

# Efficient single-survey estimation of carnivore density using fecal DNA and spatial capture-recapture: a bobcat case study

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Received: 3 April 2017 / Accepted: 11 January 2018 / Published online: 5 February 2018  
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## Abstract

Population density estimates are necessary to inform management and conservation, yet are difficult to obtain for cryptic species such as carnivores, and often require intensive sampling. We implemented a single-survey, closed session, scat sampling protocol to estimate bobcat density using fecal DNA and spatial capture-recapture at two sites over five sessions in Virginia, USA. We employed a Poisson encounter model to allow for multiple detections on scat transect segments over a single collection interval, and compared single session and multistrata (closed, multi-site, multisession) spatial capture-recapture (SCR) approaches to estimate density for each site and session. We found improved precision for most estimates using the multistrata SCR approach, sharing data on baseline detection and individual movement across sites and sessions. We suggest the summer session estimates are representative of the resident population, that differences in density between summer and winter are representative of potential net recruitment, and that differences between consecutive summer sessions are representative of the net recruitment realized for the population (dependent on survival and emigration). Finally, we assessed factors affecting precision in single session model estimates and provide recommendations to improve detection and reduce credible intervals that may be applicable across the bobcat range and to other carnivore species. The single survey transect methodology provides flexibility in monitoring carnivore populations specifically, or as part of concurrent monitoring for multiple carnivore species. This methodology has potential to dramatically increase the effectiveness of conservation and management dollars, improving our ability to monitor carnivore populations and assess conservation needs and actions.

**Keywords** Felid conservation · *Lynx rufus* · Multistrata spatial capture-recapture · Noninvasive genetic sampling · Population density · Scat transects

## Introduction

Populations of many carnivore species are decreasing (IUCN 2017), and charismatic carnivores are commonly used as flagship or umbrella species in conservation (Andelman and

Fagan 2000; Carroll et al. 2001; Sergio et al. 2006). As a result, the density and local abundance of many carnivores are critical metrics required for assessing population status and conservation actions (May 1999). The recent rapid advancements of noninvasive sampling technology and quantitative approaches have improved our ability to monitor cryptic and wide-ranging carnivores (Karanth 1995; Waits and Paetkau 2005; O’Connell et al. 2010; Kelly et al. 2012; Davidson et al. 2014). However, sampling requirements for abundance estimates are intensive, often requiring multiple detector devices (e.g., camera-traps) at each sampling station, large extent spatial coverage, and repeated visits to sites within a single session (Ruell et al. 2009; Sun et al. 2014; Wilton et al. 2014). As a result, monitoring of carnivores can be time consuming and costly (Rodgers and Janecka 2013).

Bobcats (*Lynx rufus*) are widespread across the United States (Kelly et al. 2016), and populations are reported to be stable or increasing in most states (Roberts and Crimmins

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s10144-018-0606-9>) contains supplementary material, which is available to authorized users.

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2010). Although bobcat populations in some regions are increasing, the status of bobcat populations in other areas is unknown or of concern (Riley et al. 2003; Litvaitis et al. 2006). Sound estimates of abundance are needed to monitor the status of these populations, detect possible increases or declines over time, and allow for explicit management decisions. Yet, despite the wide distribution of bobcats throughout North America, estimates of population abundance and density are scant and often are not comparable due to non-standardized methodologies and differing sources of sampling bias (Thornton and Pekins 2015). Thus, bobcats provide a suitable case study for demonstrating the difficulties, and possible solutions, to improve monitoring for carnivore populations.

Most states rely on harvest statistics to track bobcat population trends and inform management practices (Roberts and Crimmins 2010). However, the development of relatively inexpensive remotely-triggered cameras promoted studies of bobcats because, as with many other felid species, their recognizable spot patterns enabled researchers to identify individuals and generate encounter histories for capture-mark-recapture applications (CMR) to estimate abundance (Heilbrun et al. 2006; Thornton and Pekins 2015). However, accurate photographic identification of individuals typically requires images of both flanks of an animal, and maintaining sampling grids with two cameras at each station, at an extent appropriate to estimate population density of carnivores, can be intensive (both in time and financial costs). In addition, variation in bobcat pelage (Young 1978; Croteau et al. 2012), where markings are indistinct in some regions, such as the central Appalachians, can hinder accurate identification of individuals and other carnivores that may not have temporary or permanent distinguishing marks. As a result, researchers have developed several alternative camera study designs and model advancements to improve the precision of estimates and reduce required sampling effort. These include incorporating a known marked population (e.g., telemetry collars) and single-side and hybrid camera station designs (McClintock et al. 2013; Sollmann et al. 2013; Alonso et al. 2015; Augustine et al. 2018, in press).

As an alternative to camera-trap surveys, noninvasive genetics surveys have been employed to estimate population density (Russell et al. 2012; Davidson et al. 2014; Rodgers et al. 2014). A study in southern California found scat transects easy and efficient to implement and yielded reasonable amplification success for identification of individual bobcats from nuclear DNA (nDNA) microsatellites (Ruell et al. 2009). Scat transects also allow for concurrent sampling of species that are not individually identifiable on camera, but may be sampled using fecal DNA (Morin et al. 2016a). However, the abundance models employed by Ruell et al. (2009) required multiple scat detection surveys repeated over a single closed session to account for imperfect detection, which

drastically increases the field effort and cost of monitoring, and may violate assumptions of closure for single session abundance estimates.

Although often implemented with multiple sampling occasions, noninvasive genetic sampling and capture-recapture methods do not necessarily require repeated surveys to estimate density, thus reducing required sampling effort (Miller et al. 2005; Morin et al. 2016a). Spatial capture-recapture (SCR) models use the spatial information from trap locations to directly estimate density from the observed data (Royle et al. 2013). Additionally, recent development of a single microsatellite primer multiplex that distinguishes between felid species allows for rapid and efficient identification of individuals at a reduced laboratory effort per species (Wultsch et al. 2014). Thus, the integration of non-invasive genetic sampling with SCR methods may provide a useful method for monitoring felid populations, either as the target population, an umbrella species, or as a component of the carnivore guild (Carroll et al. 2001).

We employed a single occasion scat sampling protocol at two sites in the mountains of Virginia over five different time periods (from July 2011 to July 2013) to estimate closed population abundance for each session. We used a recently developed microsatellite multiplex (Wultsch et al. 2014) to identify individual bobcats from fecal samples to estimate density during each session at both sites using three different parameterizations of SCR models. First, we estimated density independently for each site and session using the basic SCR model ( $SCR_0$ ). Second, we fit a closed population, multi-site, multisession (or strata) model to estimate density for each site and session, where detection and individual movement were estimated separately for each season (summer or winter), but were constrained to remain the same for each site to allow for greater sample sizes in fitting the detection function. Finally, we fit a closed population, multistrata model, simultaneously estimating density for both sites and all five sessions, with detection and individual movement constrained to be constant across sites and seasons. We expected the multistrata approaches would improve precision in estimated parameters as long as the data were sufficient to estimate parameters, and animal movement and detection were similar across sites, and possibly seasons. Finally, we evaluated factors affecting parameter estimation using this method and provide recommendations for implementation in other regions and for other felid species.

