Efficacy of non-invasive fecal DNA-based estimation of population abundance, density, sex-ratio, and survival in the Pacific deer herd

Photo courtesy of Tom Batter

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Abstract – Recent developments in non-invasive fecal DNA technologies provide potentially cost-effective tools for estimating and monitoring mule deer (*Odocoileus hemionus*) population parameters. We investigated the efficacy of such approaches as applied to the post-fawning migratory portion of the Pacific deer herd on its summer range (~500 km^2) in the central Sierra Nevada Range during 2013 and 2014. Objectives were to (1) obtain estimates of population parameters for the Pacific deer herd, (2) use those results in combination with statistical power modeling to develop a set of guidelines for design of long-term monitoring programs, and (3) provide general cost estimates associated with such programs.

First, we applied noninvasive DNA-based methods, including non-spatial capture-recapture (CR) and spatial capture-recapture (SCR), to estimate abundance, density, sex ratio, and survival, as well as to model habitat use of the Pacific deer herd. During the field study, we collected 883 pellet samples, including 477 collected in 2013 and 406 collected in 2014. From 411 samples genotyped at 8–10 microsatellite loci, we identified 209 unique individuals sampled 1–13 times each. The sex ratio (M:F) estimated directly from genotyped individuals across the study area was 62% (95% CI: 41–93%) in 2013 and 65% (95% CI: 45–94%) in 2014. Using non-spatial Huggins closed-population CR modeling, we estimated the average abundance ($\widehat{N}$) across sites at 6.9 (95% CI: 4.4–9.4) deer per transect in 2013 and 7.9 (95% CI: 5.5–10.3) deer per transect in 2014. Based on the MMRD method of estimating effective sampling area (0.91 km^2 per transect), these estimates translated to estimated density ($\widehat{D}$) of 7.6 (95% CI: 4.8–10.3) deer/km^2 in 2013 and 8.7 (95% CI: 6.0–11.3) deer/km^2 in 2014. The precision of study-area wide abundance estimates was estimated in terms of the coefficient of variation (CV), which was 17.4% in 2013 and 14.6% in 2014. Using SCR, which estimates density directly without arbitrarily defining effective sampling area, we estimated density averaged over the study area at $\widehat{D}$ = 5.0 (95% CI: 2.3–7.7) deer/km^2 in 2013 and $\widehat{D}$ = 5.1 (95% CI: 3.0–7.2) deer/km^2 in 2014. Corresponding $\widehat{N}$ estimates for the entire study area were 2,574 (95% CI: 1,083–4,065) deer in 2013 and 2,627 (95% CI: 1,511–3,743) deer in 2014. These estimates were lower than the CR density estimates, which implied that the latter were biased upwards due to underestimation of effective sampling area. Using SCR to model habitat variables indicated that density was highest on relatively flat, north-facing slopes and in vegetation corresponding to high predicted suitability for deer based on California Wildlife Habitat Relationships (CWHR). Use of non-spatial CR, robust-design modeling produced estimates of annual survival (S) at 0.73 (SE = 0.43) for F and 0.59 (SE = 0.48) for M; the difference was not statistically significant.

Next, we estimated the precision of annual abundance estimates necessary to achieve 80% power to detect trends with varying magnitudes and type I error rates both for annual and biennial surveys. We then assessed the optimal allocation of effort with respect to numbers of transects per site versus numbers of sampling occasions per transect. We conclude that, beyond a minimum of 3 sampling occasions, increasing numbers of transects is a more efficient use of effort than increasing numbers of sampling occasions. Emphasizing numbers of transects over numbers of sampling occasions per transect also increases robustness to spatial heterogeneity. Excluding costs of permanent staff time, but including seasonal staff and laboratory analyses, costs of sufficiently intensive monitoring studies could range from $25,000 to $67,000 per year, depending on the size of the project.

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INTRODUCTION

Mule and black-tailed deer (*Odocoileus hemionus*) abundances have fluctuated significantly over the past century in the western USA, yet the causes remain unclear (Connolly 1978, Unsworth et al. 1999, Ballard et al. 2001, Hurley et al. 2011). Understanding the potentially complex processes causing fluctuations, which may include both top-down and bottom-up factors, requires accurate long-term data (Forrester and Wittmer 2013). Of central importance are accurate estimates of deer abundance. At present, few methods are available to estimate deer abundance that are equally effective in all habitat types. Consequently, there is a need to develop methods for estimation of deer abundance that are robust across the diverse habitats of the western USA (CDFW 2015).

Traditional methods to estimate abundance of deer are difficult to standardize because they vary in utility across landscape types. For example, methods relying on high visibility, such as aerial transect surveys or direct counts, provide poor estimates in areas of dense vegetation cover (Caughley 1974, Floyd et al. 1979, DeYoung 1985, Pollock and Kendall 1987). Trend indexes, which assume a constant relationship with abundance over time, enable qualitative inferences about localized population trends, but offer no ability to compare abundances among locations. These indexes, which include hunter surveys, sign counts, and spotlight surveys, also suffer from low precision and unquantifiable sampling bias (Anderson 2001, Collier et al. 2013). Statistically based sampling methods, which directly estimate abundance ($\widehat{N}$) and/or density ($\widehat{D}$), include aerial surveys of transects or quadrats, and capture-recapture (CR) or spatially-explicit capture-recapture (SCR) models. Of these methods, CR and SCR methods tend to provide the most accurate estimates and are the least prone to habitat-specific or observer biases (Keegan et al. 2011). The CR and SCR models also can incorporate and control for heterogeneity in detection probability. Because physically marking and re-capturing or re-sighting deer can be logistically and financially prohibitive, the use of noninvasive genetic approaches to "capture" and "recapture" individuals from DNA left in the environment may offer the only practical means of applying CR and SCR broadly (Waits and Leberg 2000, Schwartz et al. 2006). Such approaches utilize DNA from hair or epithelial cells remaining on the outside of fecal pellets to construct individual genotypes (Lukacs et al. 2005).

Both traditional CR and SCR methods have advantages. The SCR approaches are designed to estimate density directly based on modeling the decay of individuals' probabilities of capture with increasing distance from their activity centers. By explicitly accounting for the spatial nature of sampling and animal movements, SCR also reduces the bias due to edge effects that is associated with non-spatial CR analyses (Wilson and Anderson 1985, Efford 2004, Borchers and Efford 2008). In traditional (non-spatial) CR, $\widehat{D}$ is often calculated using a buffer strip around the sampling area, the width of which is estimated using measures such as mean maximum distance moved (MMDM; Otis et al. 1978), also referred to as mean (or median; Lounsberry et al. 2015) maximum recapture distance (MMRD; Brinkman et al. 2011). These buffer-strip methods, which are intended to reduce positive bias in estimates of $\widehat{D}$, are often inadequate in that the actual effective sampling area tends to be larger than estimated (Wilson and Anderson 1985, Parmenter et al. 2003, Ivan et al. 2013). Another attribute of SCR is to provide an integrated framework for modeling density surfaces from spatial and habitat variables (Royle et al. 2013, Efford and Fewster 2013), which facilitates inferences about wildlife-habitat relationships and the extrapolation of models to obtain $\widehat{D}$ estimates beyond the sampling sites when $\widehat{D}$ is heterogenous across the study area. On the other hand, an advantage of traditional CR methods is that they can be employed over consecutive years to estimate survival, which

facilitates development of mechanistic demographic models to complement abundance trend models in long-term monitoring programs.

The utility of CR and SCR approaches to abundance or density estimation for wildlife management ultimately depends on statistical power and cost-effectiveness, particularly as applied to long-term monitoring programs addressing populations on regional scales. In principle, these approaches can be employed over larger areas in multiple years and across a diversity of habitats to cost-effectively monitor population trends of deer over time (Brinkman et al. 2011, McCoy et al. 2014, Lounsberry et al. 2015). Planning monitoring programs that rely on this method requires knowledge of precision of annual abundance estimates and its relationship to statistical power to detect trends over time.

The primary objectives of the field study (Section I) were to use and compare non-spatial and spatial CR methods to (1) estimate $\widehat{N}$ and $\widehat{D}$ of the migratory portion of the Pacific deer herd, a partially migratory mule deer (*O. h. californicus x columbianus*) population, on their summer range in the central Sierra Nevada Mountains of California and (2) explore approaches to extrapolating $\widehat{N}$ and $\widehat{D}$ to broader scales, including use of SCR to model deer-habitat relationships in terms of $\widehat{D}$. We also estimated sex ratio and sex-specific survival. The primary objectives of Section II were to (1) determine the precision required of annual and biennial CR abundance estimates to detect multi-annual trends of varying magnitudes with specified power and type-I error rates, (2) assess optimal allocation of effort between numbers of transects and numbers of surveys per transect to maximize precision within years (and, therefore, power to detect trends across years), and (3) develop a set of tentative guidelines for study design and frequency of monitoring. Section III provides cost estimates based on results of the preceding sections to aid in planning of monitoring programs.

Section I. Estimating population abundance, density, sex-ratio, and survival

STUDY AREA

The study area comprised the summer migratory range of the Pacific deer herd, which is located on the western slopes of the central Sierra Nevada, in the El Dorado National Forest, California, USA (Fig. I-1). Elevation in the study area ranges from approximately 1600 to 2300 m. The climate is Mediterranean, with warm, dry summers and cold, wet winters, with snow present above 1600 m (Dahlgren et al. 1997). Temperatures range from -18 °C in winter to 33 °C in summer (California Department of Water Resources, 2005-2015). The predominant vegetation types are coniferous forests, chaparral, and montane hardwood forest. A variety of grasses and forbs are intermixed throughout. Principle California Wildlife Habitat Relationships (CWHR; Mayer and Laudenslayer 1988) types include Sierran Mixed Conifer, Montane Chaparral, Montane Hardwood, Mixed Hardwood Conifer, White Fir (*Abies concolor*), Red Fir (*Abies magnifica*), Jeffrey Pine (*Pinus jeffereyi*), and Ponderosa Pine (*Pinus ponderosae*). Large reservoirs occur throughout the study area, including Loon Lake (~5.2 km^2), Ice House Reservoir (~2.7 km^2), and Union Valley Reservoir (~10 km^2).

Once snowpack begins to melt in late May, deer migrate upward from their winter range to the west in the surrounding foothills to occupy the study area, taking advantage of the appearance of new growth in late spring/early summer (Hinz et al. 1981). Migratory deer display philopatric behavior with strong site fidelity in home range selection (Verme 1973, Loft et al. 1989, Livezey 1991, Lesage et al. 2000). From Jun to the time of the first winter storms in Oct, deer occupy home ranges that remain relatively stable summer after summer (T. Weist, CDFW, personal obs.), as in other migratory deer populations (Tierson et al. 1985, Garrot et al. 1987, Kufeld et al. 1989, Brown 1992). The study area is in the D-5 deer hunt zone, where seasonal antler-only harvest begins in mid Aug (archery) and late-Sep (general). Sampling ranged from Jun 3 to Aug 8 in 2013 and from Jun 23 to Sep 10 in 2014 with the intent to ensure enough time to sample the study area adequately, while minimizing overlap with fawning, seasonal migrations, and hunting season, which would violate assumptions of population closure. Although fawning can occur throughout Jun (violating the assumption of population closure to some extent), most sampling occurred after fawns were born, such that our $\widehat{N}$ estimates reflected a post-breeding survey (see Methods and Results for tests of closure).

Pacific Deer Herd

The population, referred to as the Pacific deer herd, is composed of migratory and resident California mule and Columbian black-tailed deer, and ranges over approximately 914 km^2 in El Dorado County, CA and a southern portion of Placer County, CA (Hinz et al. 1981). The winter range is located in the foothills west of the summer range, 600–1,500 m in elevation. In the summer, some individuals migrate up-slope to between 1,600 and 2,500 m in elevation, where meadows containing grasses and forbs compose an important forage habitat. The 1981 Pacific deer herd management plan indicated that the population had been experiencing significant fluctuations since the late 1960s. From 1976 to 1980, abundance estimates derived from herd composition counts and harvest data, in conjunction with change-in-ratio estimators, suggested an average $\widehat{D}$ of ~5–6 deer/km^2 on the summer range (Selleck and Hart 1957). Based on these (unverified) abundance estimates, the goal in 1981 was to increase habitat range capacity $\geq$50% by 1990 to sustain approximately 8 deer/km^2 on summer range.

METHODS

Sampling Design and Protocol

Our sampling design was similar to those of previous noninvasive CR deer studies (Brinkman et al. 2011, Lounsberry et al. 2015). Specifically, we used a blocked sampling design to ensure representative sampling of the study area. To adequately sample across the different habitat types in the study area, we (1) determined the proportional composition of the CWHR types in the study area (512 km^2), (2) overlaid a grid composed of 30-km^2 cells, and (3) selected 4 of the cells such that their combined habitat composition was proportionally similar to that of the entire study area and for which no two edges were shared. To ensure that sampling of each cell (hereafter, "site" or "block") was unbiased, we selected starting points at random for $n = 6$ transects within each of the $k = 4$ sites, representing the sample units (N = 24). We established 1.2-km long by 2-m wide belt transects from each starting point in a general direction determined by random compass bearing, except that we followed game trails when they were encountered (Fig. I-2). At intersections of trails, transects followed game trails that resulted in the closest agreement with the initial compass bearing. Where no trail or sign of deer was apparent, we continued along the direction of the compass bearing.

We sampled transects every 7–10 days to allow sufficient time for pellets to accumulate for recapture, while minimizing the time pellets were exposed to the environment. We only sampled pellets from piles that appeared sufficiently fresh for DNA extraction (i.e., with a mucous sheen or no sheen but un-cracked). From each pile, we collected 4–6 pellets in a 15 mL centrifuge tube containing a sufficient volume of 95–100% ethanol to submerge all pellets for DNA preservation. Importantly, pellets were put into ethanol on the same day as collection. Excess pellets were swept off the transect path or buried to avoid false recaptures on subsequent sampling occasions. Each transect was sampled 4 times between early Jun and early Aug in 2013 and 3–6 times between late Jun and early Sep in 2014. In 2014, we intended to compare results from 4 vs 6 sampling occasions for each transect, but a large adjacent forest fire, the King Fire in Pollock Pines, CA, burned approximately 400 km^2 west of the study area, which remained closed from mid-Sep to early Oct, 2014. As a result, the number of sampling occasions varied for each transect, with all 24 transects sampled on 3 occasions, 19 transects sampled on 5 occasions, and 17 transects sampled on all 6 occasions. In 2014, between Jun 23 and Jul 9, we also collected a subset of samples in 20 ml scintillation vials containing buffer ATL (Qiagen, Valencia, CA) to test the efficacy of buffer ATL in preserving DNA over time during storage (<1 month vs. >2 months) prior to extraction (Appendix A). Samples were covered to protect from exposure to UV radiation and transferred to the Mammalian Ecology and Conservation Unit of the University of California Davis Veterinary Genetics Laboratory for processing.

DNA Analysis

We used Qiagen DNeasy Blood and Tissue Kits to extract DNA from the surfaces of fecal pellets, using a protocol modified for deer fecal DNA extraction (Lounsberry et al. 2015; Appendix B). We removed 2–4 pellets for each sample from their respective tubes and dried them at 21°C for 45 minutes to remove residual ethanol. We placed the dried pellets in 20 ml scintillation vials and pipetted 1.5 ml buffer ATL into each vial. These vials were then placed on a rocking apparatus for 1 h to wash the epithelial cells from the surfaces of the pellets. We then followed the manufacturer's protocols designed for blood to extract DNA from the epithelial cell/buffer ATL solution. We included 1 extraction blank for every 7–11 samples as a negative control to detect potential contamination.

To genotype samples, we used 10 microsatellite markers and a sex-typing marker from a highly conserved portion of the Y chromosome, as described previously (Lounsberry et al. 2015). Specifically, we amplified markers using polymerase chain reaction (PCR) with reagents from the Qiagen multiplex PCR kit (Qiagen). Each 10 µl PCR mixture contained 1.0 ml of extracted DNA, 0.5 ml RNase-free water, 5.0 ml Qiagen Multiplex Mastermix, 1.0 ml Q-solution, and 2.5 ml of the following primer mixture: ADCYC (0.32µM), BM6506 (1.4µM), CELB9 (1.2µM), CERVID1 (0.36µM), ETH152 (0.32µM), SBTD04 (0.36µM), SBTD05 (0.92µM), SBTD06 (0.44µM), SBTD07 (0.36µM), TGLA94 (0.54µM), SRY (2.2µM). The reaction concentrations of each primer pair were 25% of the stock concentrations listed above and primer sequences and references can be found in Lounsberry et al. (2015). We used the following thermal profile: 15 min at 95°C, followed by 33 cycles of 30 sec at 94 °C, 1.5 min at 58 °C, 1 min at 72 °C, followed by 10 min at 72 °C. We used an ABI 3730 (Applied Biosystems, Grand Island, NY) and internal size standards (500-LIZ; Applied Biosystems) for electrophoresis and manually scored alleles using electropherograms visualized in Program STRand (version 2.4.89; Toonen and Hughes 2001). We used a multiple-tube approach, whereby each DNA sample was genotyped in two independent PCR reactions. All PCR sets were conducted with two negative PCR controls to detect potential contamination.

For each sample, we combined replicate multi-locus genotypes into a single composite genotype. To reduce error owing to allelic dropout from low quality fecal DNA samples, we excluded samples with <8 successfully amplified microsatellite loci (Lounsberry et al. 2015). To arrive at an individual identification for each sample, we used the Allelematch package in R, which assigns and matches genotypes to unique individual IDs based on an optimal maximum threshold number of mismatching loci (Galpern et al. 2012, R Core Team 2012).

Sex Ratio

We calculated sex ratio directly from the genotypes, i.e., the ratio of the number of M individuals to the number of F individuals. We estimated the 95% confidence limits for this ratio by first expressing them as proportions (p) of samples that were female, estimating 95% confidence limits for these proportions based on Zar's (1999) method utilizing the relationship between the *F* and binomial distributions, and then back-transforming these confidence limits to ratios [i.e., p/(1 – p)].

Non-Spatial Capture-Recapture Abundance and Density Estimation

We estimated $\widehat{N}$ for each transect in each year using Huggins closed capture models in Program MARK (Huggins 1989, White and Burnham 1999). Huggins closed capture modeling uses a conditional likelihood approach to derive $\widehat{N}$, which allows inclusion of individual covariates in a linear modeling framework to account for heterogeneity in probability of capture. Specifically, Huggins closed capture estimates two parameters: p, the probability of capture, and c, the probability of recapture, which are, in turn, used to derive $\widehat{N}$. The model assumes that the population is closed to births, deaths, immigration, and emigration; probability of capture is the same across individuals (unless a heterogeneity model is used), and individual identifications are correct. To ensure that additions and removals were negligible during each sampling period, we used the Stanley-Burnham test for closure, which is more sensitive than other tests (Stanley and Burnham 1999), using CloseTest version 3 (Stanley and Richards 2011).

We tested two pre-defined models: the null model, M0 (Otis et al. 1978), assuming capture probabilities did not differ by time or individual, and an individual heterogeneity

model, Mh2 (Pledger 2000), which allowed capture probabilities to vary between 2 random finite latent classes. We tested these models with and without sex as an individual covariate. We did not include models that tested for a behavioral effect on probability of capture because our sampling was non-invasive, nor did we test for a time effect, as our sampling was asynchronous among transects. Because only one capture per individual can be considered in a particular sampling occasion, we collapsed multiple captures of the same individual within a sampling occasion into a single capture event. In 2014, we ran models using the data from only 3 sampling occasions per transect, which enabled us to estimate abundances for all 24 transects. Final model selections were based on Akaike's Information Criterion, corrected for small sample sizes (AICc; Burnham and Anderson 2002), and models were averaged according to their AICc weights for final $\widehat{N}$ estimates. In all of these analyses, we obtained transect-specific estimates of $\widehat{N}$ to facilitate analyses of effects of year, site, sex, and interactions among these variables.

Spatiotemporal heterogeneity in abundance and density.—We conducted fully factorial analysis of variance (ANOVA) using SYSTAT (version 9.0, SPSS Incorporated, Chicago, IL) to assess differences in abundance between years and among sites. We converted transect-specific $\widehat{N}$ to $\widehat{D}$ by dividing $\widehat{N}$ for each transect by an estimate of effective sampling area using a buffer strip of width equal to full MMRD around the sampling area (Parmenter et al. 2003). Specifically, we used the mean of the maximum distance between recaptures for each individual captured in 2014 (263 m), buffered around a 1.2 km long transect of width 2 m (0.91 km^2). We used the MMRD from 2014 as it was the larger of the two MMRD measures, and thus resulted in a more conservative $\widehat{D}$.

Quantifying precision— Quantifying precision of study area-wide annual abundance estimates, including 95% confidence intervals and coefficients of variation (CV), is important to the design of monitoring programs aimed at detecting trends. We used the Wald 95% confidence interval, which is estimated using the standard error (SE) and the *t*-distribution for the corresponding degrees of freedom (*df*), and defined the CV as the SE divided by the estimate (Zar 1999). Estimation of the area-wide confidence intervals and CV, in turn, depends on the study design. In the simple case of a homogeneous landscape with a single level of replication (e.g., randomly placed transects throughout the study area), the variance would be calculated directly from the individual transects' abundance estimates, $\widehat{N}_i$, where i = transects 1 to N. However, in a blocked design, where representative sites (blocks) are chosen, within each of which *n* transects are randomly placed (as in the present study), the variance is decomposable in terms of the primary (transects within sites) and secondary (among sites) levels of analysis. The variance that arises from the *n* transect estimates within sites must be incorporated as random and is therefore (inversely) proportional to the precision. However, the among-site variance primarily reflects spatial variation in abundance (i.e., real heterogeneity). As long as each site is sampled in every annual survey and spatial variance assumed to be independent of temporal variance, the among-site variance should not significantly affect power to detect multiannual trends. Therefore, we obtained study area-wide annual estimates of precision (95% CI, CV) based solely on the within-site variance component. Specifically, we estimated the annual area-wide CV using the mean square error (MSE) from a one-way analysis of variance (ANOVA) of the transect-specific abundance estimates with site as a blocking factor. We estimated the 95% CI using the *t*-distribution corresponding to N – *k df* and the CV as follows:

$$\text{cv} = \frac{\sqrt{\text{MSE/error df}}}{\widehat{N}} \quad ,$$

where the error *df*, 20, was calculated as the total number of transects (N = 24) minus the number of sites ($k = 4$), and $\widehat{N}$ represents the average abundance estimate across all N = 24 transects..

Spatial Capture-Recapture Density and Abundance Estimation

We estimated deer density using SECR, an R program that applies maximum likelihood estimators in SCR analyses. This approach combines a "state model" and an "observation model." The state model is a probability distribution of animal locations whereas the observation model is a probability distribution describing animal detections, given animal locations. The state model assumes a Poisson point process distribution of activity centers, the intensity of which translates directly to $\widehat{D}$. This can be either homogenous, if density is assumed to vary randomly across the landscape, or heterogeneous, if density is allowed to vary across space, e.g., in relation to some set of habitat variables. The observation model estimates probability of capture at detectors, which decays with increasing distance from activity centers, according to a detection function (Efford et al. 2005, Efford et al. 2009). The parameters that are estimated are density ($\widehat{D}$), the capture probability of an individual at its activity center ($g0$), and the scale of movement (σ). The combination of $g0$ and σ in a detection function is analogous to p in non-spatial CR models. As with non-spatial closed CR, SCR analysis assumes that the population is demographically closed, but, in contrast, explicitly allows for spatial variation in detection (Efford et al. 2009). To determine an appropriate function for the decay of probability of detection as distance increases from an activity center, we compared the AICc values for null models using exponential and half-normal distributions.

In SECR, the user specifies a buffer width around the sampling detectors, which is the region of integration over which the model parameters are estimated. To prevent bias in $\widehat{D}$, the region of integration must be large enough that animals with activity centers outside of the boundary will not be detected by the detector layout. The recommended buffer width size is 4 times the "root pooled spatial variance" (RPSV), a measure of the 2-dimensional dispersion of sample points (Calhoun and Casby 1958, Slade and Swihart 1983), which for our data was approximately 1000 m. After assessing potential bias using built-in functions of SECR, we specified a final buffer width of 1500 m and a grid cell spacing of 100 m. Lakes were excluded from the region of integration as non-habitat using the El Dorado Fire Return Interval Departure GIS layer (Safford et al. 2011) in QGIS (QGIS version 2.12, http://qgis.osgeo.org); we also used QGIS to quantify CWHR type, size, and density.

We input sampling transects as a series of "count" detectors spaced every 200 m along each transect. We used all detections in analyses, including those of multiple individuals and of multiple detections of the same individual at a given detector in a particular sampling occasion (Efford et al. 2009). This contrasted with the non-spatial CR analyses, for which multiple samples from an individual on the same transect during the same sampling occasion were collapsed into a single capture. In SCR, we also incorporated a usage covariate matrix that specified the varying number of occasions (3–6) detectors were used in 2014, which enabled us to use all sampling occasions in 2014, rather than limiting analyses to the 3 sampling occasions used in CR. As in CR, we used the data only from sampling occasions 2–4 in 2013 to avoid violations of closure.

We considered both a null model (M0) and an individual heterogeneity model (Mh2) to estimate the 2-parameter probability of capture (*g0* and σ). Rather than using latent classes, as in CR analyses, we assigned the two classes (h2) by sex *a priori*, which is analogous to using sex as an individual covariate in CR analyses. For all models, we used the data from both years for analyses, but allowed *g0* and $\widehat{D}$ to vary by sampling year. To reduce the number of parameters, we kept σ constant between years.

To test for heterogeneous density across the sampling area, as well as explore deer-habitat relationships, we considered the influence of topographical and habitat variables known to impact space usage by deer (Long et al. 2008). Topographical variables included elevation (m), slope%, cosine of the aspect, and sine of the aspect. We also included distance (m) to nearest road, including actively maintained human-use trails. Lastly, we included mule-deer habitat suitability values, which ranged from 0 (poor habitat) to 1 (optimal habitat) and were estimated with CWHR version 9.0 (CDFW 2014). The values represented the average of expert-derived cover and feeding scores for habitat types of a particular size and stageWe then superimposed a grid of 150 m x 150 m cells over the study area and assigned to each grid cell the average values for each variable, which were incorporated into the region of integration for density modeling. We used AICc to select the best fitting models. We obtained $\widehat{N}$ for each year for the larger study area by discrete summation of the model-averaged $\widehat{D}$ from the top-fitting models. Specifically, we predicted $\widehat{D}$ for 22,685 150 m X 150 m grid cells across the study area, multiplied each cell-specific $\widehat{D}$ by 0.0225 km^2 (i.e., the area of one cell) to obtain a cell-specific $\widehat{N}$, and then summed $\widehat{N}$ across cells to obtain a total, study area-wide $\widehat{N}$. The study area-wide SE also was computed as the sum among cells.

Robust Design Estimation of Demographic Parameters

To estimate survival, we used a robust design model (Pollock 1982, Kendall et al. 1995), implemented in program MARK. The robust design model combines closed and open population modeling. Specifically, we used Huggins closed population modeling within each annual season (i.e., summers 2013 and 2014, termed the "secondary" sampling occasions) to estimate the probability of capture, *p*, and used open population modeling to estimate the probabilities of survival (*S*) and emigration (γ'') between the 2013 and 2014 annual seasons (i.e., the "primary" sampling occasion). We could not estimate a third parameter, the probability of the animal staying away from the study area, given that it emigrated in a prior session (γ'), as its estimation requires a minimum of 3 primary sampling occasions. We tested 4 models: (1) all parameters (*S*, γ'', *p*) varied by sex, (2) only the demographic parameters (*S*, γ'') varied by sex, (3) only *p* varied by sex, and (4) all parameters were constant (i.e., the null model).

RESULTS

Genotyping and Individual Identification

We collected a total of 883 samples, including 477 pellet groups collected in 2013 and 406 collected in 2014 (Table I-1). After eliminating samples with <8 (out of 10) loci amplifying, 411 samples remained for analyses, including 158 (33%) from 2013 and 253 (63%) from 2014. The reason for the low genotyping success in 2013 is unclear but could have resulted from samples having been stored for multiple days inside an enclosed vehicle that year. Although these samples were protected from UV radiation, ambient temperatures inside the vehicle could be very high during daylight hours. From the 411 successfully genotyped samples, we identified 209 unique individuals based on distinct multi-locus

genotypes. This equated to approximately 2 captures per individual on average (range: 1–13 captures per individual). Thirty-two individuals (30%) initially captured in 2013 were also captured in 2014. The sex ratio (M:F) estimated directly from genotyped individuals across the study area was 62% (95% CI: 41–93%) in 2013 and 65% (95% CI: 45–94%) in 2014.

Capture-Recapture Abundance Estimates

Assumption of population closure.— In 2013, we obtained 4-occasion encounter histories from all N = 24 transects (n = 6 per site) for analysis. The assumption of population closure was rejected based on the Stanley-Burnham test ($\chi^2 = 18.14$, df = 4, $P = 0.001$). In 2014, we collected pellet groups from all 24 transects for 3–6 sampling occasions, necessitating analysis based on 3 sampling occasions to make use of all 24 transects. Although we utilized the first 3 sampling occasions when possible (21 transects), we used the last 3 sampling occasions (4–6) for the remaining 3 transects (VV2, VV3, and VV5) because of poor genotyping success on samples collected during the first three sampling occasions. The assumption of population closure could not be rejected based on the Stanley-Burnham test ($\chi^2 = 1.26$, df = 2, $P = 0.534$).

In both years, all sampling was completed before rifle season start dates (but overlapped archery season in 2014). However, we began sampling earlier in the year in 2013 (June 3) than 2014 (June 23), suggesting that violation of closure in 2013 could have been due to early sampling encompassing more births or higher "immigration" associated with the unfinished seasonal migration. To investigate this possibility, we tested for closure separately for the first three occasions (1–3, when closure violations were expected to be greatest) and the last three occasions (2–4). As predicted, the analysis using occasions 1–3 exhibited significant violations of closure ($\chi^2 = 7.36$, df = 2, $P = 0.025$) but that based on occasions 2–4 did not ($\chi^2 = 3.34$, df = 2, $P = 0.189$). Therefore, we used occasions 2–4 for all CR analyses of 2013 data.

CR abundance estimation—In both years, the individual heterogeneity models (Mh2) produced unrealistic error estimates (e.g. SE = 0 or 95% CI = 0–1) and were, therefore, eliminated during the model selection process. As the M0 and M0-sex models were very close in likelihood in both years (i.e., < 2 ΔAICc), we model-averaged $\widehat{N}$ results to produce final estimates (Table I-2).

In the ANOVA, we found no significant interactions or main effects of year or site ($F_{1-3,40} < 0.56$, $P > 0.51$). In 2013, the site-averaged $\widehat{N}$ ranged from 5.6 (95% CI = 1.8–9.5) in Loon Lake to 8.0 (95% CI = 2.1–13.8) in Van Vleck and, in 2014, the site-averaged $\widehat{N}$ ranged from 5.8 (95% CI = 3.3–8.4) in Loon Lake to 7.1 (95% CI = 3.0–11.3) in Van Vleck (Fig. I-3).

The mean squared errors (MSE) of the ANOVAs with $df = 20$ (N – k) were 28.86 (2013) and 26.63 (2014), corresponding to standard errors of 1.201 (2013) and 1.154 (2014). We estimated the average abundance across sites at 6.9 (95% CI: 4.4–9.4) deer per transect in 2013 and 7.9 (95% CI: 5.5–10.3) deer per transect in 2014, which equated to average $\widehat{D}$ of 7.6 (95% CI: 4.8–10.3) deer/km^2 in 2013 and 8.7 (95% CI: 6.0–11.3) deer/km^2 in 2014, assuming the 0.91-km^2 average effective sampling area per transect estimated from MMRD (see Methods). To estimate the total number of deer for the entire study area in each year, we multiplied $\widehat{D}$ and the corresponding 95% confidence limits by the total area of the study area (512 km^2). This resulted in average estimates of 3,868 (95% CI: 2,470–5,287) deer in 2013 and 4,431 (95% CI: 3,088–5,793) deer in 2014 for the entire study area. Correspondingly, the CV was estimated at 17.4% in 2013 and 14.6% in 2014.

Spatial Capture-Recapture Density and Abundance Estimates

Based on the null model, use of the exponential detection function (ΔAICc = 0.00) greatly improved the fit of the model over the half-normal detection function (ΔAICc = 19.44). We therefore used the exponential detection function in all subsequent analyses. The highest ranking model for a homogeneous density (i.e., no habitat variables) was the null model (Table I-3). Consequently, we constructed habitat models by adding environmental variables to the density function to model heterogeneous density, while maintaining the same specifications for *g0*, σ, and *pmix* as the null model.

We considered a global habitat model (h1), which included slope%, cosine of aspect, sine of aspect, elevation, CWHR value, and distance to road; as well as the following reduced models: (h2) topography (i.e., elevation, cosine of aspect, sine of aspect, and slope%), (h3) CWHR, (h4) distance to road, (h5–h9) each topographical variable modeled in isolation, and (h10) the additive effects of CWHR and cosine of aspect. The top ranking model remained the null after incorporation of habitat covariates, but several heterogeneous density models were <2 ΔAICc from the null model (Table I-3), which was our criterion for inclusion in the final model-averaged $\widehat{D}$. The model that considered $\widehat{D}$ as a function of cosine of aspect was the next highest ranked (beta estimate = 0.20, SE = 0.17), with $\widehat{D}$ increasing as values became more positive, indicating an association of deer with north-facing slopes (Fig. I-4). The model using values derived from CWHR showed a positive relationship to $\widehat{D}$ (beta estimate = 1.20, SE = 1.52) and was ranked high by AICc, as well as the additive model which used cosine of aspect and CWHR values (cosine of aspect beta estimate = 0.20, SE = 0.16; CWHR beta estimate = 1.15, SE = 1.43). Modeling $\widehat{D}$ against slope% was also ranked high, with $\widehat{D}$ declining as slope% increased (beta estimate = -0.56, SE = 0.99). The homogeneous density models that varied the sex ratio (i.e., *pmix*) by year and *g0* by sex were also included in the model-averaged $\widehat{D}$. All other models were >2 ΔAICc from the null model, and were therefore excluded.

The model-averaged estimates of density were extrapolated to the study area in both years, enabling estimation of the number of deer on the study area (Fig. I-5). We estimated study area-wide density at $\widehat{D}$ = 5.0 (95% CI: 2.3–7.7) deer/km^2 in 2013 and $\widehat{D}$ = 5.1 (95% CI: 3.0–7.2) deer/km^2 in 2014. Corresponding $\widehat{N}$ estimates for the entire study area were 2,574 (95% CI: 1,083–4,065) deer in 2013 and 2,627 (95% CI: 1,511–3,743) deer in 2014. The precision of these annual estimates in 2013 (CV = 0.28) and 2014 (CV = 0.20) were not as high as for the corresponding CR estimates, but, in contrast with the latter, incorporated variance associated with spatial heterogeneity.

Robust Design Estimation of Demographic Parameters

We tested 4 models to estimate survival (Table I-4). The top-ranking models ($\leq$2 ΔAICc units) included (1) the full model (all parameters varied by sex) and (3) the capture-probability varying model (*p* varied by sex, *S* and γ'' were constant). The capture-probability varying model, however, was unable to estimate SE for *S* and γ'', leaving the model varying all parameters by sex as the best model. Estimates of *S* for this model were 0.73 (SE = 0.43) for F and 0.59 (SE = 0.48) for M. The emigration parameter (γ'') was estimated at 0.35 (SE = 0.45) for F and 0.23 (SE = 0.32) for M. Neither parameter estimate was sufficiently precise to confidently assess the relative survival or emigration rates for the two sexes.

DISCUSSION

Our study was aimed at estimating abundance, density, sex-ratio, and survival in the

Pacific deer herd, through noninvasive DNA CR sampling on the summer range. In the context of these objectives, we also addressed several problems of more general significance to the integration of such an approach into a statewide monitoring strategy for California. Although a previous study in the coastal mountains of the state had already demonstrated that noninvasive CR produced precise estimates of abundance and sex-ratio specific to 4 pre-selected fawning areas (Lounsberry et al. 2015), the present study extended those results in several important ways: (1) application of a similar approach to a seasonally migratory population in the Sierra Nevada Mountains, (2) use of a sampling design that allowed estimation of per-transect abundance and precision applicable to the entire study area, (3) application of spatially explicit CR methods to estimation of density and abundance for the entire study area, (4) modeling of deer density-habitat relationships, and (5) estimating survival and emigration using robust design modeling.

Our CR-based estimates of precision of study area-wide abundance varied between the two years, but both CVs were <20%, which is considered high for monitoring purposes (Gibbs et al. 1999). Given that these estimates were based on the use of only 3 sampling occasions per transect, precision could potentially be increased further by additional sampling occasions. On the other hand, allocation of effort could be better spent by increasing the number of transects rather than sampling occasions per transect, particularly when heterogeneity in deer density is expected to be high (see Section II). The design of monitoring programs ultimately requires estimates of annual CV to be evaluated in the context of specified type I and II error rates, magnitudes of trends, length and frequency of monitoring, and estimates of multiannual fluctuations in abundance over and above underlying trends (Gibbs et al. 1999, Section II).

One of the most important assumptions of application of CR abundance estimation is population closure. In 2013, we discovered a statistical violation of the population-closure assumption. In particular, the violation was associated with the early-season sampling occasions, possibly reflecting excessive births or migratory movements early in Jun. In contrast, we found no significant violations of the closure assumption for the latter-season runs in 2013 and all analyses in 2014 (for which transect runs began later in the year). Thus, our findings suggest that sampling efforts are most likely to satisfy closure assumptions if begun in late Jun/early Jul for the migratory Pacific deer herd. More generally, sampling periods may need to vary among study areas according to seasonal timing of migrations and fawning.

The two data analytical approaches employed here, non-spatial CR and SCR, produced reasonably precise estimates of abundance and density, although exclusion of inter-site variance in the CR estimates resulted in their CVs being somewhat lower than those corresponding to SCR estimates. However, the CR estimates of density were dependent upon a relatively arbitrary method of estimating the effective sampling area and, therefore, likely biased (Lounsberry et al. 2015). Because SCR estimates the density of individuals directly from the spatial location and scale of movement of the captures, this method is expected to be unbiased (Efford and Fewster 2013). Further, SCR explicitly accounts for heterogeneity in probability of capture that is associated with edge effects from the sampling layout. Individuals with activity centers farther from the transect will likely have a lower probability of capture than individuals with activity centers located on the transect, which is accounted for in SCR but not CR. The SCR density estimates (~5.0 deer/km^2) were similar to the 5–6 deer/km^2 estimated in 1981 (Hinz et al. 1981). Given that our density estimates were on

average approximately 60% higher with CR than SCR, we infer that our assumed effective sampling area (0.91 km^2) was 60% lower than actual (i.e., ~1.46 km^2). Although it is tempting to conclude that in the future, we can simply adjust our assumption and apply CR to obtain unbiased estimates, doing so entails additional assumptions that may not be valid under future conditions. Therefore, we reiterate the assertion by Lounsberry et al. (2015) that application of CR "density" estimates should be taken solely as indexes of abundance when sampling is not continuous over the population range, and in such cases, recommend that SCR be applied to obtain relatively unbiased estimates of abundance that can be compared over space as well as time (Efford and Fewster 2013).

The estimated sex ratio was 0.44–0.74 M:F, which is relatively high compared to a previous estimates of sex ratio for the Pacific deer herd (Hinz et al. 1981). The historical average for this population (1956–1981,) was 0.32, and the estimated ratio from the broader region (CDFW hunt zone D5) in 2014 was 0.34 (CDFW, http://www.dfg.ca.gov). Using the same microsatellite assay as we used for this study, Lounsberry et al. (2015) estimated a M:F ratio of 0.24 to 0.50 for a Columbian black-tailed population in Mendocino County, CA. In partially migratory mule deer populations, females often exhibit a mixed strategy, with some females remaining resident year-round at lower elevations, and others, along with most males, migrating to higher-elevation summer ranges (Nicholson et al. 1997). Such a pattern could account for our higher estimate of M:F ratio in this study, as we only surveyed the migratory component of the population.

Another advantage of SCR modeling is that it provides an integrated framework for habitat modeling, which can be used to infer habitat relationships and to increase accuracy of abundance and density estimates, particularly if density is highly heterogeneous across the study area. For the Pacific deer herd, slope and aspect appeared to affect abundance, with deer density highest on relatively flat north-east facing slopes. In the summer, north-east facing slopes receive less solar radiation, and therefore provide cooler temperatures (Urban et al. 2000) and moister environments, which deer may prefer during the dry, hot summer. Other migratory mule deer have been found to select north-facing slopes as well (Truett 1972, Koerth 1981, Ordway and Krausman 1986, Nicholson et al. 1997). The CWHR values, intended to represent habitat suitability for deer, also showed a positive relationship to density, as expected.

On the other hand, the relationships observed in this study between habitat covariates and density were relatively weak. The null model, which assumed uniform density, was ranked higher than the heterogeneous-density models. Moreover, all the highest-ranked models produced density estimates <<1 SE from one another, indicating essentially no difference with respect to density estimates for the study area as a whole. These findings suggest that density was not strongly heterogeneous in the study area, at least according to the habitat variables used in the models.

The ability to use robust design modeling in CR provided the advantage of obtaining estimates of demographic parameters, such as survival and emigration, that are helpful in managing populations and devising harvest strategies. Our finding that 30% of deer detected in the first year were resampled on the same transects in the second year indicate deer exhibited sufficient site fidelity to enable survival analysis, although at least 3 consecutive years are required for complete demographic modeling (Pollock 1982). Telemetry studies also have found that deer tend to exhibit strong fidelity to their migratory summer ranges (Garrot et al. 1987, Kufeld et al. 1989, Brown 1992).

In summary, both CR and SCR offer advantages in monitoring deer population trends over time. A significant advantage of the SCR approach is that it provides potentially unbiased estimates of density that can be compared across study sites, as well as over time. Pairing density estimation with habitat modeling in SCR also can illuminate habitat relationships. Conversely, at present, only traditional CR analysis incorporates the capability to obtain estimates of survival and other demographic parameters using robust-design modeling. In principle, similar methods can be developed for spatial approaches, which may be advantageous in the future as statistical methods are further developed for this purpose. As demonstrated in the present study, CR and SCR are not mutually exclusive and can be employed in concert if the sampling design is of proper scale and distribution.

Section I. Figures

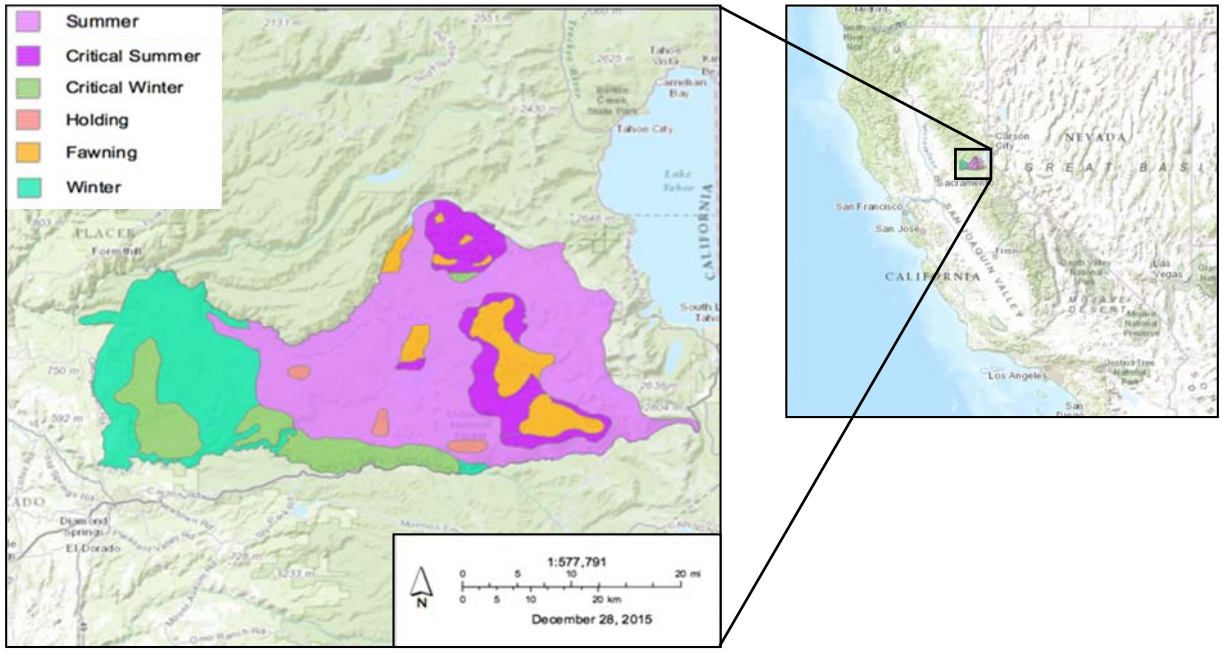

Figure I-1. Known distribution of the Pacific deer herd in El Dorado County, California, including winter, summer, fawning, and holding (intermediate) ranges for both resident and migratory deer. Migratory summer range is in higher elevation areas, along the western slopes of the Central Sierra Nevada, in El Dorado National Forest. Winter range is at lower elevations (650–1,500 m). Data were compiled in 1990 from a mixture of biotelemetry studies, California Department of Fish & Wildlife (CDFW) personal knowledge, and predicted habitat use (CDFW, Rancho Cordova, CA). The study area encompassed most of the migratory summer range, including fawning (orange) and critical summer (dark pink) ranges (elevation 1,800–2,500 m).

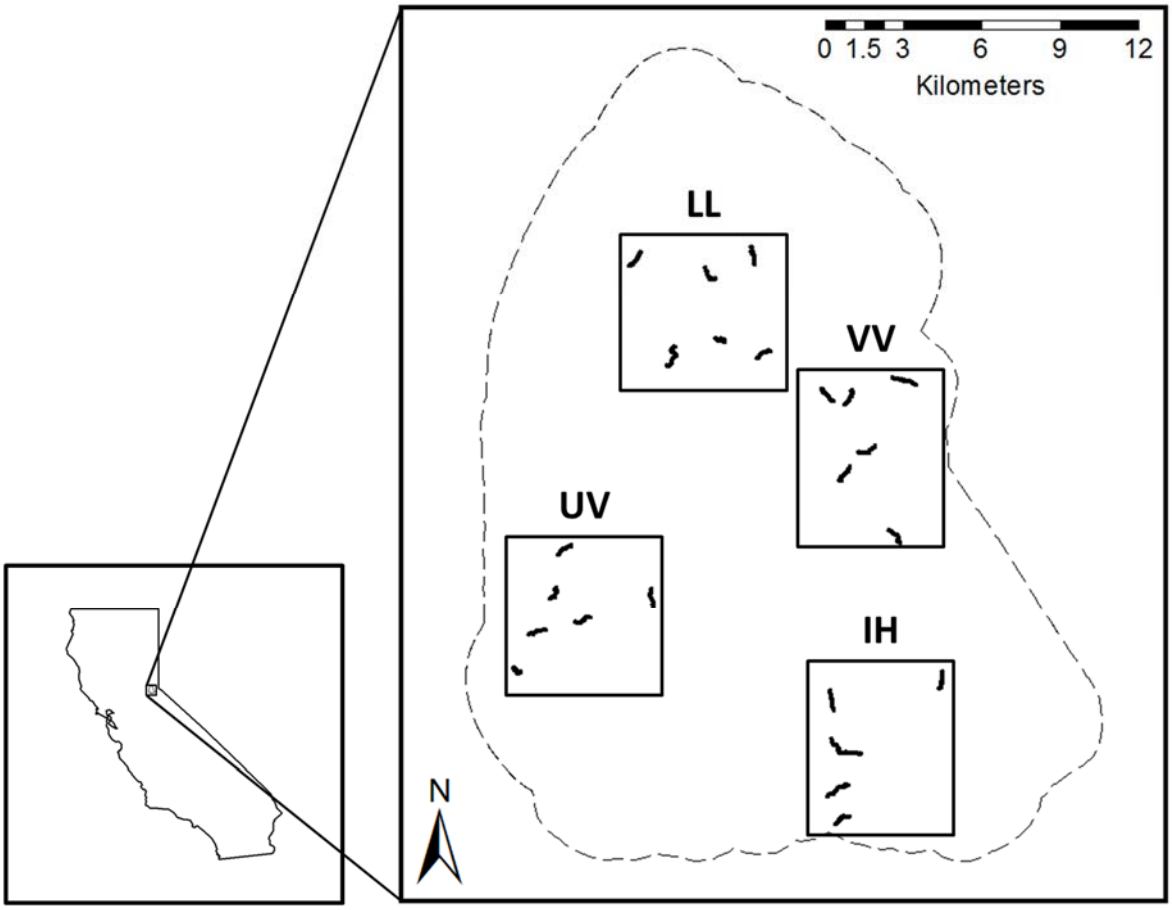

Figure I-2. Transect locations at the four sites. Transects were randomly established within four rectangular study sites (30 km^2)—Ice House (IH), Loon Lake (LL), Union Valley (UV), and Van Vleck (VV)—that were selected to represent the habitat composition of the 512 km^2 study area (polygon circumscribed by dashed line) corresponding to the summer range of the Pacific deer herd, El Dorado County, California.

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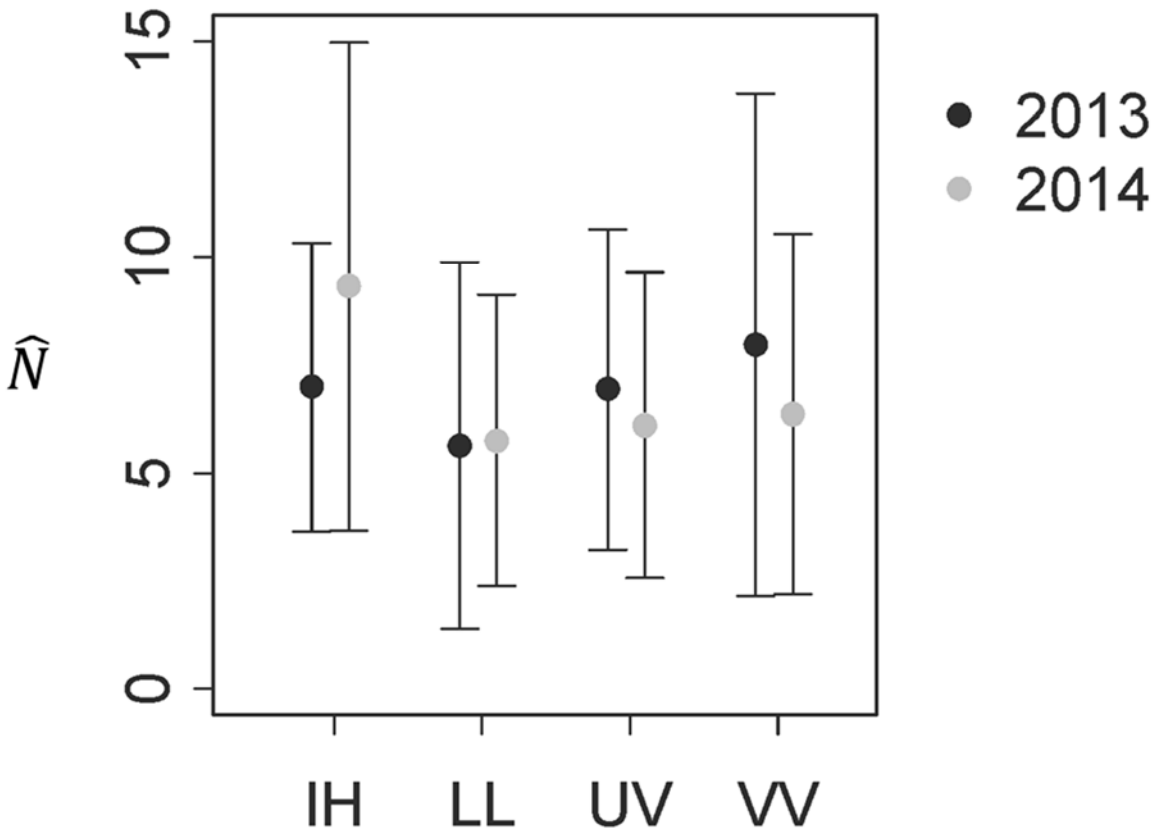

Figure I-3. Average capture-recapture estimates (and 95% confidence intervals) of per-transect deer abundance ($\widehat{N}$) for 4 fecal pellet sampling sites: Ice House (IH), Loon Lake (LL), Union Valley (UV), and Van Vleck (VV) during summer 2013 and 2014, El Dorado County, California. Results from 2013 were estimated from sampling occasions 2–4. Results from 2014 were estimated primarily from sampling occasions 1–3. However, for 3 transects with insufficient numbers of successfully genotyped samples in the first 3 sampling occasions (VV2, VV3, and VV5), we used sampling occasions 4–6.

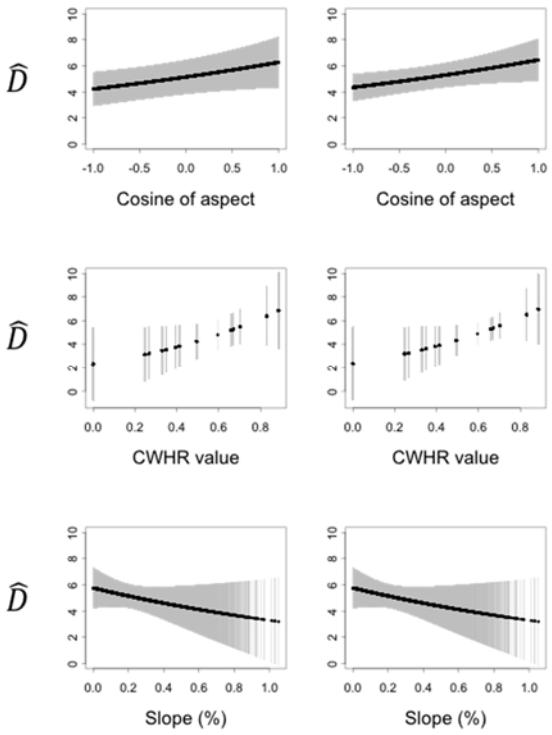

Figure I-4. Predicted numbers of deer/km^2 ($\widehat{D}$ +/- SE) as a function of habitat covariates as estimated from spatially explicit capture-recapture analysis during 2013 and 2014 in El Dorado County, California. Habitat covariates are shown from top to bottom in order of their relative strength from AICc model ranking results: cosine of aspect, California Wildlife Habitat Relationship (CWHR) value, and slope%. The cosine of aspect ranges from -1 to 1, with -1 indicating south-facing slopes and 1 indicating north-facing slopes. Only habitat covariates from models within 2 ΔAICc from the top-ranking model are presented.

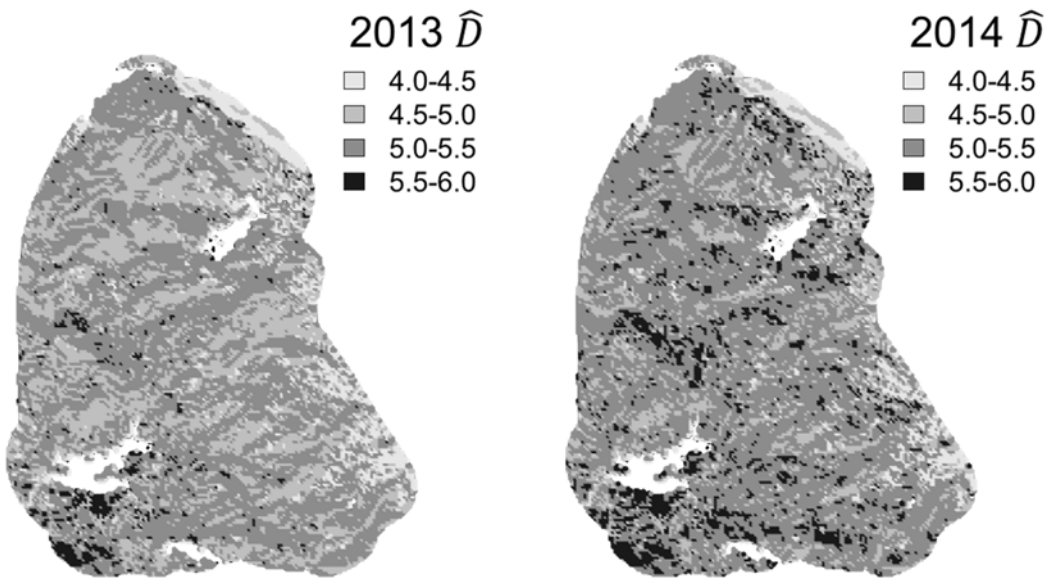

Figure I-5. Density ($\widehat{D}$) surfaces (150 m resolution) describing numbers of deer/km^2 across the range of the Pacific deer herd in El Dorado County, California during summers 2013 and 2014. Density surfaces were based on model-averaged spatially explicit capture recapture results of all models <2 ΔAICc of the top-ranking model.

Section I. Tables

Table I-1. Numbers of deer pellet samples collected, samples successfully genotyped (i.e., at >7 loci), individuals identified, individual M, individual F, and recaptures (recap) used in non-spatial capture-recapture (CR) and spatially explicit capture-recapture (SCR) analyses during summers 2013 and 2014 in El Dorado County, California.

Year	No. samples collected	No. genotypes	No. individuals	No. M	No. F	No. SCR recap[a]	No. CR recap[a]
2013	477	158	107	41	66	51	27
2014	406	253	134[b]	53[b]	81[b]	119	74
TOTAL	883	411	209[b]	85[b]	124[b]	170	101

[a] Larger numbers of SCR than CR recaptures are due to multiple captures of an individual in one sampling occasion, which were used separately in SCR but collapsed into a single capture event in CR.
[b]No. individuals identified in 2014 include 32 of the individuals (9 M, 23 F) initially sampled in 2013. Total numbers of individuals reflect actual numbers of individuals sampled (i.e., they were not double counted if sampled in both years).

Table I-2. Model rankings for capture-recapture (CR) estimation of abundance ($\widehat{N}$) in the Pacific deer herd during summers 2013 and 2014 in El Dorado County, California. Average per-transect $\widehat{N}$ are reported for each model, with the average (Avg) SE for each transect-specific estimate from each model's estimation process. The probability of capture (*p)* and the mixture proportion of the h2 latent classes (*pi)* are also reported for each model, with associated standard errors. For models incorporating h2 classes, there are two *p* values, one for each class, and for models incorporating sex as a covariate, the *p* value reported is for females.

Year	Model [a]	AICc	ΔAICc	AICc wt	No. Par	$\widehat{N}$ (Avg SE)	*P* (SE)	*Pi* (SE)
2013								
	Mh2+sex[b]	224.6	0	0	3	9.3 (61)	0.12/1.00 (0.38/2.13)	0.99 (0.09)
	Mh2[b]	225.1	0.42	0	2	69.4 (33,110)	0.01/0.55 (5.28/9.54)	0.99 (4.43)
	M0[c]	227.9	3.26	0.57	1	6.6 (3.1)	0.18 (0.04)	NA
	M0+sex[c]	228.5	3.85	0.43	2	7.2 (3.9)	0.21 (0.05)	NA
	Model Avg	-	-	-	-	6.9 (3.5)	0.17 (0.14)	NA
2014								
	M0+sex[c]	375.7	0	0.66	2	8.1(2.8)	0.31(0.05)	NA
	M0[c]	377.0	1.30	0.34	1	7.5 (2.3)	0.26 (0.04)	NA
	Mh2+sex[b]	377.7	2.04	0	3	8.1 (2.8)	0.31/0.31 (0.05/0.72)	0.98 (20.94)
	Mh2[b]	379.0	3.33	0	2	7.5 (2.3)	0.26/0.26 (0.06/0.34)	0.87 (0)
	Model Avg	-	-	-	-	7.9 (1.0)	0.29 (0.05)	NA

[a] Model notations follow Otis et al. (1978), including the null model (M0), where probability of capture is constant, and the heterogeneity model (Mh2), a mixture model with 2 latent classes to account for individual heterogeneity. Models including "sex" in the notation incorporate sex as an individual covariate.

[b] These models were excluded from model-averaged estimates of $\widehat{N}$ due to high error (see text).

[c] These models were included for the model-averaged estimate of $\widehat{N}$.

Table I-3. Model rankings for spatially explicit capture-recapture (SCR) analysis for abundance estimation in the Pacific deer herd during summers 2013 and 2014 in El Dorado County, California. Parameter estimates include density ($\widehat{D}$), probability of capture (*g0*), scale of movement (σ), and the mixture proportion (*pmix*) of the 2 sexes. The null model considered $\widehat{D}$ and *g0* to be functions of year and σ to be constant across years. Habitat covariates included sampling year, sex, cosine of aspect, CWHR value, slope%, distance to road (DTR, m), sine of aspect, and elevation (m). Topographical variables include elevation, slope%, cosine of aspect, and sine of aspect. The global model included all habitat variables. Habitat models are labeled beginning with "h."

						2013		2014	
Model Description[a]	**Rank**	**AICc**	**Δ AICc**	**AICc wt**	**No. Par**	$\widehat{D}$	**SE**	$\widehat{D}$	**SE**
Null model	1	2435.60	0.00	0.26	6	5.0	1.3	5.1	0.87
(h5) Cosine of aspect ($\widehat{D}$)	2	2436.40	0.74	0.18	7	5.1	1.4	5.3	1.1
(h3) CWHR values ($\widehat{D}$)	3	2436.80	0.19	0.14	7	4.9	1.6	5.0	1.3
Year (sex *pmix*)	4	2437.40	1.77	0.11	7	5.0	1.3	5.1	0.97
(h6) Slope% ($\widehat{D}$)	5	2437.50	1.82	0.11	7	5.0	1.4	5.1	1.1
Sex (*g0*)	6	2437.60	1.93	0.10	7	5.1	1.3	5.1	0.88
(h10) CWHR, cosine of aspect ($\widehat{D}$)	7	2437.60	1.97	0.10	8	5.0	1.7	5.2	1.5
(h4) DTR ($\widehat{D}$)	8	2437.60	2.01	0	7	-	-	-	-
(h8) Sine of aspect ($\widehat{D}$)	9	2437.80	2.14	0	7	-	-	-	-
Sex (σ)	10	2437.80	2.14	0	7	-	-	-	-
(h7) Elevation ($\widehat{D}$)	11	2437.80	2.15	0	7	-	-	-	-
Sex (*g0*, σ)	12	2440.00	4.05	0	8	-	-	-	-
(h2) Topographical vars ($\widehat{D}$)	13	2444.30	8.71	0	10	-	-	-	-
(h1) Global model ($\widehat{D}$)	14	2448.50	12.8	0	12	-	-	-	-
Model Avg	-	-	-	-	-	5.0	1.4	5.1	1.0

[a]Model descriptions indicate variables first, followed by affected parameters in parentheses

Table I-4. Robust-design model rankings for models numbered as in text, in the Pacific deer herd, during summers 2013 and 2014 in El Dorado County, California. Huggins closed capture models were used to estimate probability of capture, *p*, for the secondary occasions within each year ($p1$ for 2013, $p2$ for 2014), and an open model to estimate survival (*S*), and emigration (γ'') between primary sampling occasions, 2013 and 2014.

Model	**Rank**	**AICc**	**ΔAICc**	**No. par**	***S* (SE)**	**γ'' (SE)**
(3) *S* and γ'' constant, $p_{1,2}$ vary by sex	1	681.91	0.00	4	0.67 (0)	0.31 (0)
(1) *S*, γ'', and $p_{1,2}$ vary by sex	2	684.00	2.09	5	F: 0.73 (0.43) M: 0.59 (0.48)	F: 0.35 (0.45) M: 0.23 (0.32)
(4) *S*, γ'', and $p_{1,2}$ are constant	3	685.92	4.00	4	0.68 (30.5)	0.32 (31.0)
(2) *S* and γ'' vary by sex, $p_{1,2}$ constant	4	687.25	5.34	5	F: 0.75 (78.7) M: 0.91 (7.6)	F: 0.31 (72.4) M: 0.61 (3.2)

Section II. Study design considerations for long-term monitoring using standard non-invasive fecal genetic capture-recapture

Background and Approach

Statistical power is the probability of rejecting a false null hypothesis, which, in the context of long-term population monitoring programs, equates to detection of a systematic trend (increase or decline) in some population parameter (e.g., abundance; Gerrodette 1987, Cohen 1988, Gibbs et al. 1999). Power to detect a trend depends both on variance associated with estimates of abundance and the magnitude of the trend. Sources of variance can be decomposed into sampling variance (including measurement error), which can often be reduced by increasing sampling effort or modifying sampling design, and process variance, which stems from stochastic properties of the biological system under study. Process variance must be incorporated in the study design, and potentially includes variation in space (within years) and time (among years). The relevant component of process variance affecting power in multi-annual monitoring studies is the year-to-year fluctuation in abundance over and above any underlying long-term trend.

To obtain sufficiently high precision in annual estimates of abundance for population monitoring purposes, the sampling design should be spatially representative (Brashares and Sam 2005) and incorporate a sufficient number of sample units and sampling occasions (Otis et al. 1978). Based on use of the four 30-km^2 sites that we selected to sample the Pacific deer herd's summer range, we were interested in effects of number of transects per site and number of sampling occasions per transect on precision of abundance estimates.

We conducted a two-part analysis to investigate (1) the precision required of annual and biennial CR abundance estimates to detect multi-annual trends of varying magnitudes with specified power and type-I error rates, and (2) optimal allocation of effort between numbers of transects and numbers of surveys per transect to maximize precision within years (and, therefore, power to detect trends across years). Based on these analyses, we developed a set of tentative guidelines for study design and frequency of monitoring.

METHODS

Power as a Function of Precision and Magnitude of Trend

We examined the relationship between precision of annual closed population abundance estimates versus statistical power to detect multi-annual population changes of varying magnitudes. We used the program TRENDS (Gerrodette 1993) and employed a log-linear regression model, which is appropriate for a constant annual growth rate (e.g., $\lambda < 1$ for a negative trend). We assessed the precision required of annual abundance estimates to detect trends with the following statistical criteria: $\alpha = 0.05$, 0.10, and 0.20 and power $(1 - \beta)$ equal to 80%. A target power of 80% based on $\alpha = 0.05$ is standard for wildlife studies but α is sometimes relaxed to 0.10 to 0.20 depending on the monitoring objectives (Gibbs et al. 1999). For example, it may be a reasonable trade-off to increase risk of a type I error to 20% (i.e., $\alpha = 0.20$) in exchange for increasing sensitivity (greater power) of the monitoring program to detect a trend as early as possible. We investigated trends of magnitudes corresponding to annual

declines of 1.4%, 2.7%, and 6.7%, which, if extended into the future, would correspond to 50% population declines in 50, 25, and 10 years, respectively (e.g., Hatch 2003). We examined the precision required for both annual surveys and biennial surveys (every two years) conducted over a 10-year period (i.e., 10 vs. 5 surveys). We used a 2-tailed test and assumed a constant coefficient of variation (CV), which implies proportionality between the standard error and abundance estimate (Gerrodette 1987, Hatch 2003). For this analysis, we represented precision of annual estimates with the CV, which is the ratio of the standard error of the average abundance estimate to the untransformed value of that estimate. The procedure for estimation of the standard error corresponding to our particular study design was presented in Section I.

Assessment of Study Design

To maximize precision of closed-population abundance estimates for use in multi-annual monitoring, the study design should allocate effort in such a way as to minimize overall sampling variance. The most direct way to minimize sampling variance is by increasing sampling effort. Given a fixed number of transect sampling occasions allowable in a budget, these can be allocated to maximize numbers of transects, which would necessitate fewer runs per transect, or maximize number of sampling occasions per transect, which would necessitate fewer transects. The optimal allocation of numbers of transects versus sampling occasions per transect depends in part on the spatial heterogeneity of abundance. Deciding how to allocate effort also requires information on the functional relationship between precision and each of these two sampling elements. Thus, we used an empirical approach based on data collected in 2014 on the Pacific deer herd in El Dorado County, California (Section I).

Using the Pacific deer herd data set, we examined the relative effects of the two sampling elements (number of transects, number of occasions) on precision. Specifically, we used CR abundance estimates from the 14 transects that were sampled for all 6 sampling occasions in 2014. We additionally produced abundances estimates for 3, 4, and 5 sampling occasions to examine the effect on CV of varying number of transects and number of sampling occasions per transect. First, to quantify the effect of number of transects, we estimated the average CV from 40 subsamples each of 3–10 transects (i.e., transect-specific abundance estimates drawn randomly without replacement from 14 total transects) and plotted the functional relationship. To quantify the effect of the number of sampling occasions per transect, we additionally used CR estimates of abundance calculated from the first 3 sampling occasions, the first 4 sampling occasions, and the first 5 sampling occasions and estimated from these values the average CV from all 14 transects. We plotted these results to visualize the functional relationships of each of these elements independently. Assuming the two sources of variance were independent, we then combined relationships into a single model to predict the CV as a function of both numbers of transects and numbers of sampling occasions per transect. Because these analyses combined transects regardless of site, the estimates of CV were larger than predicted for an actual field study, for which among-site variance would have been excluded (see Section I). Therefore, to recalibrate CVs for direct comparison to those obtained for actual study area-wide annual estimates, we reduced these values by the difference between the empirically estimated CV from the 2014 field study (14.6%) and the corresponding estimate in this section based upon the same

number of transects per site (n = 6) and sampling occasions (n = 3). In essence, we removed the component of the CV estimates from the present section that were attributed to among-site variance.

RESULTS

Power as a Function of Precision and Magnitude of Trend

The relationships between precision required to detect a trend versus its magnitude were approximately linear (Fig. II-1). In general, annual surveys were able to detect weaker trends than could biennial surveys; increasing levels of α also enabled detection of weaker trends. For example, based on a CV of 14.6% (as achieved by the 2014 estimate of abundance in the Pacific deer herd; see Section I), and employing α = 0.05, biennial surveys over a 10-year period could be expected to detect a trend corresponding to an annual decline of 6.2% per year or more. Annual surveys conducted over the same period could be expected to detect a trend corresponding to an annual decline of as little as 4.2% per year. Similarly, given the same precision and monitoring duration, and adoption of a relaxed α (e.g., 0.20), surveys could detect a trend corresponding to annual declines of as little as 2.2% or 3.9% for annual or biennial surveys, respectively. Although annual surveys clearly offer greater power to detect trends, biennial surveys, which require half the effort, could nevertheless be a more efficient design the objective is strictly to detect trends in abundance.

Assessment of Study Design

Based on random subsets of the 14 transects that were sampled 6 times each in 2014, precision of study area-wide abundance estimates increased with increasing numbers of transects (Fig. II-2). Moreover, the reduction in the CV for each additional transect was 3 times greater when increasing from 3 to 4 transects (CV decreased 5.7%) than increasing from 6 to 7 transects (CV decreased 1.9%), emphasizing the importance of establishing a minimum number of transects (e.g., 6 transects per unit). A trend line conforming well to this relationship took the form $Y = 0.6588*X^{-0.582}$, where Y was the predicted CV and X was the number of transects.

Increases in the number of sampling occasions per transect also increased the precision of abundance estimates (Fig. II-3). However, this relationship was relatively constant with respect to numbers of sampling occasions. A regression line based on this relationship had a slope of -0.011, indicating a 1.1% decrease in the CV for every additional sampling occasion added beyond 3 occasions (up to 6). Thus, the difference in CV achieved for 3 vs. 6 runs would be approximately 3.3%.

These relationships were then merged and standardized to our actual estimates of precision based on CR estimates for the Pacific deer herd in 2014 (Fig. II-4), providing guidelines for CR surveys to achieve a desired precision for a given amount of sampling effort. For example, if 5 biennial surveys (i.e., a 10-year period) are planned with the goal of detecting a decline of magnitude 3% per year with power 80% and type I error rate up to 10%, a CV of approximately 10% would be required of annual estimates. Based on Figure II-4, a study with 4 sites, each of which had 6 transects run 6 times (i.e., 6 sampling occasions) would provide the necessary precision. However, this would entail a total per-site effort of 36 transect runs.

Alternatively, 8 transects per site, each run 3 times, would produce a similar degree of precision of the study area-wide estimate, but this allocation decision would only require 24 transect runs per site and would therefore be a more economical choice. Emphasizing numbers of transects over numbers of sampling occasions per transect also reduces potential bias associated with poor representation of spatial heterogeneity.

DISCUSSION

These analyses indicate that annual surveys of the scope used in Section I for the Pacific deer herd can provide sufficient precision to be employed biennially to detect moderate trends in abundance. Moreover, by varying the allocation of effort to maximize numbers of transects, surveys can provide greater precision resulting in higher power to detect trends. Figure II-4 provides a tentative guide for study design enabling predictions about the amount of precision expected and, in conjunction with Figure II-1, can facilitate planning for long-term monitoring projects. However, several caveats in our analysis warrant discussion, some of which may necessitate revisions to these guidelines as data accumulate from subsequent surveys.

Notably, in the analyses above we did not account for multi-annual variance (stochastic fluctuations) in abundance over and above any long-term trend. Because we only sampled for two years, we had no basis from which to estimate such multi-annual process variance. In general, populations of large-bodied animals (i.e., long-lived, low reproductive output, e.g., "k-strategists") tend to fluctuate much less than small-bodied animals (e.g., r-strategists), suggesting that this type of variance could be relatively minor (e.g., Gibbs et al. 1998). However, as monitoring programs accumulate data from multiple annual surveys, this assumption should be tested and, if necessary, precision recalculated to incorporate this source of variance. The effect of such temporal variance in abundance would be to reduce the power to detect any underlying trend. To be conservative in the interim, educated guesses about the magnitude of temporal variance can be used to elevate the functions in Figure II-4 up the Y axis as deemed appropriate.

In addition, the model presented in Figure II-4 relates to the actual numbers of transects and numbers of sampling occasions per transect used in data analysis. However, as was the case in our study of the Pacific deer herd in both years (Section I), unanticipated problems can arise (e.g., the King Fire) that force changes in the design or render some of the samples unusable. Indeed, other studies also have experienced unanticipated obstacles, suggesting the need to plan for such events proactively by incorporating surplus sampling in study design (Brinkman et al. 2011, Lounsberry et al. 2015). For example, although Figure II-4 suggests that studies using 3 sampling occasions per transect may be sufficient, a safer design would allow for transects to be sampled a minimum of 4 occasions annually to ensure that each transect has at least 3 usable sampling occasions at the end of the annual survey. Previous recommendations suggest the need for a minimum of 5 sampling occasions when using maximum likelihood methods to estimate abundance using closed capture CR (Otis et al. 1978). However, fecal-genetic CR studies that used a similar protocol as the present study (Section I) reported moderate precision in estimates from sampling a portion or all of the transects only 2–4 times in each season (Brinkman et al. 2011, Lounsberry et al. 2015). Because these studies utilize spatially-distributed replicates to

estimate average per-transect abundance and density, the precision of each transect-specific estimate is less influential on the study area-wide estimate and precision.

Further, the precision achieved in the Pacific deer herd study was partly a function of that particular study area and degree of habitat heterogeneity. Therefore, although a reasonable starting point, actual precision can be lower in other study areas, particularly where habitat heterogeneity is higher. In addition to habitat heterogeneity, climate and other environmental conditions, as well as the diet of the deer, can alter genotyping success or necessitate an alteration of sampling protocol (e.g., shorter sampling intervals in areas with high temperatures and UV exposure, which can degrade DNA from the pellet surface). A pilot study can also establish baseline estimates, and an estimate of sampling error, which can be used in prospective power analyses to design an effective long term monitoring program tailored to the particular region.

Most of the discussion up to now applies to the estimation of abundance and density. However, an additional consideration in deciding how frequently to survey pertains to monitoring survival and emigration. Whereas a biennial survey design could be more efficient for detecting trends in abundance, such a design might not necessarily be as amenable to estimation of other demographic parameters. To fully estimate demographic parameters (e.g., including probability of surviving after having emigrated in a previous year), a minimum of 3 consecutive years is required (Pollock 1982). Thus, the present power analysis could be extended in the future beyond annual vs. biennial surveys to include additional schemes more conducive to open population modeling, e.g., sets of 3 consecutive annual surveys alternating with 3 non-survey years.

Lastly, SCR methods can provide some advantages over the standard CR methods considered in this section, particularly when habitat correlates are incorporated into models (e.g., Section I). Although Figure II-1 provides a useful guide regardless of the approach used, Figures II-2–II-4 could eventually be replaced with analyses of other factors specific to SCR, such as whether a single sampling occasion or multiple occasions were used, length of transects, and other factors specific to SCR (e.g., McCoy et al. 2014).

Section II. Figures

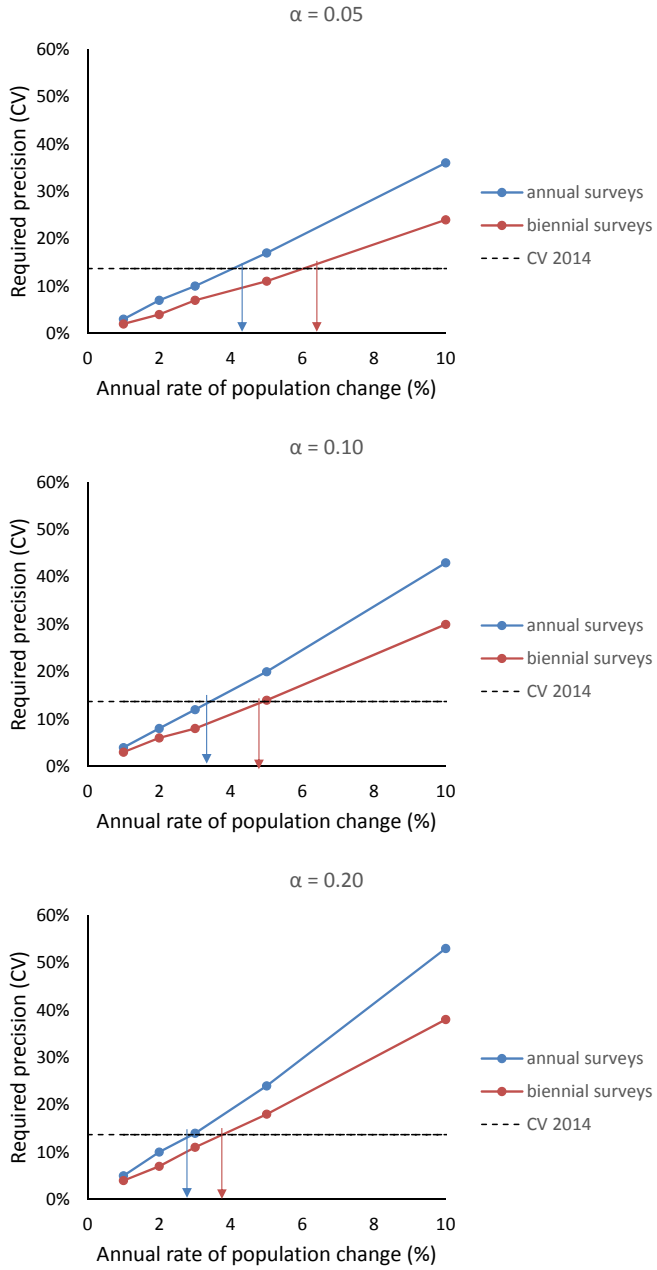

Figure II-1. Precision (CV) required to detect a trend with 80% power (1 - β) during annual and biennial closed-population noninvasive genetic CR surveys as a function of trend-magnitude for α = 0.05 (top), α = 0.10 (center), and α = 0.20 (bottom). Dashed line representing an empirically estimated CV (14.6%) for a 2014 survey is shown for reference.

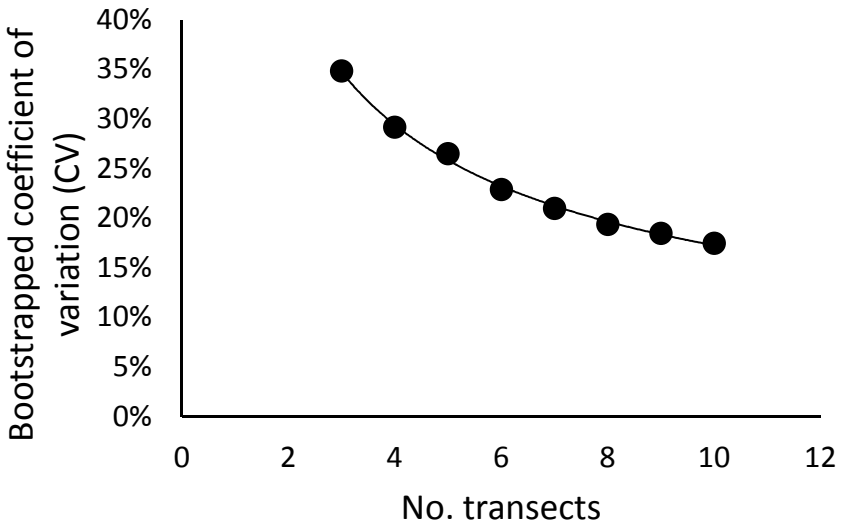

Fig. II-2. Relationship between precision (CV) of study area-wide abundance estimates and number of transects used to arrive at abundance estimates based on Huggins CR estimates from 6 sampling occasions using 40 random subsamples of 3–10 transects each (from 14 possible transects) in the Pacific deer herd, El Dorado County, California, 2014.

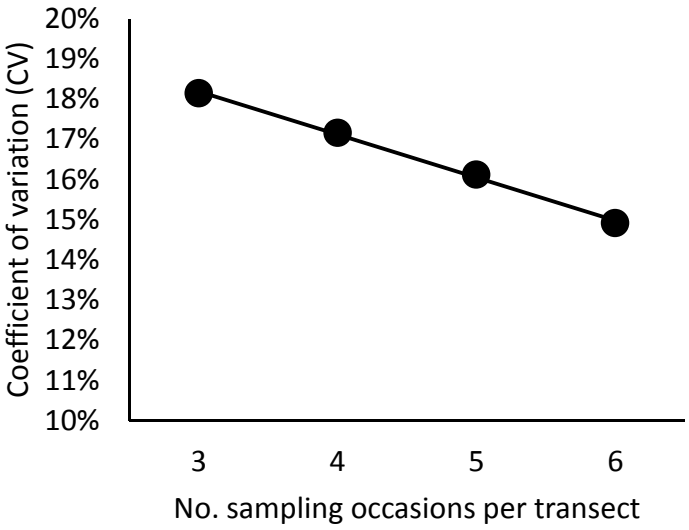

Fig. II-3. Relationship between precision (CV) of study area-wide abundance estimates and numbers of times each transect was sampled to arrive at abundance estimates based on Huggins CR analyses using 14 transects of the Pacific deer herd, El Dorado County, California, 2014.

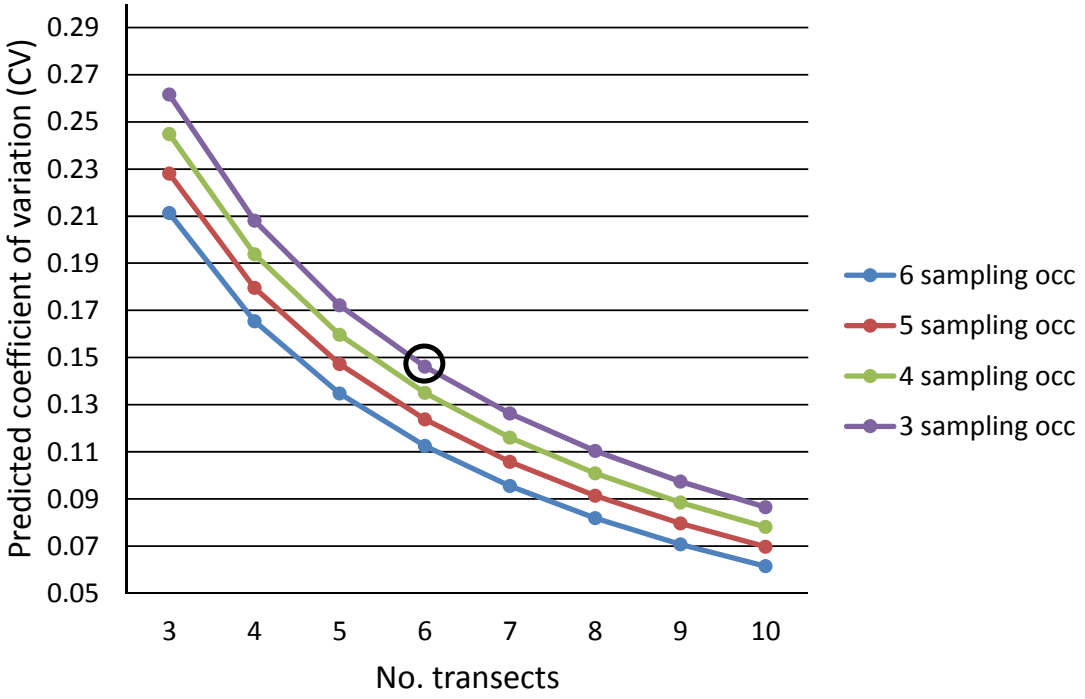

Figure II-4. Predicted coefficient of variation corresponding to ranges of numbers of transects and numbers of sampling occasions per transect in a single site, assuming a study design with 4 such sites. Relationships illustrate, for example, similar precision for 8 transects sampled 3 times (24 transect runs) each and for 6 transects sampled 6 times each (36 transect runs). The Y axis is scaled to the empirical study area-wide estimate (CV = 14.6%) based on 4 sites, each of which had 6 transects run 3 times each in 2014 (circled on the figure).

Section III. Cost of noninvasive fecal CR annual abundance estimates

The cost of annual abundance estimates depends on several factors, including numbers of transects per site, numbers of sites, numbers of sampling occasions per transect, and numbers of fecal pellets analyzed. The cost can vary significantly, depending on the number of seasonal staff hired and number of pellet groups collected and processed. Assuming seasonal staff are paid $15/ hour (+35% for benefits) and work 40 hours per week for 3 months, costs associated with each seasonal staff member total approximately $10,500, plus an additional $1000 each for travel and per-diem costs, yields $11,500 per seasonal staff member. Based on the Pacific deer herd study, 2 seasonal staff were able to conduct on average 3 sampling occasions per field day, which composed 75% of the total days employed (the others used for training, data entry, and misc. tasks). This effort amounted to approximately 150 sampling occasions allocated between transects and sampling occasions per transect. Importantly, the number of transects surveyed per day was constrained in part by logistics associated with getting to and from, often, remote locations. Because unbiased estimates of abundance depend on random placement of transects, this expectation seems reasonable in planning future studies as well. The expectation of 150 sampling occasions allows for a study with 24 transects surveyed 6 times each or, as might be a more prudent allocation of effort, 36 transects surveyed 4 times each. Alternatively, a single seasonal staff member could be hired to run 18 transects (e.g., 6 transects in 3 sites) 4 times each. Depending on the study, 300 to 1000 pellet groups can be anticipated, which, at a cost of $45 per sample would equate to $13,500 to $45,000 for laboratory costs. Therefore, in addition to permanent staff time, a survey involving a single seasonal staff member, who collected 300 samples from 18 transects, would cost approximately $25,000, whereas a survey using two seasonal staff to cover 36 transects collecting up to 1,000 pellet groups could costs as much as $67,000.

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Appendix A. UC Davis – *Mammalian Ecology and Conservation Unit*

Efficacy of storage in ATL buffer as an alternative to ethanol storage

Storing samples in 95-100% ethanol (hereafter "EtOH") has been shown to effectively preserve deer and other mammal fecal DNA for extraction (e.g., Lounsberry et al. 2015, Miles et al. 2015). However, there are a number of downsides to using EtOH related to both field and laboratory procedures. Therefore, we conducted an experiment to test an alternative approach involved storing pellets in Qiagen Buffer ATL (hereafter "ATL"), which would preclude the need to use EtOH in the field and increase efficiency of DNA extraction in the laboratory. According to our EtOH protocol, samples stored in EtOH must be first dried for 15 min and then placed in ATL and rocked for 1 hour to wash cells off the external surfaces of the pellets before extraction of DNA. Alternatively, a protocol involving storage directly in ATL could eliminate both the drying and transfer steps in the laboratory and potentially eliminate the need to rock samples (i.e., if normal motion associated with transporting samples accomplished the same effect).

To assess the feasibility of using ATL as an alternative storage solution for fecal DNA samples, we conducted 3 experiments for differential genotyping success: (1) comparison of the type of storage solution (ATL vs. EtOH), (2) effect of ATL storage time (<1 month vs. >2months), and (3) effect of rocking of ATL samples prior to extraction. We considered a sample successfully genotyped if >7 out of 10 loci successfully amplified, which would make it a usable sample for capture-recapture analyses.

For these experiments, we used a total of 93 pellet groups collected in summer 2014, 41 of which we divided and, on the same day of collection, submerged half the pellets in EtOH (15 ml centrifuge tubes) and the other half in ATL (20 ml scintillation vials). The EtOH samples were processed using the traditional approach (Lounsberry et al. 2015) as were the ATL samples, except that we omitted the drying and transfer steps in the latter. The ATL samples were never dried and when we rocked samples did so in their original vials. For all comparisons, we used the log-likelihood test of independence (Zar 1999).

Experiment 1

We first compared the standard EtOH procedure and the alternative ATL procedure using the 41 paired samples. All samples stored in ATL for this experiment were processed within 22 days of collection (9–22 days), although those stored in EtOH were extracted up to 48 days after collection (18–48 days). (This time-difference was conservative in predisposing EtOH to perform more poorly than ATL.) We used the same rocking step in both cases to isolate storage reagent as the target treatment variable. The EtOH experienced a significantly higher number of successes than ATL ($G_1^2 = 7.55$, $P = 0.006$), with 85% of the EtOH samples successfully genotyped compared to 59% of the samples stored in ATL (Table 1).

Table 1. Contingency table for experiment 1, including numbers of pellet groups amplifying 8–10 microsatellite loci (Success) or amplifying at <8 loci (Fail) for two treatments of the same 41 pellet groups.

	ATL	EtOH
Success *n*	24	35
Fail *n*	17	6
Total *n*	41	41

Experiment 2

To invesigate whether time between collection and extraction affected the efficacy ATL storage, we stored 45 samples in ATL for 9–22 days and 24 samples for 65–67 days prior to extraction. We found that the performance of ATL dropped precipitously over time ($G_1^2 = 18.13$, $P < 0.0001$). Out of 45 samples stored for 9–22 days, 58% were successful, compared to only 8% of the samples stored for 65–67 days (Table 2).

Table 2. