. Although Packsaddle's small mammal diversity and species richness was higher than James Collins, and James Collins had the lowest predicted abundances of small mammals, the James Collins study site had an abundance of gray and fox squirrels, which may make up a considerable portion of bobcat diet (Fritts and Sealander 1978, Rolley and Warde 1985).

Additional Data

Throughout the hair-snare and small mammal trapping field seasons of 2020 and 2021, we collected >240 mesocarnivore scats using an incidental collection approach. However, this method increased the proportion of scats collected that were old, and thus had highly degraded DNA, making them unsuitable for genetic analyses. However, our low success rate at amplifying DNA from scats is surprising, especially considering our ethanol preservation method in the 2021 collection season. Perhaps given more time to refine the genetic protocol (hair samples were prioritized above scats during laboratory work) we may have been able to amplify a larger proportion of samples. Any subsequent research using bobcat scats in Oklahoma, may benefit from the use of scat detection dogs. Studies have found that scat detection dogs increase genetic success, proving far more cost-effective than hair-snares (Harrison 2012,

Long et al. 2007, Ruell and Crooks 2007, Adams 2009, White 2010) and sometimes more than camera traps (Long et al. 2007, Harrison 2012).

Activity pattern analysis showed both bobcats and coyotes were more diurnal at James Collins WMA compared to western study sites. This, as with the small mammal findings, may suggest bobcats may supplement their diet with squirrels (as well as other diurnal prey) in these areas. It may also be a response to the increased vegetation cover present there, allowing bobcats to be more active during the day without being exposed. Bobcats and coyotes overlap substantially in their diel activity patterns, suggesting temporal partitioning of niche is not the primary source of niche segregation among the two mesocarnivore species. There appears to be considerable activity period differences in both species on a west-east axis; some temporal and dietary behaviors may result in differing degrees in niche overlap between bobcats and coyotes. Coyote activity overlapped greatly with rabbits at PS, suggesting this may be a common source of prey for coyotes in the study region. Bobcat activity was largely asynchronous with the activity patterns of rabbits, particularly at James Collins WMA.

MANAGEMENT RECOMMENDATIONS

Using a new hair-snare method developed for bobcats in West Virginia (Rounsville, 2018), we tested its use in Oklahoma to 1) monitor bobcat populations state-wide and 2) estimate bobcat densities on 3 WMAs representing 3 different ecoregions of Oklahoma. The hair-snare cubbies were successful in collecting mammal hair from a wide variety of locations throughout the state, and generated enthusiasm for wildlife research by involving undergraduate students from a large number of Oklahoma institutions. However, the accuracy of identifying hairs to species by technicians in the OSU lab, when tested against genetic identification, was not high enough to use as a stand-alone monitoring technique. Perhaps with a highly trained technician from a forensics or museum laboratory, the method would be more reliable. Combining both conservative morphological and genetic identifications improved our ability to use occupancy modeling at the state-wide scale and develop probability of use maps for the state, but the standard error of the occupancy predictions in the southeast in a few key areas (southeast and a swath of west-central Oklahoma) made predictions in those areas unreliable. Using more targeted placement of cubbies across the state would improve this method for monitoring, however, the low success rate of morphological and genetic identification of bobcats indicates that occupancy modeling using camera-trap methods would be a more promising monitoring method. Developing and advertising a platform on which the general public could submit trail camera photos of bobcats, supplemented with camera stations established by agency personnel could be used to gather presence/absence data for occupancy modeling statewide.

Because of the difficulty of using genetic methods with bobcats, particularly bobcat hair, a situation confirmed by other geneticists recently, we resorted to using data from our camera traps and individual identification based on unique natural markings to estimate bobcat density on Sandy Sanders, Packsaddle, and James Collins WMAs. This method would work well for targeted population/density estimates, but in particularly sparse areas, such as Sandy Sanders WMA, a greater number of cameras should be used.

Significant Deviations:
None.

Equipment Purchased:
None.

Attachments:

1. Ph.D. Dissertation for Nathan Proudman - Assessing the Distribution, Abundances, And Ecology of Bobcats (*Lynx rufus*) in Oklahoma
2. M.Sc. Thesis for Timothy McSweeny – Observations on the Genetic Diversity of Bobcat (*Lynx rufus*) Populations in Oklahoma

Prepared by: Nathan Proudman and Sue Fairbanks, Oklahoma State University

Approved by: Russ Horton, Assistant Chief of Wildlife
Oklahoma Department of Wildlife Conservation

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Oklahoma Department of Wildlife Conservation

LITERATURE CITED

Adams, L. M. 2009. Use of Non-Invasive Surveys to Validate Predicted Bobcat (Lynx rufus) Habitat Distribution in Wisconsin from Landscape-Scale GIS Information. Thesis, University of Wisconsin Stevens Point, College of Natural Resources. <https://minds.wisconsin.edu/handle/1793/81345>. Accessed 13 Feb 2023.

