# The proof is in the poop: First density estimates for a recovering bobcat population in southeast Ohio using DNA from scat

Marissa Dyck[1], Genelle Uhrig[2], Stephen Spear[2], Ruben Iosif[3], and Viorel Popescu[1]

[1]Ohio University
[2]The Wilds
[3]Foundation Conservation Carpathia

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**Abstract**

The recovery of mammalian species in the US Midwest through natural recolonization constitutes a conservation success story, yet management remains challenging due to many unknowns related to population dynamics and abundance. Abundance is a critical parameter for management decisions, and estimating the density and abundance of elusive species, such as terrestrial carnivores, remains challenging despite recent technological advances. In this study, we evaluated density and abundance of a recovering carnivore species, the bobcat (Lynx rufus) in two areas of Ohio using non-invasive DNA from scat. The target areas in eastern and southern Ohio have been shown to have uneven dynamics and recolonization success and we expected that this would be reflected in differences in density and abundance. We collected 298 bobcat scats between July 2018 and April 2019 on 150 km of repeated transects. Of these, 102 scats were successfully genotyped, and 55 individuals were identified (33 in eastern Ohio and 22 in southern Ohio). Using Spatially Explicit Capture-Recapture models, we estimated 17.9 ± 4.3 and 11.3 ± 2.9 bobcats/100 km2 in eastern and southern Ohio study areas, respectively. Our results support prior telemetry data which indicated that bobcats in eastern Ohio had smaller home-ranges than bobcats in southern Ohio, and thus could support a higher density of individuals. The higher densities were similar to other eastern US populations and are much higher than other Midwestern recovering populations. Our results provide a snapshot of the population status and can be used to determine sustainable management strategies for Ohio's bobcat population

## Introduction

Anthropogenic effects on wildlife are ubiquitous and increasing (Dirzo et al. 2014; Venter et al. 2016; Ibisch et al. 2016; Ceballos et al. 2017). Carnivores are particularly susceptible to human disturbance, due to their large home range requirements and sensitivity to habitat fragmentation (Palomares and Caro 1999; Crooks 2002). In recent history many carnivore ranges have been reduced and/or fragmented due to colonization and human expansion (Crooks 2002). The bobcat (*Lynx rufus* ) is among these carnivores; native to North America, bobcats are a medium-sized felid that occur from southern Canada to central Mexico, and historically spanned all 48 contiguous U.S. states (Reding et al. 2012). However, in the mid 1800's bobcats were extirpated from a number of states along the Northeast and the Midwest, including Ohio, due to deforestation and overharvesting (Reding et al. 2012). In recent years, bobcat populations have begun to recover in many of these states (Deems and Pursley 1978). In 1946 an adult male killed along the Ohio River in Scioto was the first record of a bobcat in Ohio after a century of extirpation (ODNR 2018), and the number of confirmed bobcat sightings has been steadily increasing since (Bobcat Management Plan 2023). Recent evidence indicates that bobcats have successfully recolonized Ohio and are expanding their range (Roberts and Crimmins 2010; ODNR 2018; Prange and Rose 2020; Popescu et al. 2021), prompting the Ohio Department of Natural Resources (ODNR) to remove them from the Ohio Endangered and Threatened Species List. Consequently, there is increased interest from recreational hunters and trappers to open a harvest season for bobcats in

Ohio. Bobcats are classified as a furbearing animal in Ohio under Ohio Revised Code Section 1531.01 and Ohio Administrative Code Rule 1501:31-1-02, while harvest is not currently permitted in Ohio, legal harvest of bobcats occurs in 39 of the 47 states within their current range. However, important population factors such as abundance and density of the Ohio populations are currently unknown and bobcat density varies widely across their range (Ferguson et al. 2009). Therefore, research is needed to investigate density and abundance of bobcats in the state to inform current and future management.

