Empirical comparisons of abundance estimators for two sympatric carnivores using noninvasive genetic sampling

Authors: Lonsinger, Robert C., Lukacs, Paul M., Gese, Eric M., and Waits, Lisette P.

Source: Wildlife Biology, 2019(1) : 1-9

Published By: Nordic Board for Wildlife Research

URL: https://doi.org/10.2981/wlb.00534

Wildlife Biology 2019: wlb.00534
doi: 10.2981/wlb.00534

Subject Editor: Al Glen. Editor-in-Chief: Ilse Storch. Accepted 15 May 2019

Empirical comparisons of abundance estimators for two sympatric carnivores using noninvasive genetic sampling

Robert C. Lonsinger, Paul M. Lukacs, Eric M. Gese and Lisette P. Waits

R. C. Lonsinger (https://orcid.org/0000-0002-1040-7299) ✉ (robert.lonsinger@sdstate.edu), Dept of Natural Resource Management, South Dakota State Univ., Brookings, SD 57007-1696, USA. – P. M. Lukacs, Wildlife Biology Program, Dept of Ecosystems and Conservation Sciences, W.A. Franke College of Forestry and Conservation, Univ. of Montana, Missoula, MT, USA. – E. M. Gese, Wildlife Services, National Wildlife Research Center, Dept of Wildland Resources, United States Dept of Agriculture, Utah State Univ., Logan, UT, USA. – L. P. Waits, Dept of Fish and Wildlife Sciences, Univ. of Idaho, Moscow, ID, USA.

Abundance estimators are often evaluated with simulations, or by comparing estimates to populations of known size. Advances in noninvasive genetic sampling have fueled an increase in the use of noninvasive genetic sampling-based capture–recapture. However, when working with free-ranging populations of unknown size, managers often lack data necessary to select the appropriate estimator. This leads to uncertainty regarding how choice of estimator or sampling design influence estimates, and managers may select estimators based on funding or logistical constraints. Alternatively, comparing estimates from multiple estimators can provide managers with greater confidence in estimates, or highlight potential differences. We used noninvasive genetic sampling to estimate the abundance of free-ranging kit foxes *Vulpes macrotis* and coyotes *Canis latrans*. We generated estimates of abundance with two non-spatial likelihood-based estimators: 1) robust design Huggins capture–recapture models and 2) single-occasion capture with replacement (CAPWIRE) models. We compared these with recently published estimates derived from spatially explicit capture–recapture (SECR) models. For both species, estimates from Huggins models were generally lower than those from SECR. Abundance estimates from CAPWIRE, which was developed specifically for noninvasive genetic sampling and generates estimates from a single sampling occasion, tended to be biased low with high precision. Our results suggest that choice of estimator and sampling design can significantly influence estimates, and that the relationship between estimators varied between species. Our results further suggest that single-occasion sampling often employed with CAPWIRE abundance estimation may produce biased results and be inappropriate for species requiring dispersed sampling strategies.

Keywords: abundance, *Canis latrans*, capture–recapture, CAPWIRE, noninvasive genetic sampling, *Vulpes macrotis*

Animal abundance is a critical parameter for management and is most reliably estimated with capture–recapture methods (Williams et al. 2002). Conventional live-capture and recapture may be impractical for estimating abundance ($\hat{N}$) for management-scale or long-term studies, particularly when species are difficult to capture, or when managers need to monitor multiple species concurrently. Noninvasive genetic sampling provides an alternative 'capture' method and involves the collection and genetic identification of biological material from the environment (Waits and Paetkau 2005, Schwartz et al. 2007). Statistical abundance estimators, or models, have been developed (Miller et al. 2005, Puechmaille and Petit 2007) or refined (Lukacs and Burnham 2005, Thompson et al. 2012) for use with noninvasive genetic sampling. These advancements have facilitated an increase in the combined use of noninvasive genetic sampling and capture–recapture to meet management goals (Stenglein et al. 2010b, Piaggio et al. 2016, Lonsinger et al. 2018).

Abundance estimators commonly used with noninvasive genetic data include non-spatial closed-population models, spatially explicit models and capture with replacement models. Non-spatial closed-population capture–recapture models assume geographic and demographic closure within primary sampling periods and require ≥2 secondary sampling occasions, during which animals are captured, released and remain available for recapture (Otis et al. 1978, Williams et al. 2002). Spatially explicit capture–recapture (SECR) models use spatially disparate captures of individuals to address capture heterogeneity among individuals associated with proximity to traps, and to estimate density ($\hat{D}$) by evaluating the effective sampling area (Borchers and

Efford 2008); $\hat{N}$ is a derived parameter ($\hat{N} = \hat{D} *$ effective sampling area) of SECR. Data collected via noninvasive genetic sampling differs from conventional live-capture data in that individuals can be captured >1 time within a sampling occasion (Miller et al. 2005, Thompson et al. 2012). While SECR models can generate estimates based on a single sampling occasion (when individuals are captured at >1 location; Efford 2011), capture with replacement (CAPWIRE) models have been developed specifically for noninvasive genetic sampling, exploiting repeat captures of individuals within a sampling event (Miller et al. 2005).

The performance of abundance estimators (i.e. accuracy and precision) is often evaluated using simulations (Petit and Valiere 2006, Borchers and Efford 2008, Lukacs et al. 2009, Efford and Fewster 2013), or by generating estimates for populations of known size (Carothers 1973, Puechmaille and Petit 2007). However, in practice, managers are interested in $\hat{N}$ for free-ranging populations of unknown size, and it is often unclear how the choice of estimator influences estimates. If $\hat{N}$ varies substantially among estimators, reliance on a single statistical model, without consideration of alternatives, may result in inaccurate estimates and misinformed management. Furthermore, differences in $\hat{N}$ can be diagnostic of departures from model assumptions (Otis et al. 1978) and provide guidance on which estimators are likely to be more robust.