Bailey, T. N. 1974. Social Organization in a Bobcat Population. The Journal of Wildlife Management 38:435–446.

Davison, A., J. D. S. Birks, R. C. Brookes, T. C. Braithwaite, and J. E. Messenger. 2002. On the origin of faeces: morphological versus molecular methods for surveying rare carnivores from their scats. Journal of Zoology 257:141–143.

Dewitz, J. (2021). National Land Cover Database (NLCD) 2019 Products [Data set]. U.S. Geological Survey. https://doi.org/10.5066/P9KZCM54

Diamond, D. D., and L. F. Elliott. 2015. Oklahoma ecological systems mapping interpretive booklet: Methods, short type descriptions, and summary results. Oklahoma Department of Wildlife Conservation, Norman, OK.

Efford, M. (2020). secr: Spatially explicit capture-recapture models. https://CRAN.R-project.org/package=secr

Fritts, S. H., and J. A. Sealander. 1978. Diets of Bobcats in Arkansas with Special Reference to Age and Sex Differences. The Journal of Wildlife Management 42:533–539.

García-Alaníz, N., E. J. Naranjo, and F. F. Mallory. 2010. Hair-Snares: A Non-Invasive Method for Monitoring Felid Populations in the Selva Lacandona, Mexico. Tropical Conservation Science 3:403–411.

Harrison, R. L. 2002. Evaluation of Microscopic and Macroscopic Methods to Identify Felid Hair. Wildlife Society Bulletin (1973-2006) 30:412–419.

Harrison, R. L. 2012. From the Field: A Comparison of Survey Methods for Detecting Bobcats. Wildlife Society Bulletin 34:548–552.

Hansen, K. 2012. Bobcat: master of survival. Oxford University Press, Oxford; New York.

Jacques, C. N., R. W. Klaver, T. C. Swearingen, E. D. Davis, C. R. Anderson, J. A. Jenks, C. S. Deperno, and R. D. Bluett. 2019. Estimating density and detection of bobcats in fragmented midwestern landscapes using spatial capture–recapture data from camera traps. Wildlife Society Bulletin 43:256–264.

Kitchener, A. C., C. Breitenmoser-Würsten, E. Eizirik, A. Gentry, L. Werdelin, A. Wilting, N. Yamaguchi, A. V. Abramov, P. Christiansen, C. Driscoll, J. W. Duckworth, W. E. Johnson, S. J. Luo, E. Meijaard, P. O'Donoghue, J. Sanderson, K. Seymour, M. Bruford, C. Groves, M. Hoffmann, K. Nowell, Z. Timmons, and S. Tobe. 2017. A revised taxonomy of the Felidae : The final report of the Cat Classification Task Force of the IUCN Cat Specialist Group. Cat News 38–40.

Knick, S. T. 1990. Ecology of Bobcats Relative to Exploitation and a Prey Decline in Southeastern Idaho. Wildlife Monographs 3–42.

Lombardi, J. V., C. E. Comer, D. G. Scognamillo, and W. C. Conway. 2017. Coyote, fox, and bobcat response to anthropogenic and natural landscape features in a small urban area. Urban Ecosystems 20:1239–1248.

Long, R. A., T. M. Donovan, P. Mackay, W. J. Zielinski, and J. S. Buzas. 2007. Comparing Scat Detection Dogs, Cameras, and Hair Snares for Surveying Carnivores. The Journal of Wildlife Management 71:2018–2025.

Macdonald, D. W. 1980. Patterns of scent marking with urine and faeces amongst carnivore communities. Symposia of the Zoological Society of London 45:107–139.

Morin, D. J., S. D. Higdon, J. L. Holub, D. M. Montague, M. L. Fies, L. P. Waits, and M. J. Kelly. 2016. Bias in carnivore diet analysis resulting from misclassification of predator scats based on field identification. Wildlife Society Bulletin 40:669–677.

Pearson, D. E., and L. F. Ruggiero. 2003. Transect versus Grid Trapping Arrangements for Sampling Small-Mammal Communities. Wildlife Society Bulletin (1973-2006) 31:454–459.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.