Genetic research conducted on bobcat samples from the early 2000s indicated that bobcat recolonization in Ohio occurred sequentially with two genetically distinct subpopulations in southern and eastern Ohio (Anderson et al. 2015). The eastern population was founded from individuals in West Virginia and was thought to be self-sustaining by 2012, whereas the southern population was dependent on continual immigration from founder animals in Kentucky (Anderson et al. 2015). Researchers also found differences in the average home-range size for bobcats between these areas; bobcats in the southern Ohio area had significantly larger home ranges and core areas then those in the eastern area (Prange and Rose 2020). These regional differences in space use could be a result of differences in habitat quality and degree of population recovery, which would ultimately affect the density of bobcats in the two areas. This has implications for management of the bobcat population in Ohio, particularly if lethal harvest is to be considered in this recovering population. For example, as part of ongoing efforts to understand the long-term viability of bobcats in Ohio, we determined that density was a critical parameter in predicting future population trajectories via spatial population simulation models (Dyck et al. In review). Thus, the applicability of these models to inform management decisions is contingent on accurate bobcat density estimates for different regions of Ohio. Although recent research shows evidence of genetic admixture between the southern and eastern populations (Heffern 2021), other sources of data (citizen sightings, roadkill, camera trap) suggest that regional differences in density and abundance likely still persist. Therefore, we predict that bobcat density will be lower in southern Ohio compared to eastern Ohio.

Harvest data are used in many states to track population trends of bobcats and inform management decisions (Roberts and Crimmins 2010). However, given that these data are not available for Ohio's population, we used non-invasive sampling to estimate density for bobcats in southeast Ohio. Non-invasive sampling is a particularly useful tool for monitoring cryptic and wide-ranging species such as carnivores (Kelly et al. 2012; Davidson et al. 2014). We used DNA from scat as opposed to motion-triggered cameras (another common non-invasive method used with capture-recapture models to estimate animal density; Karanth and Nichols 1998; Royle et al. 2009) for our study because of the tendency for bobcats in this region to have indistinct markings, thus leading to unreliable individual animal identification (Morin et al. 2018). Studies have found that non-invasive genetic surveys are an efficient alternative to camera trap surveys for estimating abundance and density of bobcats and other carnivores (Waits et al. 2001; Waits and Paetkau 2005; Ruell et al. 2009; Morin et al. 2018).

In this study, we implemented a multi-occasion scat sampling protocol at three public land areas in southeast Ohio (1 in eastern and 2 in southern Ohio) to estimate regional population abundance and density of the returning bobcat population. We used capture histories generated from the scat samples with spatially explicit capture-recapture (SECR) analysis for density estimation (Efford 2022). The SECR framework utilizes the spatial data associated with detectors to account for animal movement and generate robust density estimates by avoiding biases in calculating the effective sampling area (Efford 2004; Borchers and Efford 2008; Royle and Young 2008).

## Methods

*Study area*

Our study took place on three major public land areas in Southeast Ohio (Figure 1), Vinton Furnace State Experimental Forest (Vinton; 64 km2), Zaleski State Forest (Zaleski; 116 km2), and AEP reclaimed strip-mining lands (AEP; 239 km2). Vinton and Zaleski were in close proximity to each other and are both included in the southern population from prior research, therefore we aggregated the data from these two

areas for analysis (hereafter Vinton-Zaleski). Both study areas consist of primarily forested land (AEP = 60.1%, Vinton-Zaleski = 92.7%) interspersed with small patches of developed land (AEP = 3.8%, Vinton-Zaleski = 2.8%; Figure 1). AEP is also characterized by moderate areas of wetland habitat (11.1%), water bodies (2.3%), shrub/scrub habitat (9.0%), and open habitat, including grasslands (9.0%), pastures (10.7%), and barren land (<1%). Vinton-Zaleski has proportionally fewer wetlands (1.4%), water bodies (<1%), shrub/scrub (<1%), and open habitat (<1%).

*Scat collection surveys*

Scat collection took place continuously from August 2018 to June 2019 in Vinton, Zaleski, and AEP. Scats were collected daily along trail transects at the field sites (AEP = 64.0 km and Vinton-Zaleski = 82.8 km); transects included dirt roads, gated access roads, and hiking trails in each study area. Transects were re-surveyed two times (three surveys in total) during the study. Weather prevented re-surveys for a limited number of transects (<5%) during the winter. Trained field technicians searched for scats, collected samples, recorded GPS locations, and tracked survey effort using Avenza Maps (Avenza Systems Inc.). Scats were collected using a different set of latex gloves for each sample to prevent transfer of DNA between samples and samples were frozen at -10°C the day of collection to preserve DNA.