We used noninvasive genetic sampling data from sympatric kit foxes *Vulpes macrotis* (hereafter foxes) and coyotes *Canis latrans* to generate likelihood-based estimates of abundance with two non-spatial statistical abundance estimators: 1) robust design Huggins closed-capture models and 2) single-occasion CAPWIRE models. We then compared these estimates to one another, and to estimates generated by Lonsinger et al. (2018) using multi-session SECR models. Abundance estimates generated from all three modeling frameworks represented the same spatio–temporal extent and we aimed to compare these estimates within and between species in relation to model assumptions and evaluate if the choice of estimator and/or sampling design significantly influenced results. We hypothesized that for each species within each session, $\hat{N}$ would exceed the minimum number known alive (MNKA) for all estimators. Capture heterogeneity, if unaccounted for, can result in underestimation of abundance (Otis et al. 1978). Therefore, we expected $\hat{N}$ from SECR models to be higher than non-spatial estimates, as SECR accounts for heterogeneity not addressed by non-spatial models. Single-occasion sampling designs can be appealing to managers due to decreased effort and costs and have been demonstrated with simulations to produce reliable estimates of abundance. Thus, we hypothesized that CAPWIRE estimates would be similar to those of the other estimators.

Material and methods

Terminology

Using a robust design, sessions were primary periods within which there were multiple secondary sampling occasions and populations were assumed to be closed (Pollock et al. 1990; Fig. 1); populations were assumed to be open between sessions. For single-occasion CAPWIRE models, we incorporated only samples from the first sampling occasion within each session. Season indicated sessions representing the same climatic season across years (Fig. 1). Robust design and multi-session are used to indicate that parameters were estimated across sessions within a single modelling framework, but are generally used for non-spatial and spatial models, respectively. Finally, we used 'capture' and 'recapture' to describe the identification of an individual (a unique genotype) through noninvasive genetic sampling.

Study site and sample collection

Our study site was ~3015 km^2, including portions of the U.S. Army Dugway Proving Ground and surrounding federal lands (collectively hereafter, Dugway) in Utah, USA (Lonsinger et al. 2018). Dugway was characterized as Great Basin Desert with low-lying basins separated by mountains (~1200–2100 m). Land cover included cold desert playa, cold desert chenopod shrubland, vegetated and unvegetated dunes, and non-native invasive grasslands at lower elevations, and arid shrubland and open woodland at higher elevations (Lonsinger et al. 2017).

We conducted carnivore fecal DNA surveys along dirt and gravel roads during four sessions from 2013 to 2014: two winters (January to March) and two summers (July to August). We surveyed 30 randomly selected 5 km transects multiple times per session (hereafter, multi-occasion transects), with a sampling interval of ~14 days between occasions (Lonsinger et al. 2015a; Fig. 1). Additionally, we surveyed 2-km of transects (composed of four 500 m transects) within each of 60 sites once per session (hereafter, single-occasion transects); sites were randomly selected from a grid of 576 cells (each 6.25 km^2) superimposed over the study area (Lonsinger et al. 2017). We collected ~0.6 ml of fecal material from the side of each carnivore scat detected (Stenglein et al. 2010a) into 1.4 ml of DETS buffer (20% DMSO, 0.25 M EDTA, 100 µM Tris, pH 7.5 and NaCl to saturation; Seutin et al. 1991), and collected remaining portions for diet analyses (Gosselin et al. 2017, Byerly et al. 2018). Sampling methods are further detailed in Lonsinger et al. (2018).

Based on sample accumulation rates (Lonsinger et al. 2015a), we surveyed multi-occasion transects four times in winter 2013 and three times in summer 2013. We then performed power analyses to evaluate the number of occasions required to achieve a coefficient of variation (CV) <10% for $\hat{N}$ in each season for closed-capture analyses. For each analysis, we ran 1000 simulations in program MARK (White and Burnham 1999) using estimates of capture probability (p) from preliminary closed-capture models considering temporal variation in p and the number of individuals captured per session. We assumed no behavioral response to sampling: recapture probabilities (c) = p. Power analyses indicated our effort was insufficient to achieve desired levels of precision for fox $\hat{N}$, so we increased sampling in 2014 to five winter and four summer occasions (Fig. 1). During the final occasion of summer 2014, we only collected scats identified as fox based on size (Lonsinger et al. 2015b).

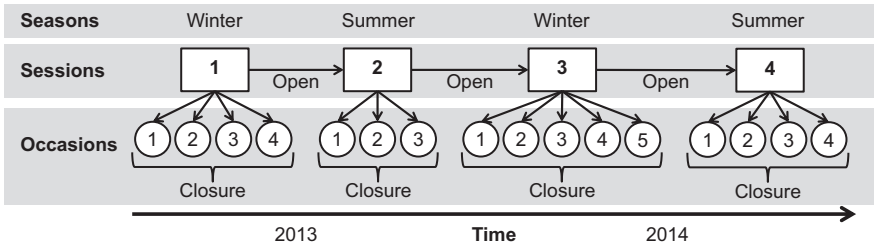

Figure 1. Graphical representation of the temporal sampling scheme (robust design) employed along multi-occasion transects for kit foxes *Vulpes macrotis* and coyotes *Canis latrans* in the Great Basin desert, Utah, 2013–2014. Populations were assumed to be geographically and demographically closed within sessions, and open between sessions.