Rolley, R. E. 1983. Behavior and population dynamics of bobcats in Oklahoma. Doctoral dissertation, Oklahoma State University. <https://shareok.org/bitstream/handle/11244/19427/Thesis-1983D-R752b.pdf?sequence=1>. Accessed 2 Jul 2019.

Rolley, R. E., and W. D. Warde. 1985. Bobcat Habitat Use in Southeastern Oklahoma. The Journal of Wildlife Management 49:913.

Rounsville Jr., T. F., R. E. Rogers, A. B. Welsh, C. W. Ryan, and J. T. Anderson. 2022. Novel hair snare and genetic methods for non-invasive bobcat detection. Ecology and Evolution 12:e8435.

RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC. Boston, MA http://www.rstudio.com/.

Rucker, R. A., M. L. Kennedy, G. A. Heidt, and M. J. Harvey. 1989. Population Density, Movements, and Habitat Use of Bobcats in Arkansas. The Southwestern Naturalist 34:101–108.

Ruell, E. W., and K. R. Crooks. 2007. Evaluation of Noninvasive Genetic Sampling Methods for Felid and Canid Populations. The Journal of Wildlife Management 71:1690–1694.

Symmank, M. E., C. E. Comer, and J. C. Kroll. 2008. Estimating Bobcat Abundance in East Texas Using Infrared-triggered Cameras. 7.

Thornton, D. H., and C. E. Pekins. 2015. Spatially explicit capture–recapture analysis of bobcat (Lynx rufus) density: implications for mesocarnivore monitoring. Wildlife Research 42:394.

Thurmond, L. M. 2014. Spatial ecology of bobcats in a Texas High Plains ecosystem. Master's Thesis, West Texas A&M University, Canyon, Texas. USA.

White, J. M. 2010. Development of noninvasive genetic techniques to monitor elusive carnivores; A case study of bobcats (Lynx rufus) in the Northern Lower Peninsula, Michigan. M.S., Michigan State University, United States -- Michigan. <https://search.proquest.com/docview/871202075/abstract/9CEFBBCD66D442BEPQ/1>. Accessed 7 Nov 2020.

APPENDICES

Appendix I. Landcover types of Sandy Sanders, Packsaddle, and James Collins WMAs reclassified from Diamond and Elliot (2015).

Sandy Sanders WMA

Landcover Type for this Project	Vegetation Classification (OKECOS)
Juniper/ Mesquite Shrubland	Canyon: Deciduous Shrubland
	Canyon: Gyp Deciduous Shrubland
	Canyon: Gyp Juniper Shrubland
	Canyon: Gyp Mesquite Shrubland
	Canyon: Juniper Shrubland
	High Plains: Mesquite Shrubland
	High Plains: Sandy Deciduous Shrubland
Prairie/Grassland	Canyon: Grassland
	Canyon: Gyp Grassland
	Central Mixedgrass: Prairie/Pasture
	Central Mixedgrass: Sandy Prairie/Pasture
Riparian/Bottomland	Eastern Great Plains: Herbaceous Wetland
	High Plains: Bottomland Barrens
	High Plains: Bottomland Deciduous Shrubland
	High Plains: Bottomland Eastern Redcedar Woodland and Shrubland
	High Plains: Bottomland Hardwood Forest
	High Plains: Bottomland Herbaceous Wetland
	High Plains: Depression Herbaceous Wetland
	High Plains: Riparian Barrens
	High Plains: Riparian Deciduous Shrubland
	High Plains: Riparian Eastern Redcedar Woodland and Shrubland
	High Plains: Riparian Hardwood Woodland
	High Plains: Riparian Herbaceous Wetland
	High Plains: Riparian Mixed Hardwood - Eastern Redcedar Woodland
Ruderal Deciduous Shrubland	Ruderal Deciduous Shrubland and Young Woodland
	Ruderal Deciduous Woodland
	Ruderal Eastern Redcedar Woodland and Shrubland
	Ruderal Mesquite Shrubland
	Ruderal Mixed Deciduous - Eastern Redcedar Woodland
	Ruderal Plains Shrubland