*DNA extraction and species identification*

DNA from collected scats were initially extracted using the QIAamp Fast DNA Stool Mini Kits (Qiagen, Hilden, Germany) following the manufacturer protocol. Subsequent re-extractions were performed using a modified protocol to increase DNA quantity and included the following additional steps: 1) duplicate samples were taken from each scat and combined downstream, 2) samples were incubated overnight at 60°C prior to homogenization, 3) 100ul of Buffer ATE was used for eluting DNA, 4) samples were incubated at 60°C for 5 minutes prior to centrifuging for elution, and 5) eluate was pipetted back onto the spin column, incubated for 5 minutes, and centrifuged again. To prevent cross-contamination, scats were extracted in a dedicated low-quality DNA processing lab and gloves were changed between each sample. We designed species-specific primers and probes for bobcat (mitochondrial genome subunit 5 gene; GenBank: KP202285.1) and coyote (*Canis latrans)* (mitochondrial genome isolate 1 USA; GenBank: DQ480510.1) using Primer3 (Koressaar and Remm 2007; Untergasser et al. 2012; Koressaar et al. 2018) and checked for species specificity using NCBI's Primer-BLAST (Ye et al. 2012) (Table 1). Quantitative Polymerase Chain Reactions (qPCR) for species identification were performed in 15µl reactions containing 7.5µl Quantitect Multiplex NO-Rox Master Mix (Qiagen), 0.75µl primer-probe mix (primer concentration 8µM each, probe concentration 4µM), 0.3µl TaqMan 50X Exogenous IPC DNA (Applied Biosystems), 0.6µl TaqMan 10X Exogenous Internal Positive Control (IPC) Block (Applied Biosystems), and 2.85µl di$H_2O$. Samples were run in triplicate on a 7500 Real-Time PCR System (Applied Biosystems) using standard dilutions made from tissue DNA extracts for each species. Amplification curves were visualized using the 7500 RT PCR System software. Negative controls were included in each step downstream to identify cross-contamination. bobcat positive samples were identified and DNA was used in subsequent steps.

*DNA Amplification and Genotyping*

A suite of 8 dimorphic microsatellite primers originally designed for domestic cats (Menotti-Raymond et al. 1999, 2005) and previously amplified with bobcats (Morin et al. 2018) were optimized using bobcat tissue DNA (Table 1). We used a multitube process to test the quality of scat DNA and extraction success and eliminate samples of low quality. All bobcat identified or potential samples were first amplified with a primer multiplex containing 4 loci (*FCA096, FCA275, FCA391, and FCA126;* i.e. Multiplex 1). Samples that amplified at [?]1 locus were re-extracted and samples that amplified at 2 loci (including re-extractions) were re-amplified. Samples that amplified at 3-4 loci then moved onto a subsequent multiplex (i.e., Multiplex 2) containing 4 primers (*FCA090, FCA043, FCA124, and F124)* plus a sex identification primer (*AmelX/Y* ; Pilgrim et al. 2005). DNA was amplified in 15µl polymerase chain reactions (PCR) containing 4µl DNA plus 7.5µl 2X Multiplex Master Mix (Qiagen), 1.5µl Q Solution (Qiagen), 0.375µl Bovine Serum Albumin, primer mix at 0.2µM concentration of each forward and reverse primer, and 0.425µl of di$H_2O$. Reactions were

initiated at 95°C for 15 minutes to activate hot-start TAQ, then denatured at 94°C for 30 seconds, annealed at 55°C for 3 minutes, and extended at 72°C for 1 minute for 40 cycles; then finished with a final extension at 60°C for 30 minutes. Amplified product was plated with 9.5µl formamide and 0.5µl GeneScan 500 LIZ dye Size Standard (Applied Biosystems) and genotyped on an ABI 3730xl at the Genomics Shared Resource in The James Comprehensive Cancer Center at The Ohio State University (NCI Cancer Center Support Grant P30CA016058). Genotypes were scored using Geneious Prime software (version 2021.0, Dotmatics, Boston MA). Samples were amplified 2-9 times at each locus and included in the dataset used for individual analyses if they had at least 2 consensus genotypes for homozygotes and at least 3 consensus amplifications of each allele for heterozygotes at 5 or more loci.