## Methods

### Field methods

We monitored two study areas, one each in two counties in western Virginia positioned along the eastern divide in the

northern Ridge and Valley Province of the central Appalachian Mountains (Bath and Rockingham Counties, Fig. 1). The forest structure and species composition in both study areas is primarily mature deciduous forest canopy including chestnut oak (*Quercus prinus*), red oak (*Q. rubra*), white oak (*Q. alba*), and tulip poplar (*Liriodendron tulipifera*), and the understory included rhododendron (*Rhododendron maximum*) and eastern mountain laurel (*Kalmia latifolia*). Public lands comprised large swaths of the study areas including the George Washington National Forest, state Wildlife Management Areas, and the Nature Conservancy Warm Springs Mountain Preserve. Elevation ranged from 350 to 1365 m (Bath), and 363 to 1335 m (Rockingham) and mean daily temperature ranged from a minimum of  $-4.6^{\circ}\text{C}$  in January to a mean maximum of  $31.6^{\circ}\text{C}$  in July (NOAA, public data 2012). Average annual precipitation was 97.79 cm, with most precipitation occurring between March and September. In addition to bobcats, there was a diverse carnivore guild, including coyotes (*Canis latrans*) and black bears (*Ursus americanus*).

Each study area was comprised of  $>200$  km of scat collection transects within a  $250\text{ km}^2$  area. Transects included dirt roads, hiking trails, and well-defined game trails on publicly managed lands (predominantly National Forest) in each study area: 213 km in Bath County, 208.5 km in Rockingham County. Trained field technicians searched transects for carnivore scats, collected fecal DNA samples, and recorded GPS locations for five closed sessions from July 2011 to July 2013 (3 summer sessions, and 2 winter sessions). Each closed session consisted of a single survey to allow adequate scat accumulation between clearing of transects and sample collection, and we cleared transects of scat 1 month prior to closed sessions to ensure temporal and geographic closure as described in Morin et al. (2016a). In addition to the samples collected for the closed sampling sessions to estimate density, we also collected samples during separate surveys in the same study areas for a diet study (Morin et al. 2016b), which we refer to as concurrent sampling sessions. For the purposes of increasing sample sizes for genetic analyses and individual matching, we supplemented the closed capture survey samples with additional bobcat genotype samples from the concurrent sampling sessions. However, these additional samples were not included in the closed spatial encounter histories used to estimate density over closed sessions.

## Genetic analysis methods

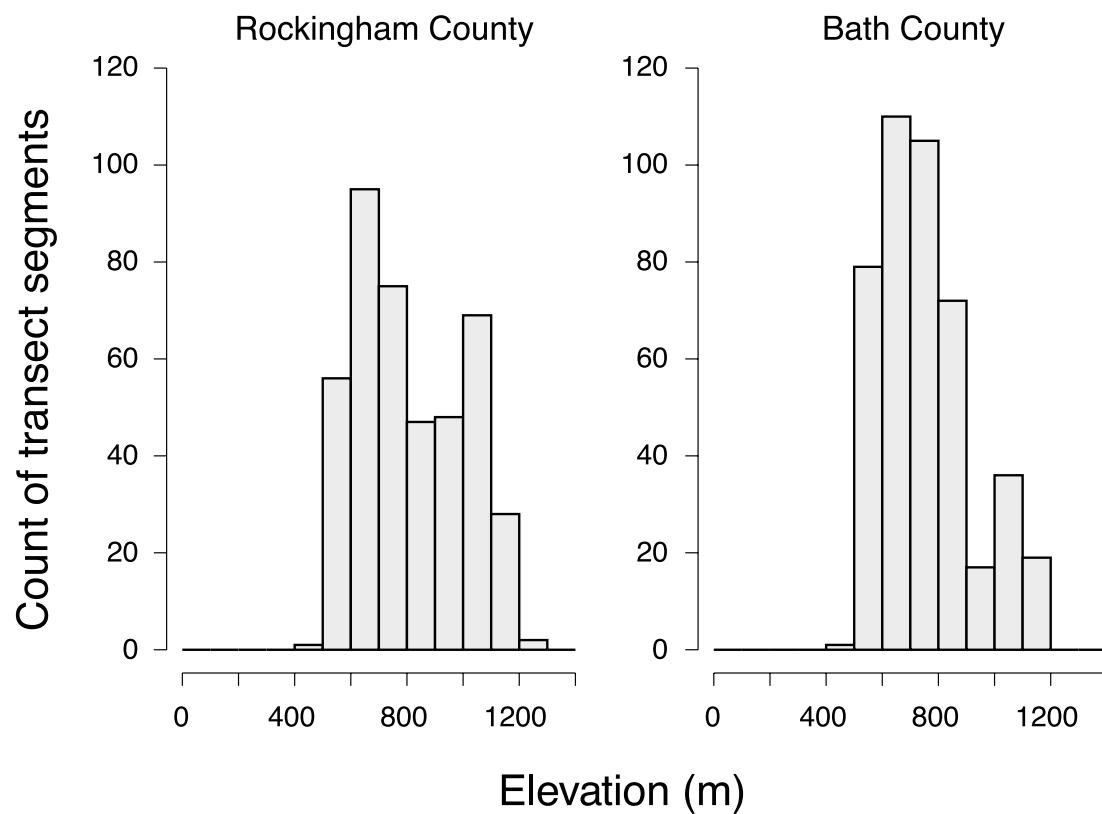
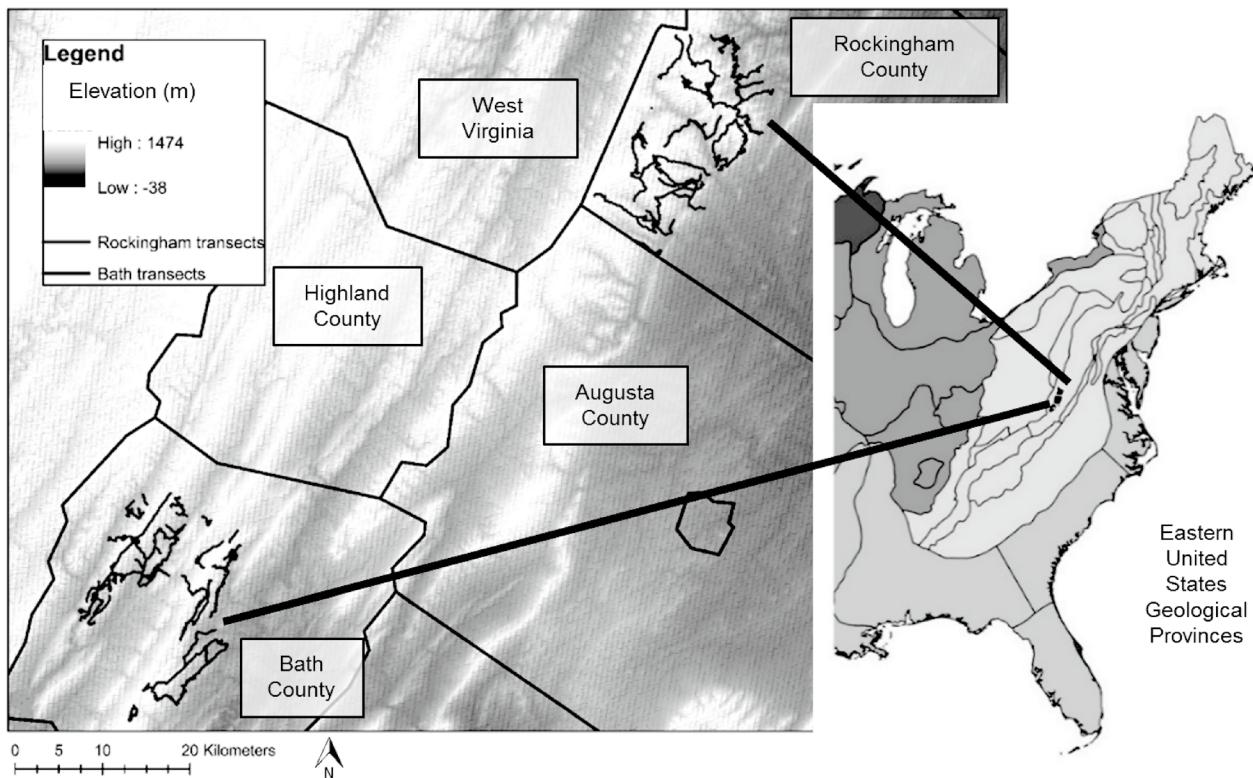
We extracted DNA from feces using Qiagen QIAmp DNA stool kit (Qiagen, Valencia, CA, USA) in a lab designated for low-quality, low-quantity DNA and screened all samples using mitochondrial DNA species identification multiplex (Davidson et al. 2014; De Barba et al. 2014) as described