Genetic analyses

We restricted DNA extraction and polymerase-chain reaction (PCR) amplification to a laboratory dedicated to low quantity and quality DNA to minimize contamination risk. We used mitochondrial DNA (mtDNA) to identify species (De Barba et al. 2014) and multiplexes with nine nuclear DNA (nDNA) loci and one sex identification locus for fox and coyote individual identification. Extraction methods, DNA storage and mtDNA PCR conditions are detailed in Lonsinger et al. (2015a), while nDNA PCR conditions and scoring methods are detailed in Lonsinger et al. (2018). We minimized the influence of genotyping errors by using a multi-tubes approach (Taberlet et al. 1996), culling low quality samples (those failing mtDNA amplification or at ≥50% of nDNA loci during initial replicates; Kohn et al. 1999, Paetkau 2003), and requiring heterozygous and homozygous genotypes be observed ≥2 and ≥3 times, respectively (Lonsinger and Waits 2015). To reliably distinguish individuals (i.e. probability that two siblings have identical multilocus genotypes <0.01; Waits et al. 2001), foxes and coyotes required genotypes at six and five loci, respectively (Lonsinger et al. 2018). We performed up to eight nDNA PCR replicates for foxes, and six replicates for coyotes, and used ConGenR (Lonsinger and Waits 2015) to compare replicates and establish consensus genotypes. We then established matches (i.e. multiple 'captures' of an individual) by comparing samples with identical or near identical multilocus genotypes (Lonsinger et al. 2018). For multilocus genotypes observed only once (i.e. potential single-capture individuals), we evaluated reliability with RELIOTYPE (Miller et al. 2002) and retained samples with a reliability ≥ 99%.

Capture–recapture analyses

Capture–recapture data were analysed using maximum likelihood methods applied in non-spatial 1) Huggins closed-capture models (Huggins 1989) and 2) CAPWIRE models (Miller et al. 2005), and $\hat{N}$ from these non-spatial models were compared to $\hat{N}$ previously derived from multi-session SECR models (Lonsinger et al. 2018). The Huggins closed-capture models were fit using the entire noninvasive genetic sampling data set (i.e. all samples with individual identification); for comparison, Lonsinger et al. (2018) used the same complete data set to generate SECR-based estimates. For Huggins models, multiple captures of an individual within an occasion were collapsed to a binary response for encounter histories. The number of times a transect was surveyed (i.e. effort) varied among sites and seasons. To account for p on single-occasion transects in Huggins models, we distinguished males and females captured on multi-occasion transects from those captured only on single-occasion transects (i.e. multi-occasion males, multi-occasion females, single-occasion males, single-occasion females), and for each sex applied the mean p estimated from multi-occasion transects to single-occasion transects.

Non-spatial Huggins models were fit using a robust design (Huggins 1989, Pollock et al. 1990) in program MARK (White and Burnham 1999). Although this modelling framework provides estimates of survival (S), p, recapture (c), temporary immigration ($1-\gamma''$) and temporary emigration (γ'), for purpose of comparing abundance estimators, we describe the model set, but focus on reporting estimates related to p and derived $\hat{N}$. We modelled apparent survival considering models with constant, time-varying or trend in survival (Otis et al. 1978, Williams et al. 2002). We also considered models in which apparent survival varied by season or was influenced by an extreme winter (2013). We considered the effects of sex, individual heterozygosity and distance to water on apparent survival. For fox apparent survival, we also considered a covariate of coyote activity. See Supplementary material Appendix 1 for details on individual covariates. We considered three movement models: random ($\gamma' = \gamma''$), constant but different ($\gamma' \neq \gamma''$) and no ($\gamma' = \gamma'' = 0$) movement. We did not expect a behavioural response to capture when using noninvasive genetic sampling and set c = p. We modeled p as constant or varying by time, trend and sex within sessions, and considering additive models of sex with both time and trend. We combined each model for S, with each combination of models for movement and p. We used Akaike's information criterion with small sample size correction (AIC_c) and Akaike weights to compare the relative fit of models (Burnham and Anderson 2002). Parameter estimates accounting for model-selection uncertainty were achieved by model-averaging (Burnham and Anderson 2002). We calculated variances and confidence intervals for model-averaged estimates with the delta method (Williams et al. 2002). We tested for closure with CLOSETEST (Stanley and Burnham 1999).

CAPWIRE assumes equal effort across sites (Miller et al. 2005). We fit separate CAPWIRE models for each session with a reduced data set that met the equal effort assumption and was intended to represent how managers would sample if using this estimator (single-occasion formulation). Specifically, we identified portions of the multi-occasion transects contained within each of the 576 grid cells used to select single-occasion

transects, and that were ≥2 km in length, allowing four 500 m nested transects to be identified. For each session, we then considered captures from single-occasion transects and only the first occasion of multi-occasion transects, restricting captures to those on nested transects.

CAPWIRE models were fit independently for each session with the R package 'capwire' (Pennell et al. 2013, <www.r-project.org>). CAPWIRE assume either that all individuals have equal p (equal capture model; ECM) or that two capture classes exist (two-innate rates model; TIRM) representing individuals with relatively low and high p (Miller et al. 2005). For each species, we fit single-occasion formulations of the ECM and TIRM for each session, and compared model fit using a likelihood-ratio test implemented in 'capwire' with 1000 simulations; the ECM was rejected when $p < 0.1$. We generated 95% confidence intervals for the estimate of the TIRM using 1000 parametric bootstraps (Miller et al. 2005, Pennell et al. 2013).

Initial CAPWIRE abundance estimates for both species were generally lower across sessions than $\hat{N}$ from multi-session models. To determine if CAPWIRE produced estimates more comparable to the multi-session models with a more complete dataset, we conducted a post-hoc analysis in which we increased the number of captures included in the analysis by including captures from all occasions and dividing the number of captures by the number of occasions to standardize effort (multi-occasion formulation); models were fit following the same procedures as for the single-occasion formulation.

Results

Sampling and genetic identification

We surveyed multi-occasions transects 3–5 times per session (Table 1, Fig. 1). Survey effort totaled 720 km in winter 2013, 570 km in summer 2013 and 870 km in winter 2014 for both species. In summer 2014, survey effort was 720 km for foxes and 570 km for coyotes (Fig. 1). We collected 3752 scats, had high species identification success (87.3%), and identified 810 fox and 2374 coyote scats. We identified 109 unique foxes (60% male), with 36–50 individuals captured per session and 37 captured in ≥2 sessions. We identified 302 unique coyotes (53% male), with 128–151 individuals captured per session and 140 captured in ≥2 sessions.