*Individual bobcat identification*

Individual bobcats were identified in program COLONY (version 2.0.6.7, Jones and Wang 2010) using the clone method to identify and group matching genotypes (Wang 2016). Parameters used included male and female monogamy with inbreeding and with clone; diploid and dioecious options selected; the Full-Likelihood method with long run time selected; no updating of allele frequencies; sibship scaling; and no sibship prior. Probability of identity ($P_{ID}$) and probability of identity for siblings ($P_{IDsib}$) were calculated in GenAlEx (version 6.503; Peakall and Smouse 2006, 2012) to assess the power of the microsatellite marker set to identify individuals.

*Spatially explicit capture-recapture*

We used a spatially explicit capture-recapture (SECR) framework to estimate bobcat density (Efford and Fewster 2013). Because we performed the sampling on pre-determined transects revisited three times during the study area, we created "detectors" by splitting the study areas into 1 km x 1 km grid cells. Only grid cells that overlapped the transects were retained and we defined 'detectors' as the centroid of each grid cell. We then assigned all bobcat-identified scats collected on the transect/s in a given cell the unique code of that cell (or detector) (Royle et al. 2014). Bobcats can move large distances (several km in a day) and have large home ranges averaging 15.83 to 39.70 $km^2$ (Ferguson et al. 2009); the distance between the center of each cell and locations of scats were therefore negligible from a bobcat movement and space use perspective and assigning the scats location to the cell centroid facilitated the development of capture history data and data analysis.

The following modeling framework and workflow used package *secr*(Efford 2022) implemented in the program R (R Core Team 2022). We used ArcGIS (ESRI, Redlands CA) to create the habitat mask used as an effective sampling area in our analysis. To model detection, we calculated the sigma ($\sigma$) model parameter using a root pooled variance function as a measure of 2D dispersion of the centroids, pooled over individuals (Efford 2022). We found that a buffer width of $5 \times \sigma$ around our detector array reduced the probability of capturing a bobcat outside this buffer to zero and increasing buffer width beyond this value had no discernable effect on the estimated density (Figure 2). This area is thus typically used as the effective sampling area in spatial capture-recapture models (Borchers and Efford 2008). The value of $\sigma$ was 1230 m. To investigate differences between the 2 study areas, AEP and Vinton-Zaleski, we built a habitat mask by creating buffers around the detectors equal to $5 \times \sigma$ (6152 m) in ArcGIS; the resulting mask had two different polygons, corresponding to the two study areas, and they had an area of 543 $km^2$ (AEP) and 580 $km^2$(Vinton-Zaleski).

We tested several detection functions and selected a '*cumulative lognormal* ' detection function to use in subsequent analyses, as this function performed better than other detection functions based on Akaike Information Criterion corrected for small sample size (AICc) (Akaike 1998) comparisons of SECR models fit with half-normal, compound half-normal, cumulative gamma, and cumulative lognormal (Table 3). We also compared several predictor variables for detection including length of transect per grid cell (*t_length* ), and various habitat variables (proportion of developed, forest, open, and wetland habitat) against a constant detection (null) model. We found that the constant detection model performed the best, but several other models were <2 $\Delta$AICc from this model (Table 4). The model that included detection as a function of the length of transect per grid cell failed some variance calculations and thus was not included in model

comparison.

We fit a SECR state (observation) model using a spatial Poisson process for animal activity centers (Borchers and Efford 2008) and included a categorical predictor (study area: AEP or Vinton- Zaleski), as we expected differences in density between the two areas based on preliminary studies (Prange and Rose 2020; Popescu et al. 2021). We compared this model to a constant density model (null) using AICc. Lastly, because the data were collected within a single year (July 2018 to April 2019), it included a single birth pulse and each survey was conducted over the course of several months, we did not investigate potential differences in density between the three surveys. Instead, we quantified the overall bobcat density and abundance during the study period and differences between the two focal areas.