in Morin et al. (2016a). To determine bobcat individual genotypes, we combined 8 nuclear microsatellite loci identification primers, F124, F85, FCA043, FCA090, FCA096, FCA126, FCA275, and FCA391 (Menotti-Raymond and O'Brien 1995; Menotti-Raymond et al. 2005), in a multiplex for polymerase chain reaction (PCR) amplification and analyzed samples using the Applied Biosystems 3130xl ABI capillary machine (Applied Biosystems, Foster City, CA, USA) and associated software as described in Wultsch et al. (2014). We included a PCR positive (known bobcat tissue sample) and PCR negative control in each PCR plate to identify PCR failure or potential contamination.

Genotyping errors are common in low quality-low quantity DNA samples from allelic dropout and polymerase errors (Taberlet et al. 1996). Thus, we used a two-step multtube process to cull poor quality nDNA samples. We initially performed PCR for each sample twice and removed samples with  $<50\%$  amplification across the eight microsatellite loci. Then we repeated PCR 3 more times for each remaining sample to confirm alleles for each locus. We required two repetitions to confirm heterozygous loci and three repetitions to confirm homozygous loci to minimize potential genotyping errors. Additionally, we used RELIOTYPE (Miller et al. 2002) to confirm  $>95\%$  accuracy of genotypes observed in only a single sample.

Finally, we matched genotypes from different scat samples to the same individual bobcat using GenAIEx 6.501 (Peakall and Smouse 2006, 2012). We used the individual bobcat samples confirmed at all 8 loci to estimate  $\text{PID}_{\text{sibs}}$  (Waits et al. 2001) in GenAIEx 6.501 to conservatively distinguish between genetically similar siblings ( $\text{PID}_{\text{sibs}} < 0.001$  at 6 loci required for a match,  $\text{PID}_{\text{sibs}} = 0.0008$  at all 8 codominant loci). We then matched all samples with confirmed alleles at 6 or more loci, resolved mismatch alleles, and removed samples that could not be confirmed as a match or a single genotype due to poor sample quality.

To ensure we only included bobcat samples in our spatial capture-recapture data set, we screened for domestic cat (*Felis catus*) samples in two ways. First, during the initial species identification screening we were able to identify an additional fragment in the mtDNA control region (amplified at both 104–106, and 129–130 bp ranges) that amplified for domestic cats, but not bobcat samples (only amplified at 104–106 bp range). We removed all samples that amplified at the species-specific fragment size for domestic cats. Second, following identification of individuals based on nDNA genotypes, we compared all individuals to genotypes from 19 samples identified as domestic cats in a previous study using 7 of the same 8 markers (Mesa-Cruz et al. 2016). We screened the felid genotypes in STRUCTURE version 2.3.4 (Pritchard et al. 2000) with 7 loci, 2 assumed populations (bobcat and domestic cat), 100,000 burnin and 200,000 iterations, and a parameter set including no admixture and an



**◀Fig. 1** Approximately 400 km of scat collection transects were located in Bath and Rockingham Counties in Virginia, USA (>200 km in each county). Transects were designated on establish dirt roads, hiking trails, and well used game trails and divided into 0.5-km segments to use as detectors for spatial capture-recapture models. This region of Virginia is in the Ridge and Valley Province of the Appalachian Mountains, and there was differential variability in elevation of transect segments between the two sites, as shown in the histograms

independent allele model. Once we finalized the confirmed bobcat individual genotype database, we used samples from the 5 closed-session population monitoring surveys for each site to construct spatial capture-recapture encounter histories (10 SCR data sets) to estimate density.

## Density estimation

We used three different spatial capture-recapture (SCR) models to estimate bobcat density (Electronic Supplementary Material (ESM) S1). First, we estimated density independently for each site and session with ten separate closed session models ( $\text{SCR}_0$ , Royle et al. 2013, Chap. 5). For each site and session, we constructed a spatially-indexed encounter history using 0.5-km transect segments as “traps”, or detectors ( $j$ ), and assigning each scat sample to the nearest detector using the Near function in the Proximity toolbox in ArcGIS (ESRI 2011). The model is hierarchical, consisting of a process model assuming that individual animals ( $i$ ) use space around a latent individual activity center ( $s_i$ ), and that probability of detection decreases monotonically with distance away from the individual activity center (detection kernel,  $p$ ), represented by a scaling parameter ( $\sigma$ ). Thus, detection of an individual along a transect segment ( $j$ ) is dependent on the distance of the transect segment from the individual’s activity center,  $p_{ij} = \lambda_0 \times e^{-\left(\frac{1}{2\sigma}\right)^2 d_{euc}(s_i, x_j)^2}$ , where  $x_j$  is the location of trap  $j$ , and  $\lambda_0$  is the baseline encounter rate, or expected rate of detection if a transect segment is at the same location as an individual’s activity center.

We used data augmentation to estimate the count of individuals that were present in the state space during a sampling session, but not detected (Royle and Young 2008). We set a large upper bound ( $M$ ) for the maximum number of possible individuals within  $S$  for each site and session (Table 1). We represented undetected individuals with encounter histories consisting of all zeros, as they were not detected during the survey. We associated an indicator variable ( $z_i$ , the outcome of a Bernoulli trial) with each possible unobserved activity center to estimate if it was a real individual present but not detected during the study ( $z_i = 1$ ), or if the augmented encounter history was a structural zero and not part of the population ( $z_i = 0$ ), with a binomial distribution

characterizing all trials ( $\psi$ ). Density ( $\hat{D}$ ) is derived by summing the number of individuals detected and the proportion of possible undetected individuals estimated to be real ( $\hat{N}$ ), and dividing by the area of  $S$ . We used a Poisson encounter model to allow for multiple detections of an individual at a single detector, and for each site, we used a state space size representative of a buffer  $\geq 2\sigma$  (Table 1), as density no longer scales with  $S$  beyond this threshold (Royle et al. 2013).

We formatted data using the scrbook package (Royle et al. 2014) in R (R Core Team 2015) and implemented each model using the rjags (Plummer 2014), and coda (Plummer et al. 2006) packages in R. Model settings for each independent site and session  $\text{SCR}_0$  model included 3 Markov chain Monte Carlo (MCMC) chains with 50,000 adaptations for the Metropolis-within-Gibbs algorithm to reduce slow mixing in chains and increase effective sample sizes. After adaptation, we sampled 100,000 iterations from the posterior distributions of each monitored parameter ( $\hat{\lambda}_0$ ,  $\hat{\sigma}$ ,  $\hat{\psi}$ ,  $\hat{N}$ , and  $\hat{D}$ ) at a thinning rate of 1 (no thinning), and discarded the first 50,000 iterations for burn-in. These settings were excessive in effort. However,  $\text{SCR}_0$  models for two sessions at one site displayed poor mixing and unidentifiable parameters, and the increased effort allowed us to fully evaluate the lack of fit for these instances while keeping settings for all single session models consistent. We assessed MCMC convergence by visually inspecting trace plots for each monitored parameter, and comparing  $\hat{R}$  statistics to 1.1 (Gelman and Rubin 1992). We reported the posterior means and standard deviation, medians, and 95% credible intervals (CRI) for  $\hat{\lambda}_0$ ,  $\hat{\sigma}$ , and  $\hat{\psi}$ , but reported the preferential, unbiased posterior mode for  $\hat{D}$  (Chandler and Royle 2013), estimated using the MCMCgIImm package (Hadfield 2010). We also monitored estimated locations of activity centers ( $\hat{s}_i$ ), and the outcome of each Bernoulli trial ( $z_i$ ) for of the final 50,000 iterations of the independent fits of the  $\text{SCR}_0$  models. This allowed for estimation of realized density surfaces for each site and session with a fitted model (ESM S2).

Although single session models are useful when only one site or season of data is available, sharing detection information across sites and sessions should improve estimation of detection and scaling parameters as a result of increased sample size and result in improved precision in density estimates for each site and session (Royle and Converse 2014). Thus, we fit two different multistrata SCR models constraining baseline detection and scaling parameter ( $\sigma$ ) across sites or sessions. The multistrata model approach simultaneously estimates density for each site and season (a multi-site, multisession model). First, we used dummy variables for season to estimate a beta coefficient for both baseline detection and  $\sigma$  separately for each season (summer or winter for the 5 sessions), but constraining the parameters to be the same

**Table 1** Capture and spatial recapture rates, model settings, and parameter estimates for spatial capture-recapture ( $\text{SCR}_0$ ) model to estimate bobcat density for each site and session in Bath and Rockingham Counties, Virginia, July 2011–July 2013

Site session	Individuals captured (total captures)	Count of individuals recaptured (spatial recaptures)	M <sup>a</sup>	S <sup>b</sup> (km <sup>2</sup> )	$\hat{D}$ <sup>c</sup> (95% CRI)	$\hat{\sigma}$ <sup>d</sup> (95% CRI)	$\hat{\lambda}_0$ <sup>e</sup> (95% CRI)	$\hat{\psi}$ <sup>f</sup> (95% CRI)
Bath Jul 2011	16 (21)	4 (2 <sup>4</sup> ) <sup>g</sup>	650	874.29	13.88/100 km <sup>2</sup> (7.78, 61.76)	1.43 km (0.77, 3.04)	0.02 (<0.01, 0.07)	0.34 (0.10, 0.83)
Bath Feb 2012	33 (48)	6 (2 <sup>4</sup> , 3, 5)	500	874.29	25.84/100 km <sup>2</sup> (13.95, 50.21)	1.70 km (1.13, 2.70)	0.02 (0.01, 0.05)	0.50 (0.24, 0.88)
Bath Jul 2012	13 (19)	5(2 <sup>5</sup> )	300	874.29	7.08/100 km <sup>2</sup> (3.77, 25.62)	1.68 km (0.98, 2.96)	0.03 (0.01, 0.08)	0.30 (0.11, 0.75)
Bath Mar 2013	30 (56)	12 (2 <sup>6</sup> , 3 <sup>3</sup> , 4 <sup>3</sup> )	250	759.08	13.96/100 km <sup>2</sup> (9.62, 22.40)	1.27 km (0.98, 1.70)	0.08 (0.04, 0.14)	0.45 (0.28, 0.68)
Bath Jul 2013	18 (26)	5 (2 <sup>3</sup> , 3 <sup>2</sup> )	400	759.08	12.35/100 km <sup>2</sup> (7.38, 35.31)	1.11 km (0.72, 1.78)	0.06 (0.02, 0.13)	0.31 (0.14, 0.67)
Rockingham Jul 2011	14 (29)	4 (2 <sup>2</sup> , 4, 6)	200	887.85	6.65/100 km <sup>2</sup> (3.38, 11.60)	1.50 km (1.03, 2.26)	0.08 (0.03, 0.17)	0.29 (0.14, 0.52)
Rockingham Feb 2012	11 (15)	2 (2 <sup>2</sup> )	NA	NA	NA	NA	NA	NA
Rockingham Jul 2012	18 (26)	7 (2 <sup>6</sup> , 3)	300	948.69 <sup>2</sup>	10.84/100 km <sup>2</sup> (6.64, 26.14)	1.58 km (1.04, 2.59)	0.03 (0.01, 0.07)	0.44 (0.21, 0.83)
Rockingham Mar 2013	9 (12)	2(2 <sup>2</sup> )	NA	NA	NA	NA	NA	NA
Rockingham Jul 2013	19 (41)	8 (2 <sup>3</sup> , 3 <sup>5</sup> )	250	887.85	9.35/100 km <sup>2</sup> (5.29, 16.11)	1.63 km (1.63, 2.40)	0.05 (0.02, 0.10)	0.33 (0.18, 0.58)

<sup>a</sup> Model setting for data augmentation—the maximum number of possible individuals (detected and undetected) in the state space

<sup>b</sup> Area of the state space within which all possible individuals (detected and undetected) are located

<sup>c</sup> The model estimates the total number of individuals ( $\hat{N}$ ) within the state space ( $S$ ). Density ( $\hat{D}$ ) is derived by  $\hat{N}/S$

<sup>d</sup> Scaling parameter related to home range size that estimates distance an individual may be detected from its center of activity ( $s_i$ )

<sup>e</sup> Baseline encounter rate—the expected detection rate of an individual if a trap is located at the animal's activity center

<sup>f</sup> Data augmentation parameter—the probability that proposed undetected individuals ( $M$  individuals detected) are actual individuals in the population

<sup>g</sup> Number of spatial recaptures number of times observed

across sites ( $\text{SCR}_{\text{seas}}$ ). This allowed for greater information about individual home range movement shared across sites, expected to improve estimation of density for the site with very low number of detected individuals, but assumes individuals display similar home range movements in the two study areas, and increased the number of estimated parameters. Second, we fit a multistrata model that estimated density and data augmentation parameter ( $\psi$ ) for each site and session, but constrained baseline detection and  $\sigma$  to be the same across sites, and also across all sessions ( $\text{SCR}_{\text{ms}}$ ). This model allowed for the greatest shared information across sites producing the most detection of individuals to improve estimation of density for each session and site, and reduced the number of parameters required to be estimated. It assumes individuals across study sites demonstrate similar movement from home range activity center resulting in detection regardless of season. This assumption might be reasonable for fecal DNA sampling of territorial animals such as bobcats that may mark the extent of individual

territories across seasons, even if home range utilization changes within a territory, or species that do not have differential seasonal home range use. For both multistrata models, we included three MCMC chains with 2000 adaptations for the Metropolis-within-Gibbs algorithm and sampled 10,000 iterations from the posterior distributions of each monitored parameter at a thinning rate of 1, and discarded the first 2000 iterations for burn-in. For the multistrata approach, state space and trap locations were different between sites, but held constant between sessions (1128.71 km<sup>2</sup> state space area for Bath County and 1211.97 km<sup>2</sup> state space area for Rockingham County),  $M$  was held constant for all sites and seasons (450 maximum individuals detected and not detected within the state space for each site and season), and  $\psi$  was estimated for each site and session (ESM S1).

Finally, to guide future sampling and monitoring efforts, we were interested in assessing factors that could improve precision in model estimates of  $D$  and  $\sigma$ . Using the output from the independent runs of the  $\text{SCR}_0$  models for each site

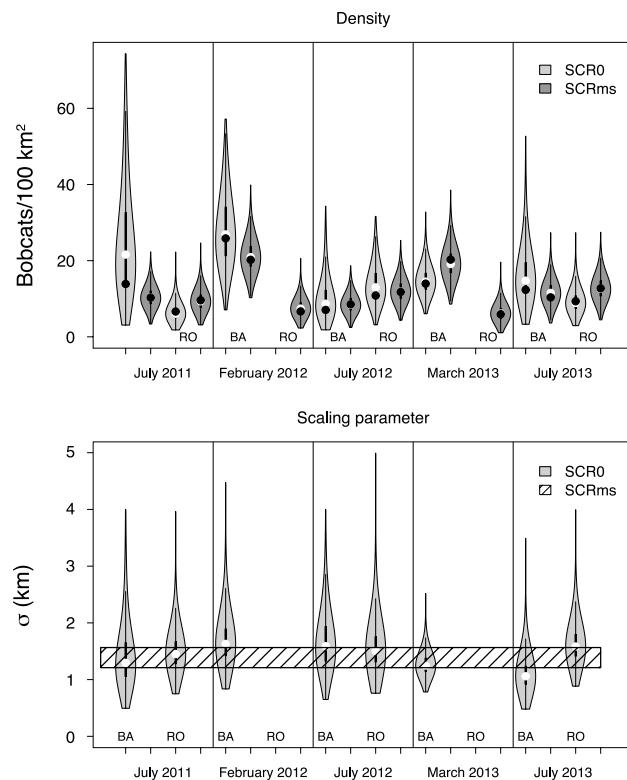
and season, we calculated the coefficient of variation (CV;  $\frac{SE}{mean} * 100$ ) for  $\hat{D}$  and  $\hat{\sigma}$ . The CV is a measure of dispersion around the mean, and lower CV values indicate better precision. We compared CVs by three metrics: (1) total number of individuals detected, (2) an index of spatial recapture success (number of spatial recaptures/individuals with spatial recaptures), (3) the CV for  $\lambda_0$ , the baseline encounter rate, and (4)  $\bar{\lambda}_0$  from model estimates from each sites and session. We expected that CVs for  $\hat{D}$  and  $\hat{\sigma}$  would decrease reflecting improved precision, with increased spatial recapture success and with greater baseline detection, and we used the results to make recommendations to refine future sampling and inform study design recommendations for implementation.

## Results

We collected a total of 2025 fecal samples in the two study areas during the five closed sessions for density estimation. Of those samples, we identified 54.57% as bobcat using the mtDNA species multiplex, and we genotyped these 1105 samples, and an additional 78 bobcat fecal samples collected during concurrent sampling within both study areas for a total of 1183 samples. We successfully amplified nDNA at 50% of loci (4 loci) for 480 samples (0.41 amplification success rate), and confirmed genotypes at 6 loci for 373 samples resulting in 0.32 confirmed genotype success rate (295 confirmed genotype samples from the 2 sites over the 5 density sampling sessions). Mean allelic dropout/sample for confirmed genotypes was 0.19 (range 0–0.59), and the mean number of false alleles/sample for confirmed genotypes was 0.03 (range 0–0.28). We detected one set of samples mismatched at 1 locus that we were unable to resolve, and we removed the sample with lower amplification success and greater number of false alleles (poor sample quality). No individuals mismatched at 2 out of 8 loci. In addition, no samples were identified as domestic cat in the STRUCTURE analysis, confirming our ability to screen for domestic cats using the mtDNA multiplex. In total, we identified 118 individual bobcat genotypes across the 2 study sites. For the closed session population monitoring, we successfully confirmed 108 individual bobcats over 5 sessions at the 2 study sites (63 individuals/171 confirmed samples in the Bath County study area, and 45 individuals/124 confirmed samples in the Rockingham study area).

For the independent SCR<sub>0</sub> models, we were unable to adequately fit SCR models to data for the two winter sessions at the Rockingham site. Due to the insufficient number of recaptures at this site (11 individuals with 2 spatial recaptures in February 2012, and 9 individuals with 2 spatial recaptures in March 2013), the data augmentation parameter was unidentifiable (returned the uniform distribution of the prior), and resulting density estimates increased with even

unrealistic increases in  $M$  (the model was not able to distinguish structural zeros for these two data sets, and therefore could not produce reliable density estimates). However, we were able to fit SCR<sub>0</sub> models and estimate density for all other sessions across the two study areas (Table 1). Although posterior distributions and 95% credible intervals overlapped for all sessions, density estimates were generally lower in Rockingham compared to Bath County (Fig. 2). Posterior modes for density estimates for the summer sessions in Rockingham County were relatively consistent. Density in



**Fig. 2** Violin plots of posterior distributions of bobcat density estimates (top) and SCR scaling parameter  $\sigma$  (bottom). The five sampling sessions are shown in panels left to right for the Bath County (BA: left in each panel) and Rockingham County (RO: right in each panel) study areas, Virginia, USA. Light colored violins represent estimates from independent single session SCR<sub>0</sub> models and dark colored violins represent estimates from the multistrata SCR<sub>ms</sub> model, where baseline detection and  $\sigma$  are constrained to be constant across sites and sessions. Thick lines for each violin plot represent the interquartile range, and thin lines represent the 95% credible intervals (CRI). Mean parameter estimates are represented by white circles. In the top plot, posterior modes for density estimates are dark circles. In the bottom plot, the 95% CRI for the single estimate of  $\sigma$  from the SCR<sub>ms</sub> are represented by the hatched polygon. Encounter histories were generated from individual bobcats identified using nDNA microsatellites extracted from fecal samples collected while walking transects. Density was estimated using a single survey sampling protocol and spatial capture-recapture models for each site, over 5 sessions from July 2011 to July 2013. The winter scat collection sessions did not result in sufficient detections to fit SCR<sub>0</sub> models, and estimates for those sessions are omitted (February 2012 and March 2013)

Bath County was greatest in February 2012, but also relatively consistent across summer sessions.

We were unable to fit the SCR<sub>seas</sub> model, estimating beta coefficients to allow baseline detection and  $\sigma$  to differ between summer and winter due to the greater number of parameters, relatively small sample size, and poor MCMC mixing. However, we were able to fit the SCR<sub>ms</sub> model and estimate density for each session, including the two winter sessions for the Rockingham site that were inestimable with the single session model approach (Table 2). The SCR<sub>ms</sub> density posterior distributions were more precise and showed less spread compared to the SCR<sub>0</sub> density posterior distributions, and the SCR<sub>ms</sub> 95% CRI for  $\sigma$  overlapped the SCR<sub>0</sub> 95% CRI of  $\sigma$  for all estimable sessions (Fig. 2). Density estimates from the SCR<sub>ms</sub> model were again higher in the winter compared to relatively consistent summer density estimates in the Bath County study area and ranged from

8.52 to 20.27 bobcats/100 km<sup>2</sup> (posterior modes) across seasons. However, SCR<sub>ms</sub>  $\hat{D}$  was lower in the winter in Rockingham County study area and ranged from 5.93 to 12.72 bobcats/100 km<sup>2</sup> across seasons, resulting in similar summer density estimates between sites (Fig. 2; Table 2).

Coefficients of variation demonstrated how precision in  $\hat{\sigma}$  and  $\hat{D}$  both increased with all metrics evaluated for single session model estimates (Fig. 3). A decrease in precision, or spread around the mean, resulted in an increase in CV values, and smaller CV values indicated better precision. Increasing numbers of individuals detected and increasing spatial recaptures/individuals with spatial recaptures both decreased spread around the mean of the posterior distributions of  $\hat{\sigma}$  and  $\hat{D}$ . Mean baseline encounter rate ( $\lambda_0$ ) also improved precision, and increasing precision in baseline detection estimates displayed the tightest relationship with increasing precision in  $\hat{\sigma}$  and  $\hat{D}$ .

## Discussion

We were able to estimate bobcat densities by fitting SCR models to encounter histories generated using noninvasive genetic sampling and single-survey scat collection transects over multiple seasons and sites, despite finding that bobcat densities were relatively low in the region. This novel protocol provides a flexible methodology for monitoring carnivores compared to previous sampling protocols, by reducing the required multiple survey, single session sampling effort, and instead, allowing for additional monitoring sessions for the same approximate effort. By sharing information over multiple seasons and sites, instead of investing in repeated surveys within a single session, we increased number of samples available to confirm genotype matches, improved precision in model estimates, and compared density estimates for both sites over time that could be used to evaluate population growth and potential recruitment. We assess limitations in the approach and provide recommendations for future implementation for bobcats and other carnivores below.

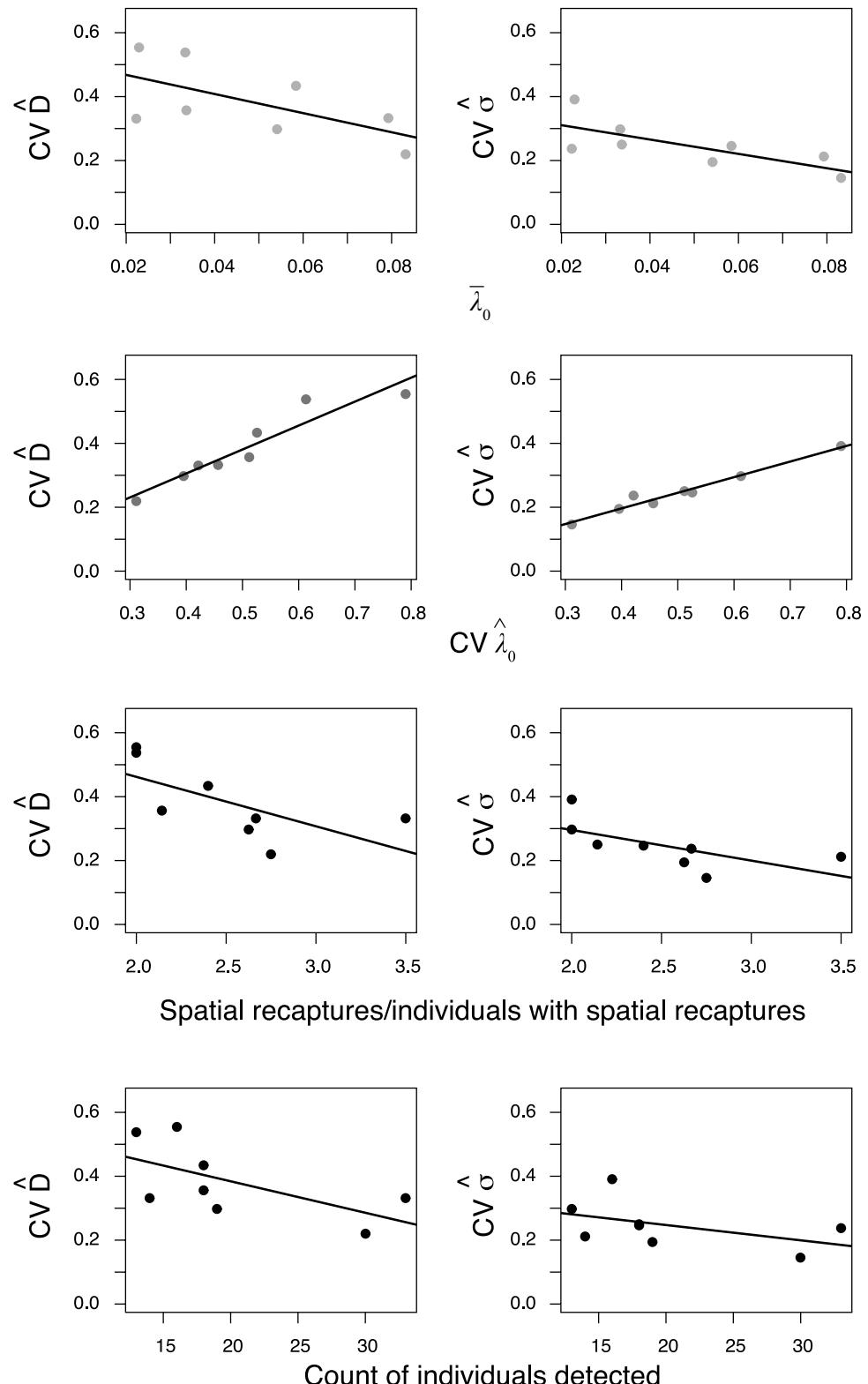
Bobcat density estimates from the Bath and Rockingham County study areas were at the low end of those reported across the bobcat range (Thornton and Pekins 2015). Previous studies across the two study areas have found low densities of both coyote populations (approximately 4–8 coyotes/100 km<sup>2</sup>; Morin et al. 2016a) and white-tailed deer (*Odocoileus virginianus*) populations (approximately 20–150 deer/100 km<sup>2</sup>; Montague et al. 2017), indicative of the generally low carrying capacity of the regional habitat (DeCalesta 1997; Diefenbach et al. 1997). Low population density can result in poor precision or inestimable parameters (Williams et al. 2002). However, by including detections over multiple sessions, we were able to improve

**Table 2** Parameter estimates for the multistrata spatial capture-recapture (SCR<sub>ms</sub>) models to estimate bobcat density for each site and session in Bath and Rockingham Counties, Virginia, July 2011–July 2013

Site session	$\hat{D}_{ms}$ (95% CRI)	$\hat{\psi}_{ms}$ (95% CRI)
Bath Jul 2011	10.28/100 km <sup>2</sup> (6.20, 16.30)	0.26 (0.15, 0.41)
Bath Feb 2012	20.25/100 km <sup>2</sup> (14.62, 29.86)	0.53 (0.36, 0.75)
Bath Jul 2012	8.52/100 km <sup>2</sup> (4.87, 13.73)	0.21 (0.12, 0.35)
Bath Mar 2013	20.27/100 km <sup>2</sup> (13.11, 28.00)	0.48 (0.32, 0.70)
Bath Jul 2013	10.37/100 km <sup>2</sup> (7.00, 18.07)	0.29 (0.17, 0.46)
Rockingham Jul 2011	9.59/100 km <sup>2</sup> (5.45, 15.02)	0.25 (0.14, 0.41)
Rockingham Feb 2012	6.67/100 km <sup>2</sup> (4.04, 12.71)	0.20 (0.11, 0.35)
Rockingham Jul 2012	11.80/100 km <sup>2</sup> (7.34, 18.65)	0.32 (0.19, 0.51)
Rockingham Mar 2013	5.93/100 km <sup>2</sup> (3.14, 10.89)	0.17 (0.08, 0.30)
Rockingham Jul 2013	12.72/100 km <sup>2</sup> (7.84, 19.64)	0.34 (0.21, 0.53)

SCR<sub>ms</sub> constrained  $\lambda_0$  and  $\sigma$  to be constant across sites and seasons. The model estimates the total number of individuals ( $\hat{N}$ ) within the state space ( $S$ ). Density ( $\hat{D}$ ) is derived by  $\hat{N}/S$ . Data augmentation parameter—the probability that proposed undetected individuals ( $M$  individuals detected) are actual individuals in the population. For the multistrata approach, state space and trap locations were different between sites, but held constant between sessions (1128.71 km<sup>2</sup> state space area for Bath and 1211.97 km<sup>2</sup> state space area for Rockingham), and  $M$  was held constant for all sites and seasons (450 maximum individuals detected and not detected within the state space for each site and season), and  $\psi$  was estimated for each site and session. For the multistrata model,  $\hat{\sigma}_{ms} = 1.37$  km, 95% CRI (1.21, 1.56), and  $\hat{\lambda}_{0ms} = 0.04$ , 95% CRI (0.03, 0.06)

**Fig. 3** Scatterplots showing effects of four different metrics on precision of the posterior distributions for density ( $\hat{D}$ , left column) and the scaling parameter ( $\sigma$ , right column) estimates, as measured by coefficients of variation (CV). Metrics evaluated included mean baseline encounter rate ( $\bar{\lambda}_0$ , top row), CV for the posterior distribution of  $\hat{\lambda}_0$  (second row), number of spatial recaptures/individuals with spatial recaptures in a session at a site (a measure of spatial recapture success, third row), and number of individuals detected in a session at a site (last row). As precision increases, CV values decrease, and lower CV values are desirable



precision and estimate density. Density estimates from the SCR<sub>ms</sub> model suggest there is an increase in number of individuals at the Bath County site in the winter sessions, compared to the summer sessions. As suggested for coyotes

(Morin et al. 2016a), it is possible more individual bobcats are detected at this site during the winter session when bobcats are more mobile and potentially attempting to disperse from their natal range. Little information is available

on bobcat dispersal (Kamler et al. 2000) since dispersal is difficult to observe. However long distance dispersal movements (> 100 km) have been reported (Nielsen and Woolf 2003). Thus, the winter session may include not only resident bobcats with established home ranges, but also individuals attempting to immigrate into the local Bath County population, still detected during a short foray into the study area. If we consider the summer session estimates to be representative of the adult resident population, any difference in density between summer and the following winter session could represent potential recruitment into the population for the following year, while the difference between consecutive summer sessions would be the net recruitment realized for the population (dependent on survival, successful immigration, and emigration). Conversely, density estimates from the Rockingham study site remained relatively consistent across seasons and years (wide overlap of 95% CRI). This may reflect an unchanging population, low potential recruitment, or the very low density may represent a limitation in the approach to detecting population changes over short periods of time at the current sampling intensity for the study area extent.

The monitoring and estimation methods demonstrated provide multiple advantages to previous efforts to estimate bobcat densities, and have potential to improve monitoring of carnivore populations worldwide. The single-survey, scat collection protocol reduced field effort/km of transects surveyed compared to those protocols and estimation methods that require multiple survey occasions within a closed session (Ruell et al. 2009). Thus, instead of repeated visits within a single session, we were able to monitor transects for additional seasons. The field costs to cover the same area we surveyed with cameras at an adequate spacing to estimate density ( $< 2\sigma$ , Sun et al. 2014) would likely have been much greater than the costs to implement a single scat survey, when considering costs of cameras at each station and field effort to maintain the camera trap grid during a closed session. For example, if we wanted to estimate density for the Bath County study area in summer 2012, based on the mean  $SCR_{ms}$  estimate of  $\sigma$  (1.37 km), spacing between camera stations would need to be  $< 2.74$  km. To cover the same area surveyed using scat transects (a 250 km<sup>2</sup> grid), a minimum of 40 camera stations would be required (2 cameras/station to identify individuals using both flanks, but see Augustine et al. 2018, in press), and this would likely be insufficient to estimate density with reasonable precision (see recommendations on spatial extent below). Additional site visits may also be required for a 1 month survey, depending on the battery life and memory capacity of the cameras. Ultimately, the costs of field effort and equipment must be balanced against the costs of compiling data, including DNA extraction and PCR, postprocessing of photographs, and success rates of identification of individuals for both approaches. However,

the use of noninvasive genetics also allows for additional population and landscape genetics analyses, including efforts to describe dispersal and immigration (Janečka et al. 2007; Croteau et al. 2010; Wultsch et al. 2016), providing valuable additional information about population dynamics.

We provide several recommendations on general sampling implementation, and suggest refinements and modifications to improve on resulting estimates for cryptic carnivore species. First, a limitation to the scat transect methodology is that sampling is restricted to existing trails and roads. As a result, some areas may not be surveyed. For example, we were unable to fit single session  $SCR_0$  models to data sets for the two winter sessions at the Rockingham site due to poor detection. Because we were able to adequately sample bobcats in winter at the Bath County site, poor detection does not appear to be related to general climate conditions, but perhaps to differential space use during the winter in relation to our sampling transects. While the overall elevation between the sites was generally equivalent, the spatial arrangement of established dirt roads and hiking trails used as transects, resulted in a greater proportion of transect segments at higher elevations at Rockingham (Fig. 1). Thus, if bobcats spend more time in more protected areas at lower elevations during harsh winter months, detection would decrease. There is also a greater number of hound hunts in the Rockingham area in the fall and winter months. Bobcats may respond by retreating from frequently used roads and trails along ridgelines, decreasing opportunities for detection on scat transects at that study site. Thus, greater stratification of transect segments across the elevation gradient, incorporating more game trails, and supplemental use of cameras in areas without trails and roads, could improve detection and allow for density estimation in the Rockingham study area (Harmsen et al. 2010). Differential use of areas with available transects should be considered when implementing scat transect surveys. If suitable transects are not available, alternative sampling methods such as using detection dogs, sampling multiple data types, and unstructured spatial sampling should be considered, but will likely result in greater effort and financial cost (Russell et al. 2012; Sollmann et al. 2013; Davidson et al. 2014).

Second, precision of both the single session and multi-strata models could be improved. As precision was directly related to number of individuals detected, increasing the overall survey area could allow for better estimation, as could increasing the density of transects in some areas to increase potential for spatial recaptures (Royle et al. 2013, Chap. 10; Sun et al. 2014; Wilton et al. 2014). As in Morin et al. (2016a), we also suggest extending the closed session allowing for more scat deposition that would increase spatial recapture rates, as long as demographic closure is not violated. Sampling strategies that increase DNA amplification rates could also increase baseline detection of individuals,

which would improve precision for density estimates. While our results demonstrate that a 1-month sampling interval can be effective for estimating population density for this species in this geographic region, this interval will vary by species, region, local environmental conditions, population density, and detection rates (Lonsinger et al. 2015; Woodruff et al. 2015). We were able to amplify samples with a 32% success rate for confirming genotypes over a 1-month interval. However, while the single-survey method decreased effort compared to multiple occasion surveys, it is possible that collecting samples every 2 weeks in our study area could decrease environmental degradation and improve amplification rates. The resulting dataset could then be treated as a single occasion (summed over all occasions) or multiple occasion sampling protocol, due to the use of the Poisson encounter model. We suggest an initial pilot study evaluating DNA degradation and scat deposition rates would aid in identifying the appropriate length of closed sessions and whether additional surveys may be required (Lonsinger et al. 2015; Woodruff et al. 2015). In addition, home range movements for felids can differ depending on sex, which can influence detection probabilities and resulting precision of parameters (Sollmann et al. 2011). Including sex-specific estimation of  $\lambda_0$  and  $\sigma$  could improve model estimates if there is sufficient data to support estimation of additional parameters, but handling of individuals with unknown sex identification, which can result from partial genotypes from low-quality DNA, should be carefully considered.

Finally, timing and frequency of closed sessions utilizing this method will depend on study objectives. If only one session is planned, greater sampling intensity over a larger extent is necessary to estimate parameters with precision. Similarly, as we were unable to fit the more complex SCR<sub>seas</sub> model with our data, sampling should be intensified to increase sample sizes over multiple sessions if there are large differences in seasonal movement and detection. However, if multiple sites or sessions are monitored, and some parameters can be constrained to be the same across sites and sessions, intensity of each single session may be reduced if a multistrata SCR approach is employed. If the primary objective is to monitor density for a region, we recommend a summer survey for our study sites to represent the resident adult bobcat population. If assessing trends in population growth is the primary objective, we also recommend summer sessions, but large intervals including multiple years between surveys will be required due to the nature of demographic stochasticity, as well as precision in estimates, even if improved. However, there are also benefits to winter sampling of bobcats in our region, separate or in conjunction with, summer sampling. These include higher individual identification success rates, estimating potential net recruitment, providing realized density surfaces (ESM S2) for use in

spatially-explicit winter harvest, evaluating seasonal habitat associations with local bobcat densities, and identifying areas with higher potential for predation pressure on prey populations compromised by snowfall. Thus, monitoring objectives, individual identification success rates, species life history and local ecology should be carefully considered in planning the timing of sampling sessions.

Conservation and monitoring are expensive and resources are limited (McCarthy et al. 2012). As a result, new, efficient methodologies are needed for monitoring low-density populations including many endangered carnivore populations worldwide. Our single-survey, scat transect sampling method, combined with a single microsatellite multiplex and SCR hierarchical models, reduces sampling effort and provides an advantage to previously employed methods of density estimation for felid populations. Employing single survey sessions increases the number of sessions that can be conducted including surveys across more sites, as repeated surveys within a session at a site are no longer required. In addition, density estimates for felids without visually identifiable marks are obtainable using this method, and because only one microsatellite multiplex is required, multiple felids can be sampled simultaneously (Wultsch et al. 2014) without additional field or laboratory costs. As a result, this methodology has potential to dramatically increase the effectiveness of conservation dollars and improve understanding of wild felid ecology and the ability to monitor carnivore populations and assess conservation needs and actions.

**Acknowledgements** We appreciate the comments of two anonymous reviewers to a previous draft that substantially improved the quality of the manuscript. Financial support provided by the Virginia Department of Game & Inland Fisheries came from the US Fish & Wildlife Service through the Wildlife and Sport Fish Restoration Program, Project WE99R. We appreciated additional funding and support from the Appalachian members of the Virginia Deer Hunter Association, The Nature Conservancy, the Edna Bailey Sussman Foundation, the Student Undergraduate Research Foundation, and the Cunningham Fellowship (Virginia Tech Graduate School). We thank our collaborators C. Wultsch, and J.R. Adams, B. Mesa-Cruz, and R.S. Alonso; the Virginia Department of Game and Inland Fisheries: M. Fies, D.E. Steffen, A. Bourgeois, and N. Lafon; the US Forest Service: C. Croy, S. Tanguay, T. Slater; the Nature Conservancy: M. Smith at the Warm Springs Mountain Preserve; and numerous field and lab technicians for assistance and collection of data.

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