Power analyses indicated four occasions in winter 2013 achieved a CV >10% for foxes. Observed p increased as snow melted (Table 1); nearly all snow had melted by the fourth occasion and we assumed that p of a fifth occasion would have been comparable to the fourth, so we set them equal while assessing power with five occasions. Five winter occasions produced a CV = 6.5%. Power analyses indicated three summer occasions failed to achieve desired precision. We again assumed the p of a final occasion would be comparable to that observed during the subsequent occasion and set them equal. Four occasions in summer produced a CV = 9.7%. Consequently, we increased sampling in 2014 to five winter and four summer occasions (Table 1, Fig. 1). For coyotes, power analyses indicated our initial sampling design was sufficient, with four winter occasions producing a CV = 7.7% and three summer occasions producing a CV = 6.5%. We elected to sample coyotes for the same number of occasions as foxes in winter 2014 but stopped sampling suspected coyote scats after three occasions in summer 2014 to reduce costs (Table 1, Fig. 1).

Robust design non-spatial capture–recapture analysis

Program CLOSETEST supported closure for foxes in 2013 (winter: $\chi^2 = 3.43$, df = 3, p = 0.329; summer: $\chi^2 = 1.19$, df = 2, p = 0.550), but not 2014 (winter: $\chi^2 = 17.08$, df = 4, p = 0.002; summer: $\chi^2 = 8.38$, df = 3, p = 0.006). Component and subcomponent tests suggested closure violations may have resulted from losses following the second occasion in

Table 1. Model-averaged estimates of capture probability (p) and unconditional standard error (SE) produced by program MARK by sex for kit foxes *Vulpes macrotis* and coyotes *Canis latrans* surveyed with noninvasive genetic fecal sampling over two winter (W) and two summer (S) sessions in western Utah, USA, 2013–2014. Behavioral response was not expected with noninvasive sampling and thus recapture probability (c) was modeled as p = c.

		Kit fox				Coyote			
		Male		Female		Male		Female	
Session[a]	Occasion[b]	p	SE	p	SE	p	SE	p	SE
W 2013	1	0.207	0.068	0.207	0.069	0.321	0.055	0.332	0.054
	2	0.236	0.064	0.236	0.065	0.271	0.046	0.281	0.049
	3	0.414	0.074	0.414	0.075	0.277	0.045	0.288	0.049
	4	0.536	0.093	0.536	0.094	0.330	0.055	0.340	0.054
S 2013	1	0.432	0.094	0.431	0.095	0.426	0.056	0.411	0.055
	2	0.369	0.072	0.368	0.074	0.400	0.058	0.386	0.068
	3	0.322	0.081	0.321	0.083	0.269	0.044	0.258	0.049
W 2014	1	0.489	0.074	0.489	0.074	0.541	0.045	0.543	0.045
	2	0.413	0.057	0.413	0.057	0.455	0.040	0.457	0.040
	3	0.373	0.084	0.373	0.084	0.378	0.041	0.380	0.040
	4	0.259	0.048	0.259	0.048	0.265	0.038	0.267	0.038
	5	0.186	0.053	0.186	0.053	0.221	0.034	0.223	0.034
S 2014	1	0.276	0.088	0.272	0.087	0.405	0.045	0.406	0.045
	2	0.368	0.099	0.363	0.097	0.432	0.040	0.433	0.040
	3	0.415	0.088	0.409	0.087	0.466	0.047	0.467	0.047
	4	0.408	0.096	0.403	0.095				

[a]Sessions represent primary sampling periods within a robust design.
[b]Occasions represent secondary sampling periods within a robust design.

both 2014 sessions. For coyotes, CLOSETEST supported closure in 2013 (winter: $\chi^2 = 1.16$, df = 4, p = 0.884; summer: $\chi^2 = 3.69$, df = 2, p = 0.158), but not 2014 (winter: $\chi^2 = 35.97$, df = 6, $p < 0.001$; summer: $\chi^2 = 15.33$, df = 2, $p < 0.001$). Component and subcomponent tests suggested closure may have been violated by additions and losses in winter, and additions in summer.

We compared the fit of 31 non-spatial models for fox and coyote S (Supplementary material Appendix 2); we also evaluated five models for fox S including an index of coyote activity (Supplementary material Appendix 2). When fit with each combination of the six detection and three movement models (Supplementary material Appendix 2), each survival model was represented 18 times in initial model sets. We excluded models for which S or p were confounded, or where boundary effects resulted in estimates of S or p fixed at 1 (SE = 0).

For both species, multiple models among the most supported shared similar structures for S but differed in structure for p and movement (Supplementary material Appendix 3, 4). Model-averaged estimates of fox p were similar between sexes (Table 1) and the best-fit models suggested a trend in p within sessions (Supplementary material Appendix 3). We observed only slight differences in p between male and female coyotes (Table 1) and top models supported time or trend variation in p (Supplementary material Appendix 4).

Model-averaged $\hat{N}$ from Huggins models suggested that there were 2.7–3.6 times more coyotes than foxes (Fig. 2). Fox $\hat{N}$ ranged from 60.1 to 73.2, whereas coyote $\hat{N}$ ranged from 198.1 to 230.7. Confidence intervals (95%) suggested that abundance of both species was relatively stable over the four sessions and that estimates were comparable to those derived from SECR models (Fig. 2).

Single-occasion formulation CAPWIRE analysis

We included captures from 103 transects of equal effort for CAPWIRE analyses resulting in 206 km of surveys per session. We identified 21–30 foxes and 72–103 coyotes across sessions. We detected a greater proportion of the MNKA for coyotes (56.3–71.6%) than foxes (55.3–62.5%), within each session. We failed to detect a greater proportion of the MNKA due to the reduction in occasions (foxes = 27.8–36.8%; coyotes = 11.3–34.4%), than due to decreased transect length from identifying nested transects (foxes = 7.5–13.9%; coyotes = 10.2–17%). The number of captures per individual was similar between species (range: foxes = 1.6–2.3; coyotes = 1.8–2.3).

For foxes, likelihood-ratio tests rejected the ECM for 2013 sessions (both $p < 0.03$), but not for 2014 sessions (both $p > 0.1$). Because likelihood ratio tests may fail to reject the ECM when sample sizes are small and/or capture heterogeneity is present (Miller et al. 2005), we report results under the TIRM, but include ECM point estimates when it was supported (Fig. 2). Likelihood-ratio tests for coyote models rejected the ECM across sessions (all $p < 0.001$).

Fox $\hat{N}$ ranged from 30 to 53 (Fig. 2), were substantially lower (27.5–59.2%) than those from Huggins and SECR models (Lonsinger et al. 2018), and were lower than the MNKA in three sessions. Generally, fox CAPWIRE estimates had higher precision than alternative approaches, and 95% confidence intervals failed to overlap multi-session point estimates in all but one session (Fig. 2). For coyotes, $\hat{N}$ from single-occasion CAPWIRE models were generally lower (13.7–49.0%) than multi-session estimates (Fig. 2); winter 2014 CAPWIRE $\hat{N}$ was more similar to multi-session estimates.

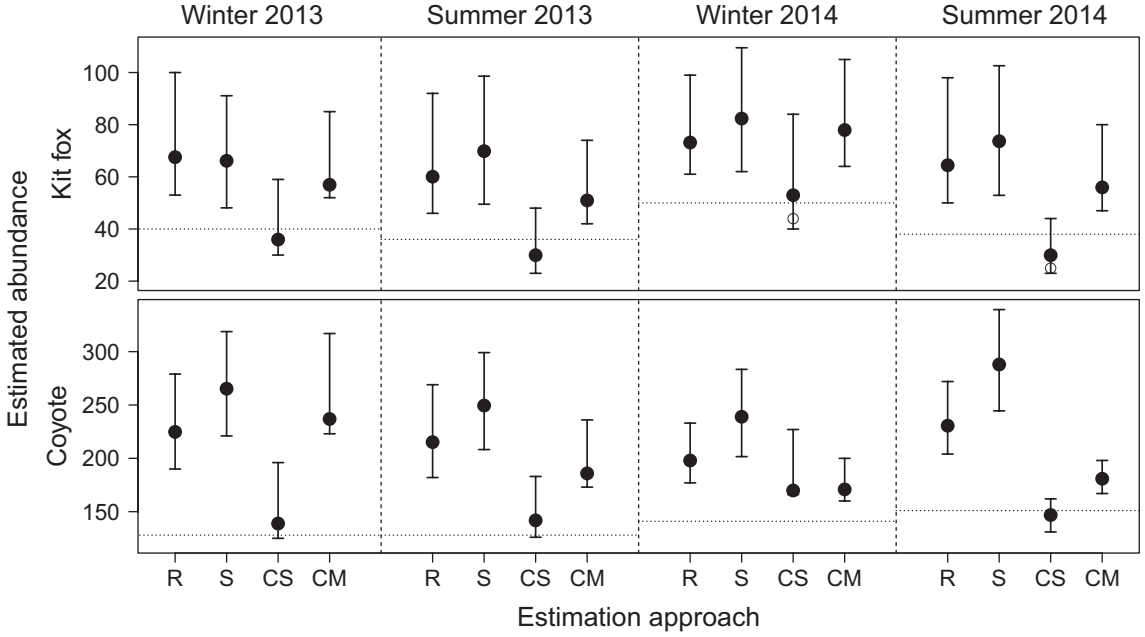

Figure 2. Estimated abundances and 95% confidence intervals for kit foxes *Vulpes macrotis* and coyotes *Canis latrans* in western Utah over four sessions, 2013–2014, resulting from robust design non-spatial Huggins closed-capture models (R) and two-innate rates capture with replacement models under single-occasion (CS) and multi-occasion (CM) formulations. Open circles represent capture with replacement point estimates under an equal capture model, where likelihood ratio tests failed to reject equal capture. The dashed horizontal line indicates the number of unique individuals identified within each session based on nuclear DNA. Results were plotted along with previously published multi-session spatially explicit capture–recapture model (S) estimates (Lonsinger et al. 2018).

Multi-occasion formulation CAPWIRE analysis

We included captures from 103 transects, but effort varied for the multi-occasion formulation from 378 to 550 km surveyed, reflecting variation in number of occasions per session (Fig. 1). Multi-occasion CAPWIRE surveys identified ≥86% of known foxes and ≥83% of known coyotes within each session. The number of captures per individual was higher for multi-occasion sampling (range: foxes = 1.9–2.4, coyotes = 1.8–3.1).

For both species, likelihood-ratio tests rejected the ECM across sessions (foxes: all $p < 0.02$; coyotes: all $p < 0.001$). Fox multi-occasion CAPWIRE point estimates were lower than multi-session estimates (except winter 2014), but confidence intervals overlapped considerably (Fig. 2). The relationship between multi-occasion CAPWIRE estimates and multi-session estimators was more variable for coyotes than foxes (Fig. 2).

Discussion

Many carnivores use linear features for movements and noninvasive surveys along roads or trails are commonly used to monitor carnivores (Kohn et al. 1999, Dempsey et al. 2015). Sampling linear features may bias estimates (i.e. convenience sampling; Anderson 2001). We attempted to avoid the pitfalls of convenience sampling at broad scales by randomly selecting sites, and then delineating transects within sites. Due to the high mobility of canids relative to the size of sites, it is unlikely that individuals occupying a site would fail to encounter a transect. In California, foxes did not avoid roads and deposited scats equally along and away from roads (Cypher et al. 2009), and coyote defecation along linear features was not influenced by sex, age, or social status (Kohn et al. 1999). Still, individual variation in road use or proximity of an animal's activity center to transects may influence p (Otis et al. 1978, Borchers and Efford 2008). We therefore considered individual covariates (e.g. sex) and compared our results to those derived from SECR models that account for variation in proximity (Lonsinger et al. 2018).

Efforts to maximize p and sample size can further minimize the influence of unaccounted for individual heterogeneity (Carothers 1973, White et al. 1982, Kendall 1999, Lukacs and Burnham 2005). For canids, surveys along linear features may yield larger sample sizes than those away from linear features with equal intensity (Güthlin et al. 2012). At Dugway, scat surveys produced higher detection rates for foxes than live-capture (Dempsey et al. 2015). Searcher ability to detect scats may vary by road substrate (Kluever et al. 2015), but it is likely higher along linear features than in vegetative cover. Transects along linear features may also be easier to access and can be surveyed more quickly, increasing survey effort given available resources.

Capture–recapture models assume that individuals are identified correctly (Otis et al. 1978, White et al. 1982) and genotyping errors can be a problem when employing noninvasive genetic sampling to estimate abundance (Mills et al. 2000, Lukacs et al. 2009). Genotyping error rates in our dataset were low (Lonsinger et al. 2018). Petit and Valiere (2006) found that error rates similar to ours had minimal effects on $\hat{N}$ (i.e. bias ≤2.5%). For most individuals, consensus genotypes were achieved at more loci than required to discriminate among siblings (Lonsinger et al. 2018), and we believe this, combined with efforts to minimize errors, effectively eliminated misidentification.

True abundances were unknown for our target populations and we cannot explicitly infer bias for each estimator. Our abundance estimates from Huggins models showed a high level of agreement with multi-session SECR estimates (Lonsinger et al. 2018). In general, our Huggins estimates were slightly (but not significantly) lower than SECR estimates (with one exception, fox winter 2013). Blanc et al. (2013) found SECR models tended to overestimate abundance for small populations but produced estimates closer to the true abundance for larger populations (defined as n = 50). Individual heterogeneity in capture, if unaccounted for, can bias $\hat{N}$ downward (Otis et al. 1978, White et al. 1982). SECR models address variation resulting from an individual's proximity to survey sites, a form of heterogeneity unaccounted for in non-spatial models (Borchers and Efford 2008). Our MNKA and abundance estimates suggested that our target populations were >50 individuals, and thus, $\hat{N}$ from non-spatial models may be biased low due to the result of unaccounted for capture heterogeneity.

Our Huggins estimates differed from SECR estimates to a greater degree for coyotes than foxes, and this may relate to the proportion of individuals on the periphery of the survey area (Blanc et al. 2013). Our sampling design was motivated primarily by fox monitoring and was centered on the low-lying basin. Consequently, the study area was bounded by mountains (north, east and south) and salt desert playa inhospitable to both species in the west. The study boundaries were more likely to bisect the home ranges of coyotes than foxes, and this may have resulted in the greater disparity between non-spatial and spatial estimates for coyotes. While both Huggins and SECR models assume population closure (Otis et al. 1978, Efford 2011), SECR relaxes the assumption by considering an animal's activity center. Population losses or gains that violate closure assumptions can negatively or positively bias estimates, respectively (Kendall 1999). For foxes, closure tests suggested population losses in 2014 sessions following the second occasion. While losses could have resulted from changing behaviours (e.g. denning in summer, or dispersal initiation in summer), our 2013 sessions included the same relative period (i.e. beyond two occasions) and we did not find evidence of similar potential closure violations. Closure test results should be viewed with caution, as they assume no individual heterogeneity in p and closure is often rejected in closed populations when heterogeneity exists (Stanley and Burnham 1999). Furthermore, we observed a similar magnitude in the differences between $\hat{N}$ from Huggins and SECR models in 2013 and 2014 for foxes. These patterns, combined with knowledge that concurrent research of telemetered foxes did not detect any movements beyond our study extent (EMG), lead us to believe the fox population was effectively closed. For coyotes, closure tests again supported closure in 2013, but not 2014. Although the temporal sampling frame

increased in winter from 2013 to 2014, winter 2014 closure violations were detected within the time frame that aligned with 2013 sampling. Summer temporal sampling was equivalent for coyotes across years. Consequently, we suspect individual heterogeneity in p likely influenced closure test results.

Non-spatial Huggins models do not account for 'holes' in the sampling frame (Efford and Fewster 2013), and this may also contribute to the lower $\hat{N}$ resulting from Huggins models. Our broad-scale random sampling resulted in several holes within our sampling frame (Lonsinger et al. 2018), from which animals likely had low (or possibly zero) p due to their distance from transects. By accounting for proximity to animal activity centers, SECR models effectively handle holes (Borchers and Efford 2008).

Multi-session Huggins and SECR models produced relatively consistent results, and we used these as a standard to evaluate the performance of CAPWIRE. The MNKA nearly always underestimates abundance (Mills et al. 2000), and therefore we regarded $\hat{N}$ at or below the MNKA as biased. In practice, funding constraints may force managers to seek cost-efficient sampling strategies. Consequently, there has been considerable interest in single-occasion methods, which have practical advantages (e.g. ease of implementation, cost; Miller et al. 2005, Petit and Valiere 2006, Williams et al. 2009). Reliable $\hat{N}$ have been reported for a range of taxa using CAPWIRE (Petit and Valiere 2006, Puechmaille and Petit 2007, Stenglein et al. 2010b), but in some cases, CAPWIRE estimates do not align with alternative estimates (Williams et al. 2009, Stansbury et al. 2014). Simulations have suggested that single-occasion sampling can produce reliable abundance estimates when the number captures per individual is >1.7 (Miller et al. 2005, Petit and Valiere 2006, Stenglein et al. 2010b). Our captures per individual were >1.7 for both species across sessions, with one exception (fox winter 2014 = 1.6). Still, single-occasion CAPWIRE estimates were substantially lower than multi-session estimates for both species across sessions. For foxes, single-occasion estimates fell below the MNKA for three of four sessions; all were below the MNKA when employing the ECM when it was supported (Fig. 2). A similar pattern was observed for coyotes, though only one estimate was less than the MNKA.

CAPWIRE assumes independence among captures and equal effort (Miller et al. 2005). Independence among captures may be violated when individuals are captured >1 time within a site. Restricting recaptures to spatially disparate sites can minimize this concern (Stenglein et al. 2010b), but reduces already limited datasets for difficult to capture species and will likely result in fewer captures per individual (Stansbury et al. 2014). CAPWIRE is robust to violations of independence among captures (Miller et al. 2005), and consequently, many researchers have opted to include all captures (Williams et al. 2009, Stansbury et al. 2014) as we have.

CAPWIRE is based on an urn model (Miller et al. 2005) and may best apply to sampling conditions that mimic this, such as sampling congregation areas (e.g. colonies). Our sampling was dispersed and temporal variation in space-use may have limited the number of individuals available for capture during a single occasion, biasing CAPWIRE estimates (Kendall 1999). Ensuring that >1 single-occasion transect is within each potential home range may alleviate this concern, but may be impractical or restrict the spatial extent of surveys. Alternatively, combining the results from multiple occasions (i.e. a multi-occasion formulation), while accounting for variable effort to meet model assumptions, may increase the probability of capturing individuals with temporal variation in space-use. Indeed, a post-hoc multi-occasion CAPWIRE analyses for both species increased the individuals captured ≥1× and produced results that were more similar to multi-session estimates.

The CAPWIRE model estimates a ratio α, between the probabilities of capture for 'seldom' and 'often' captured individuals. Outlier individuals captured many more times than the mean inflate p of the 'often' class and can severely bias results and artificially increase precision (Miller et al. 2005, Stansbury et al. 2014). For example, the coyote summer 2014 multi-occasion CAPWIRE estimate was significantly lower than those of multi-session estimators and had high precision (Fig. 2). The capture history contained two outlier individuals captured ≥19 times. Removing these outliers increased the population estimate, decreased precision and resulted in confidence interval overlap with of multi-session confidence intervals (results not shown).

Management implications

Carnivores are notoriously difficult to monitor (Gese 2001), and this is a primary challenge for estimating abundance and trends. Employing noninvasive genetic sampling can alleviate some of these challenges (Kohn et al. 1999, Petit and Valiere 2006, Piaggio et al. 2016) and facilitate concurrent monitoring of multiple species at broad scales (Williams et al. 2009). CAPWIRE was developed to compliment noninvasive genetic sampling and may further reduce costs by generating abundance estimates from a single sampling event. When a single-occasion sampling was used, CAPWIRE performed poorly: estimates were biased low with high precision. Our dispersed sampling design and the spatial ecology of canids (e.g. territoriality) both likely contributed to our failure to capture a sufficient portion of the population with a single sampling event. Early comparisons of CAPWIRE to real data ignored temporal sampling information (i.e. data were simplified to 'the total number of times each individual was caught in the study;' Miller et al. 2005). Thus, these data may not accurately reflect results under a single sampling occasion design, despite the appeal of CAPWIRE for this purpose. Our results demonstrated that choice of estimator and sampling design significantly influenced resulting estimates, the relationship between estimators varied between species, and that despite perceived benefits, CAPWIRE may not be appropriate for all scenarios.

Acknowledgements – B. Kluever, E. Burke, J. Decotis, T. Edwards, M. Melham, C. Perkins, M. Richmond and M. Smith assisted with field work. J. Adams and Waits Lab technicians assisted with DNA extractions and PCR. R. Knight provided logistical support and the Utah Division of Wildlife Resources provided housing.

Funding – Funding was provided by the U.S. Army Research Laboratory and the U.S. Army Research Office through the U.S. Department of Defense Environmental Security Technology Certification (grant: 12 EB-RC5-006) and Legacy Resource Management (grant: W9132T-12-2-0050) programs, the National Geographic Society's Conservation Trust (grant: C248-13).
Author contributions – RCL and LPW conceived the project. All authors contributed to the study design. RCL, EMG and LWP contributed to data collection. RCL, PML, and LPW conducted analyses. RCL led manuscript preparation. All authors contributed to manuscript revisions and approval for publication.

References

Anderson, D. R. 2001. The need to get the basics right in wildlife field studies. – Wildl. Soc. Bull. 29: 1294–1297.

Blanc, L. et al. 2013. Abundance of rare and elusive species: empirical investigation of closed versus spatially explicit capture–recapture models with lynx as a case study. – J. Wildl. Manage. 77: 372–378.

Borchers, D. L. and Efford, M. G. 2008. Spatially explicit maximum likelihood methods for capture–recapture studies. – Biometrics 64: 377–385.

Burnham, K. P. and Anderson, D. R. 2002. Model selection and multimodel inference: a practical information–theoretic approach. – Springer US.

Byerly, P. A. et al. 2018. Resource partitioning between kit foxes (*Vulpes macrotis*) and coyotes (*Canis latrans*): a comparison of historical and contemporary dietary overlap. – Can. J. Zool. 96: 497–504.

Carothers, A. D. 1973. Capture–recapture methods applied to a population with known parameters. – J. Anim. Ecol. 42: 125–146.

Cypher, B. L. et al. 2009. Effects of roads on endangered San Joaquin kit foxes. – J. Wildl. Manage. 73: 885–893.

De Barba, M. et al. 2014. Molecular species identification for multiple carnivores. – Conserv. Genet. Resour. 6: 821–824.

Dempsey, S. J. et al. 2015. Evaluation of scat deposition transects versus radio telemetry for developing a species distribution model for a rare desert carnivore, the kit fox. – PLoS One 10: e0138995.

Efford, M. G. 2011. Estimation of population density by spatially explicit capture–recapture analysis of data from area searches. – Ecology 92: 2202–2207.

Efford, M. G. and Fewster, R. R. M. 2013. Estimating population size by spatially explicit capture–recapture. – Oikos 122: 918–928.

Gese, E. M. 2001. Monitoring of terrestrial carnivore populations. – In: Gittleman, J. L. et al. (eds), Carnivore conservation. Cambridge Univ. Press, pp. 372–396.

Gosselin, E. N. et al. 2017. Comparing morphological and molecular diet analyses and fecal DNA sampling protocols for a terrestrial carnivore. – Wildl. Soc. Bull. 41: 362–369.

Güthlin, D. et al. 2012. Faecal sampling along trails: a questionable standard for estimating red fox *Vulpes vulpes* abundance. – Wildl. Biol. 18: 374–382.

Huggins, R. 1989. On the statistical analysis of capture experiments. – Biometrika 76: 133–140.

Kendall, W. L. 1999. Robustness of closed capture–recapture methods to violations of the closure assumption. – Ecology 80: 2517–2525.

Kluever, B. M. et al. 2015. The influence of road characteristics and species on detection probabilities of carnivore faeces. – Wildl. Res. 42: 75–82.

Kohn, M. H. et al. 1999. Estimating population size by genotyping faeces. – Proc. R. Soc. B 266: 657–663.

Lonsinger, R. C. and Waits, L. P. 2015. ConGenR: rapid determination of consensus genotypes and estimates of genotyping errors from replicated genetic samples. – Conserv. Genet. Resour. 7: 841–843.

Lonsinger, R. C. et al. 2015a. Balancing sample accumulation and DNA degradation rates to optimize noninvasive genetic sampling of sympatric carnivores. – Mol. Ecol. Resour. 15: 831–842.

Lonsinger, R. C. et al. 2015b. Evaluating the reliability of field identification and morphometric classifications for carnivore scats confirmed with genetic analysis. – Wildl. Soc. Bull. 39: 593–602.

Lonsinger, R. C. et al. 2017. The roles of habitat and intraguild predation by coyotes on the spatial dynamics of kit foxes. – Ecosphere 8: e01749.

Lonsinger, R. et al. 2018. Estimating densities for sympatric kit foxes (*Vulpes macrotis*) and coyotes (*Canis latrans*) using noninvasive genetic sampling. – Can. J. Zool. 96: 1080–1089.

Lukacs, P. M. and Burnham, K. P. 2005. Review of capture–recapture methods applicable to noninvasive genetic sampling. – Mol. Ecol. 14: 3909–3919.

Lukacs, P. M. et al. 2009. Extending the robust design for DNA-based capture–recapture data incorporating genotyping error and laboratory data. – In: Thomson, D. L. et al. (eds), Environmental and ecological statistics, 3rd edn. Springer US, pp. 711–726.

Miller, C. R. et al. 2002. Assessing allelic dropout and genotyping reliability using maximum likelihood. – Genetics 160: 357–366.

Miller, C. R. et al. 2005. A new method for estimating the size of small populations from genetic mark–recapture data. – Mol. Ecol. 14: 1991–2005.

Mills, L. S. et al. 2000. Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. – Ecol. Appl. 10: 283–294.

Otis, D. L. et al. 1978. Statistical inference from capture data on closed animal populations. – Wildl. Monogr. 62: 3–135.

Paetkau, D. 2003. An empirical exploration of data quality in DNA-based population inventories. – Mol. Ecol. 12: 1375–1387.

Pennell, M. W. et al. 2013. Capwire: a R package for estimating population census size from non-invasive genetic sampling. – Mol. Ecol. Resour. 13: 154–157.

Petit, E. and Valiere, N. 2006. Estimating population size with noninvasive capture–mark–recapture data. – Conserv. Biol. 20: 1062–1073.

Piaggio, A. J. et al. 2016. A noninvasive method to detect Mexican wolves and estimate abundance. – Wildl. Soc. Bull. 40: 321–330.

Pollock, K. H. et al. 1990. Statistical inference for capture–recapture experiments. – Wildl. Monogr. 107: 3–97.

Puechmaille, S. J. and Petit, E. J. 2007. Empirical evaluation of non-invasive capture–mark–recapture estimation of population size based on a single sampling session. – J. Appl. Ecol. 44: 843–852.

Schwartz, M. K. et al. 2007. Genetic monitoring as a promising tool for conservation and management. – Trends Ecol. Evol. 22: 25–33.

Seutin, G. et al. 1991. Preservation of avian blood and tissue samples for DNA analyses. – Can. J. Zool. 69: 82–90.

Stanley, T. R. and Burnham, K. P. 1999. A closure test for time-specific capture–recapture data. – Environ. Ecol. Stat. 6: 197–209.

Stansbury, C. R. et al. 2014. A long-term population monitoring approach for a wide-ranging carnivore: noninvasive genetic

sampling of gray wolf rendezvous sites in Idaho, USA. – J. Wildl. Manage. 78: 1040–1049.
Stenglein, J. L. et al. 2010a. Impacts of sampling location within a faeces on DNA quality in two carnivore species. – Mol. Ecol. Resour. 10: 109–114.
Stenglein, J. L. et al. 2010b. Efficient, noninvasive genetic sampling for monitoring reintroduced wolves. – J. Wildl. Manage. 74: 1050–1058.
Taberlet, P. et al. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. – Nucleic Acids Res. 24: 3189–3194.
Thompson, C. M. et al. 2012. A framework for inference about carnivore density from unstructured spatial sampling of scat using detector dogs. – J. Wildl. Manage. 76: 863–871.
Waits, L. P. and Paetkau, D. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. – J. Wildl. Manage. 69: 1419–1433.
Waits, L. P. et al. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. – Mol. Ecol. 10: 249–256.
White, G. C. et al. 1982. Capture–recapture and removal methods for sampling closed populations. – Los Alamos Natl Lab. LA-8787-NERP, NM, USA.
White, G. C. and Burnham, K. P. 1999. Program MARK: survival estimation from populations of marked animals. – Bird Study 46: S120–S139.
Williams, B. K. et al. 2002. Analysis and management of animal populations. – Academic Press.
Williams, B. W. et al. 2009. Noninvasive hair sampling and genetic tagging of co-distributed fishers and American martens. – J. Wildl. Manage. 73: 26–34.

Supplementary material (available online as Appendix wlb-00534 at <www.wildlifebiology.org/appendix/wlb-00534>). Appendix 1–4.