## Results

*Scat collection and genotyping*

We surveyed approximately 146.8 km of trails between the two study areas (AEP = 64.0 km and Vinton-Zaleski = 82.8 km) for each survey event. We collected a total of 813 scats during the three combined survey events and were able to extract DNA from 789 scats for species identification. Of those samples, we identified 37.4% (295 scats) as bobcat for density estimation. The remaining scats were identified as either coyote (*Canis latrans* ) 32.3% (255 scats) or were of unknown species 30.3% (239 scats). There were 25 samples identified as both coyote and bobcat which were included in the first round of amplifications; none of these samples amplified at any of the four MP1 loci and were removed and classified as unknown species. Of the initial 295 bobcat samples amplified at Multiplex 1, 164 reached the criteria to move onto amplification with Multiplex 2. We achieved consensus genotypes at a minimum of 5 loci for 102 samples (34.6% genotype success rate) of which 48 samples reached consensus at all 8 loci. Overall PCR amplification success across all samples was typical for scat studies at 50.9% and ranged from 27.2%-75.9% per locus (Table 2. The probabilities that two individual genotypes ($P_{ID}$) and two sibling genotypes ($P_{IDsibs}$) are identified as the same individual for the 8-locus dataset was low at 8e-8 and 1.5e-3, respectively, illustrating the high power of the locus set to distinguish individuals.

We identified a total of 55 individual bobcats from 102 unique captures (genotyped scats); the AEP study area had a higher number of individuals (n = 33) compared to the Vinton-Zaleski study area (n = 22). Overall, we identified more female bobcats than male bobcats (female = 28, male = 19, unknown = 8). We also identified more female bobcats in AEP (female = 20, male = 8, unknown = 5), while Vinton-Zaleski had a more even distribution of sexes (female = 8, male = 11, unknown = 3). Recapture rates (those with multiple scats) were similar between sites (average of 1.97 ± 0.17 SE detections per individual in AEP and 2.31 ± 0.34 SE detections per individual in Vinton-Zaleski), and sexes (average of 2.42 ± 0.35 SE detection per individual for males and 2.04 ± 0.18 SE detections per individual for females). The maximum number of detections for any individual was 9 (male in Vinton-Zaleski), and 47 bobcats were recaptured at least once. Of the 47 bobcats that were recaptured, the average distance between successive recaptures was 1.68 km ± 0.25 SE. The minimum distance between successive recaptures was 0 km (bobcats recaptured within the same grid cell), and the maximum distance between successive recaptures was 9.06 km (a male bobcat in AEP; Figure 3).

*Bobcat density and abundance*

The top SECR model for estimating the density of bobcats in our study areas included all individuals (n = 55) and estimated separate densities per location with constant detection and a cumulative lognormal detection function. This model performed significantly better than the null model ($\Delta$AICc = 4.65). Based on this model, we estimated a density of 17.9 ± 4.3 SE (13.6 – 22.2) bobcats/100 km$^2$ in AEP and 11.3 ± 2.9 SE (8.4 - 14.2) bobcats/100 km$^2$ in Vinton-Zaleski.

## Discussion

We provide the first assessment of bobcat density for the recovering population in Ohio using eDNA from scats and SECR analysis. Bobcat density differed between the AEP and Vinton-Zaleski study areas (17.9

± 4.3 SE and 11.3 ± 2.9 SE bobcats/100 km$^2$respectively). Our results support prior telemetry data which indicated that bobcats in eastern Ohio (AEP area) had smaller home-ranges than bobcats in southern Ohio (Vinton-Zaleski area) and thus could support a higher density of individuals (Prange and Rose 2020) and camera trap studies that indicated lower habitat occupancy by bobcats in the Vinton-Zaleski area (Bencin 2018; Rich et al. 2018). However, the mechanism/s driving these differences are not fully understood. Prange and Rose (2020) hypothesized that differences in home-range sizes between the two areas were a result of differences in food availability, habitat quality, and body size as other studies have shown (Litvaitis et al. 1986; Anderson 1987; Knick 1990). While Rich et al. (2018) found landscape variables had little effect on occupancy, but coyote presence had a strong negative influence on bobcat occupancy. Bobcats and coyotes are sympatric throughout most of North America and interference competition between the two predators is rare (Dyck et al. 2022). However, bobcats and coyotes have only recently begun to co-occur in the US Midwest (Deems and Pursley 1978; Woolf and Hubert 1998). The concomitant recolonization by bobcats (Reding et al. 2012) and range expansion by coyotes (Hody and Kays 2018) represents a unique situation as species are co-occurring that have shared evolutionary histories but have not coexisted in recent time. While our study provides additional support for regional differences in bobcat density and abundance, it does not address the mechanisms driving these differences, and additional research into this topic is needed and would provide useful insights for bobcat management.

When comparing density estimates across the bobcat range from SECR analyses, our estimates for AEP are at the higher end of those reported elsewhere, while estimates from Vinton-Zaleski are within the average (Clare et al. 2015; Thornton and Pekins 2015; Rounsville Jr 2018; Morin et al. 2018; Jacques et al. 2019; Greenspan et al. 2020). However, when comparing SECR density estimates for another recovering Midwest bobcat population, the estimates from both study areas are much higher than those reported for Illinois (1.4 individuals/100 km$^2$; Jacques et al. 2019). There are several factors that may explain differences in bobcat density between our study and Illinois.

First, there are distinct differences in the type and distribution of habitat between the two studies. Our study areas are characterized primarily by forested habitat (60.1% - 92.7%) with minor areas of developed land (2.8% - 3.8%; Figure 1), while the study site in Illinois was dominated by agriculture (53.2%) and pasture-hay (12.6%) with a lesser-degree constituting forest (27.3%; Jacques et al. 2019). Prior investigation into habitat suitability for the bobcat population in Ohio indicated that bobcats select for forest habitat, natural herbaceous vegetation habitat, and areas of low road density (Popescu et al. 2021). Bobcat habitat suitability is also highest in southern and southeast Ohio (Popescu et al. 2021), given our estimates are from high suitability habitat it is likely that they represent the higher end of density for the state.

Second, field methods between the two studies differed. Jacques et al. (2019) used infrared-triggered cameras which are a common non-invasive tool that can be used with capture-mark-recapture methods to estimate density and abundance of bobcats and other felid species due to their unique recognizable pelage patterns (Karanth and Nichols 1998; Silver et al. 2004; Greenspan et al. 2020; Iosif et al. 2022). However, this method requires quality images of both flanks of an animal and sufficient variation in pelage/individual markings for observers to accurately identify individuals. Jacques et al. (2019) deployed cameras for 77 days and had 139 unique bobcat events but had to discard 18.7% due to low image quality and a small subset (6.2%) of the remaining images were classified as tentative identifications. Bobcat pelage varies across their range (Young 1978; Croteau et al. 2012) and some areas are known to have individuals with less distinct markings (Morin et al. 2018).

Third, harvest of bobcats (hunting or trapping) is currently not permitted in Ohio, while Illinois has had regulated hunting or trapping season for bobcats since 2016 (Illinois DNR). The protected status of bobcats in Ohio has played a role in their recovery and may act as a mechanism for 'mesopredator release' allowing population densities to exceed those in states without protection. Legal harvest accounts for a large portion of bobcat mortality in exploited populations (Rolley 1985; Knick 1990; Fuller et al. 1995; Chamberlain et al. 1999; Blankenship et al. 2006) and therefore if harvest mortality is additive with other sources of mortality it is expected that population size would be higher for unexploited populations such as Ohio.

*Management and conservation implications*

Our results corroborate previous work suggesting uneven recovery of bobcats in Ohio (Prange and Rose 2020), but that bobcat density is relatively high in areas of suitable habitat. These findings highlight the successful self-repatriation of a large carnivore after decades of absence due to habitat recovery and improved land management practices. Bobcats were extirpated from Ohio by 1850 coinciding with massive forest clearing which reduced forest cover in the state from ˜95% to 10% by the early 1900s. The development of the first state forestry agency (now Division of Forestry) in 1885 and continued efforts from this agency to purchase and protect Ohio forests resulted in a 2.5-fold increase in forested land (˜33%) by 2011 (Widmann et al. 2014). These efforts in combination with the protection of bobcats under Ohio's state list of threatened and endangered species were major factors contributing to the recovery of this species.

Estimates of bobcat density are needed to validate and supplement ongoing research into population viability for this recovering carnivore. Outcomes of bobcat population simulation models were heavily influenced by density (Dyck et al. In review) and our results can be incorporated into these models to project future population dynamics more accurately. These data can also be used by wildlife managers in combination with prior habitat suitability analysis (Popescu et al. 2021) to inform delineations of harvest zones and regional-specific quota limits to ensure sustainable management practices.

However, our results represent a snapshot in space and time during the continual recovery process of bobcats in the Midwest and effective bobcat management in Ohio requires continuous population monitoring, including periodic estimates of density as the population continues to expand (Popescu et al. 2021). Our study outlines a feasible, efficient, and fully transparent method for estimating bobcat density that can be repeated and applied to other areas and habitats. Results from this study are also relevant at a regional level for other recovering populations in the US Midwest and can be compared to indirect measures of density such as maximum clique analysis (Jones et al. 2022) in neighboring Indiana. Overall, the results from this study provides critical information on density for recovering bobcats, outline a feasible monitoring scheme to evaluate population density as recovery continues, and can be used in combination with a variety of other quantitative tools (e.g., population simulation models, habitat suitability models) to improve management and conservation decisions for recovering bobcats.

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Table 1: Microsatellite and species ID marker information for bobcat (*Lynx rufus* ) and coyote (*Canis latrans* ). The upper area of the table contains information for 8 microsatellite loci including number of samples (n), number of alleles (NA), observed heterozygosity (Ho), expected heterozygosity (He), unbiased expected heterozygosity (uHe), probability of identity ($P_{ID}$), and probability of identity siblings ($P_{IDsibs}$). The lower area of the table contains information for the species-specific primers and probes for bobcat (LRUF) and coyote (CLAT).

| Locus | Sequence/Forward | Sequence/Reverse | Range (Bp) |
|---|---|---|---|
| FCA096 | CACGCCAAACTCTATGCTGA | CAATGTGCCGTCCAAGAAC | 165-227 |
| FCA275 | TTGGCTGCCCAGTTTTAGTT | ACGAAGGGGCAGGACTATCT | 106-146 |
| FCA391 | GCCTTCTAACTTCCTTGCAGA | TTTAGGTAGCCCATTTTCATCA | 160-276 |
| FCA126 | GCCCCTGATACCCTGAATG | CTATCCTTGCTGGCTGAAGG | 107-163 |
| FCA090 | ATCAAAAGTCTTGAAGAGCATGG | TGTTAGCTCATGTTCATGTGTCC | 100-112 |
| FCA043 | GAGCCACCCTAGCACATATACC | AGACGGGATTGCATGAAAAG | 112-124 |
| FCA124 | CCATTCCCTCCCTGTCTGTA | GCCTCAAGCCTCATTGCTAC | 125-145 |
| F124 | TGCTGGGTATGAAGCCTACT | ATTGCCTCAACTACCTAGGC | 150-190 |
| Amel X/Y | CGAGGTAATTTTTCTGTTTACT | GAAACTGAGTCAGAGAGGC | 195, 216- Male |
| LRUF ID | AGTCACCGCTAACAACCTATTC | TGCATCTGTTCGGCCATATC | |
| LRUF ID Probe | ACTGTTCATTGGCTGAGAAGGAGTAGG | x | |
| CLAT ID | CAATCTCATGGGCTCCCTAA | GTCACAAGTGGAGGCCGTAT | |
| CLAT ID Probe | TTGGCATGCATGATAGCATT | x | |

| Locus | Multiplex | Samples ran (n) | Total Amplification Attempts | Total Amplifications | Total Non-Amplifications |
|---|---|---|---|---|---|
| FCA096 | 1 | 318* | 1234 | 336 | 896 |
| FCA275 | 1 | 318* | 1241 | 799 | 440 |
| FCA126 | 1 | 318* | 1237 | 715 | 522 |
| FCA391 | 1 | 318* | 1241 | 478 | 761 |
| FCA090 | 2 | 164 | 862 | 654 | 208 |
| FCA043 | 2 | 164 | 862 | 520 | 342 |
| FCA124 | 2 | 164 | 857 | 436 | 421 |
| F124 | 2 | 164 | 861 | 427 | 434 |
| Amel | 2 | 164 | 864 | 352 | 512 |
| | | **Totals** | 9259 | 4717 | 4536 |

Table 2: Polymerase Chain Reaction (PCR) amplification rates for bobcat (*Lynx rufus* ) scats at 8 loci. Scats were collected on public lands in southeast Ohio between July 2018 and April 2019.

**Includes 25 samples that amplified as both L. rufus and C. latrans*

Table 3: Model comparison of detection functions for spatially explicit capture-recapture (SECR) models to determine the density of bobcats (*Lynx rufus* ) in southeast Ohio using eDNA from scats. For all models, density (D), detection (g0), and movement (sigma) were constant. Model fit was evaluated with Akaike Information Criterion correct for small sample size (AICc).

| Model | Detection function | Parameters | Log likelihood | AICc | ΔAICc | Weight |
|---|---|---|---|---|---|---|
| D ~ 1 g0 ~1 sigma ~1 | Cumulative lognormal | 4 | -275.8 | 560.3 | 0.00 | 0.93 |
| D ~ 1 g0 ~1 sigma ~1 | Cumulative gamma | 4 | -278.3 | 565.3 | 5.04 | 0.07 |
| D ~ 1 g0 ~1 sigma ~1 | Halfnormal | 3 | -288.8 | 584.1 | 23.79 | 0.00 |
| D ~ 1 g0 ~1 sigma ~1 | Compound halfnormal | 4 | -288.8 | 586.4 | 26.12 | 0.00 |

Table 4: Model comparison of detection variables (g0) for spatially explicit capture-recapture (SECR) models to determine the density of bobcats (*Lynx rufus* ) in southeast Ohio using eDNA from scats. Habitat detection variables represent the proportion of that habitat per grid cell (1 km x 1 km). All models were fitted with the cumulative lognormal detection function and both density (D) and movement (sigma) were constant. Model fit was evaluated with Akaike Information Criterion corrected for small sample size (AICc).

| Model | Detection variable | Parameters | Log likelihood | AICc | ΔAICc | Weight |
|---|---|---|---|---|---|---|
| D ~ 1 g0 ~1 sigma ~1 | Null | 4 | -277.9 | 564.6 | 0.00 | 0.26 |
| D ~ 1 g0 ~forest sigma ~1 | Forest | 5 | -276.8 | 564.8 | 0.26 | 0.23 |
| D ~ 1 g0 ~open sigma ~1 | Open | 5 | -276.8 | 564.9 | 0.34 | 0.22 |
| D ~ 1 g0 ~wetland sigma ~1 | Wetland | 5 | -276.9 | 565.1 | 0.53 | 0.21 |
| D ~ 1 g0 ~developed sigma ~1 | Developed | 5 | -277.9 | 566.9 | 2.375 | 0.08 |

Figure 1: Study area for scat surveys of bobcats (*Lynx rufus* ) in southeast Ohio from August 2018 to June 2019. Solid black lines represent transects which were surveyed three times each over the study period. Yellow dots represent scat locations (n = 102) of genotyped bobcat.

Figure 2: Effect of varying buffer width (meters) on estimated density for spatially explicit capture-recapture (SECR) model of bobcats (*Lynx rufus* ) in southeast Ohio. Vertical lines represent various buffer widths that were tested; 3691 m (orange), 4922 m (black), 6152 m (red), and 7383 m (blue). We selected a buffer 6152 m ($5 \times \sigma$).

Figure 3: Successive movements (meters) of individual bobcats (*Lynx rufus* ) with >1 capture identified by DNA from scat and based on 'detector' locations. 'Detectors' were set as the centroid of each grid cell that was surveyed, and spacing between 'detectors' was 1000 m.