Packsaddle WMA

Landcover Type for this Project	Vegetation Classification (OKECOS)
Riparian/Bottomland	Eastern Great Plains: Herbaceous Wetland
	High Plains: Bottomland Deciduous Shrubland
	High Plains: Bottomland Hardwood Forest
	High Plains: Bottomland Herbaceous Wetland
	High Plains: Riparian Deciduous Shrubland
	High Plains: Riparian Hardwood Woodland
Ruderal Deciduous Shrubland	Ruderal Deciduous Shrubland and Young Woodland
	Ruderal Deciduous Woodland
	Ruderal Eastern Redcedar Woodland and Shrubland
	Ruderal Mixed Deciduous - Eastern Redcedar Woodland
	Ruderal Plains Shrubland
Sandy Prairie/Grassland	Canyon: Grassland
	Central Mixedgrass: Prairie/Pasture
	Central Mixedgrass: Sandy Prairie/Pasture
	High Plains: Sand Prairie
Shinnery Shrubland	Canyon: Deciduous Shrubland
	Canyon: Juniper Shrubland
	High Plains: Sandhill Shinnery Shrubland
	High Plains: Sandhill Shrubland
	High Plains: Sandy Deciduous Shrubland

James Collins WMA

Landcover Type for this Project	Vegetation Classification (OKECOS)
Oak – Pine Forest	Osark-Ouachita: Dry Mixed Oak – Evergreen Woodland
	Osark-Ouachita: Dry Oak Woodland
	Osark-Ouachita: Dry Oak Woodland Young Regrowth
	Osark-Ouachita: Dry-Mesic Mixed Oak – Evergreen Forest
	Osark-Ouachita: Dry-Mesic Oak Woodland Young Regrowth
	Osark-Ouachita: Shortleaf Pine – Oak Forest
Bottomland/ Riparian	Osark-Ouachita: Riparian Deciduous Shrubland and Young Woodland
	Osark-Ouachita: Riparian Evergreen Woodland and Shrubland
	Osark-Ouachita: Riparian Hardwood Wetland

	Osark-Ouachita: Riparian herbaceous Wetland
	Osark-Ouachita: Riparian Mixed Evergreen – Hardwood Wetland
	South Central Interior: Bottomland Barrens
	South Central Interior: Bottomland Eastern Redcedar Woodland
	South Central Interior: Bottomland Hardwood Forest
	South Central Interior: Bottomland Mixed Evergreen – Hardwood
	South Central Interior: Bottomland Shrubland and Young Woodland
	South Central Interior: Riparian Eastern Redcedar Woodland and Shrubland
	South Central Interior: Riparian Hardwood Woodland
	South Central Interior: Riparian Mixed Evergreen – Hardwood Forest
	South Central Interior: Riparian Shrubland and Young Woodland
Ruderal Deciduous Woodland	Ruderal Deciduous Shrubland and Young Woodland
	Ruderal Deciduous Woodland
	Ruderal Eastern Redcedar Woodland and Shrubland
	Ruderal Mixed Deciduous – Eastern Redcedar Woodland

Appendix II. Thermal profiles used in PCR for 5 primers.

Primer	Initial	Thermal Profile
FurND1	95°C 5min	35 cycles of; 95°C 30sec, 53°C 30 sec, 72°C 2min. Followed by 72°C 5min
BOBCATSPID	94°C 5min	35 cycles of; 94°C 1min, 50°C 1min, 72°C 1min20. Followed by 72°C 10min
BC50	94°C 2min	35 cycles of; 94°C 1min, 50°C 1min, 72°C 1min. Followed by 72°C 7min
BOBCATNEW22	94°C 5min	30 cycles of; 94°C 30sec, 50°C 20sec, 72°C 1min. Followed by 72°C 5min
CYTB52	94°C 2min	40 cycles of; 92°C 15sec, 52°C 1min, 72°C 1min10. Followed by 72°C 10min

Appendix III. Genotyping results for hair and scat samples, showing peak heights at each genetic locus. Question marks indicate uncertainty.

		locus									
Sample ID	Sample Type	BCE5T	BCE5T	FCA77	FCA77	FCA90	FCA90	BC1AT	BC1AT	FCA96	FCA96
JC007 4A98 20	hair sub-sample			139	139			318	318		
JC007 4A99 20	hair sub-sample			139	139			342	349		
JC015 B1? 19	hair sub-sample	268	268	139	139	101	101	340?	340?		
JC021 3A 20	hair sub-sample	296	296					324	324	177	177
JC021 3B 20	hair sub-sample	289	289?	114	114?			324?	349?	185	200
JC031 5A 21	hair sub-sample			142	142						
JC034 2B 20	hair sub-sample	273	276	142	142			302	318	182	182
JC034 2C 20	hair sub-sample	273	276	142	142	106	109	302	318	176	184
JC034 2D 20	hair sub-sample	273	276	142	142			302	310	190	190
PS041 1A1 20	hair sub-sample			141	141			306	306	180	194
PS043 2B2 21	hair sub-sample					98	106				
PS043 2D 21	hair sub-sample			142	142	98	106	306	306		
PS049 1A 21	hair sub-sample			140	142						
PS059 4B8 20	hair sub-sample					106?	110?				
PS059 5A1 20	hair sub-sample					106?	106?				
PS064 2A 21	hair sub-sample	283	283			102	106?			182	182
PS064 2B 21	hair sub-sample	283	283			102	106			182	182
PS067 1A 21	hair sub-sample			139	139			310	310	190	190
PS067 4B2 20	hair sub-sample	257	257	139	141					180	180
PS069 1A 21	hair sub-sample			137	141	101	101	302	310		
PS069 1B 21	hair sub-sample			137	141	107	107			186	192
PS072 2D2 21	hair sub-sample			128	139	101	103				
SS091 3A 21	hair sub-sample			142	142						
SS096 1A 19	hair sub-sample			137	139	101	101			190	194
SS111 1A1 20	hair sub-sample	273	273	130	130			306	306		
SS111 1A1P 20	hair sub-sample			142	137						
SS111 4A 20	hair sub-sample			142	142						

SS111 6A1 21	hair sub-sample			142	142	108	108			182	198?
SS116 5A 19	hair sub-sample	265	265	139	146					166	166
SS120 4D2 20	hair sub-sample			134	144	106?	106?	318	318	182	182
SS120 4D2(2) 20	hair sub-sample	257	268	134	144	106	106	318	318	182	182
SS120 6A1 21	hair sub-sample			144	144					182	182
SS120 6B1 21	hair sub-sample					104	104				
MJ109	scat sub-sample			139	144	103	103			188?	194?
MJ125	scat sub-sample	261	273								
MJ207	scat sub-sample			139	141	99	101?			182?	190?
MS208	scat sub-sample			136	141			300	300		
MS214	scat sub-sample			138	141	107	107	291	331	285?	301

Appendix IV. Number of camera trap observations of wildlife species from James Collins, Packsaddle and Sandy Sanders Wildlife Management Areas in Oklahoma, during 2019, 2020 and 2021 field seasons. White-tailed deer and avian species observations were not quantified, except potential bobcat prey species; quail, roadrunner and wild turkey. Species/groups are ordered by total detections over all three field seasons (N = 1710 observations).

Species/Group	**Common Name**	**2019**	**2020**	**2021**	**Total**
Procyon lotor	Raccoon	64	111	136	311
Sylvilagus spp.	Rabbit	119	74	91	284
Canis latrans	Coyote	77	66	115	258
Rodentia	Rodent	55	74	29	158
Mephitis mephitis	Striped Skunk	46	60	40	146
Lynx rufus	Bobcat	43	41	48	134
Sciurus spp.	Squirrel	19	60	21	100
Sus scrofa	Feral Pig	28	28	15	71
Didelphis virginiana	Opossum	26	22	16	64
Dasypus novemcinctus	Armadillo	15	27	21	63
Erethizon dorsatum	Porcupine	5	9	37	51
Lepus californicus	Black-tailed Jackrabbit	0	0	37	37
Geococcyx californianus	Roadrunner	9	0	5	14
Urocyon cinereoargenteus	Gray Fox	6	0	0	6
Meleagris gallopavo	Wild Turkey	0	2	3	5
Taxidea taxus	American Badger	1	2	2	5
Colinus virginianus	Bobwhite Quail	1	1	0	2
Spilogale putorius	Spotted Skunk	0	0	1	1

Appendix V. Number of rodent individuals captured at different habitat types within each study site 2020-2021. Rodents are grouped by classifications and listed alphabetically.

species classification	James Collins WMA					Packsaddle WMA			Sandy Sanders WMA		
	grassland	mixed ruderal	oak wood	pine forest	riparian	riparian	sand prairie	shrubland	grassland	riparian	shrubland
deer mouse	47	13	33	19	53	25	53	89	42	63	132
grasshopper mouse							20	16			
ground squirrel							7	3			
harvest mouse	89	5	3	1	5	4	14	3	8		
hispid cotton rat	18			8	2	1	1			4	
hispid pocket mouse							3	2	2		
kangaroo rat							7	2			
marsh rice rat	1										
m. jumping mouse								1			
shrew			1	2					1		
small pocket mouse									5	1	11
wood rat					5					3	4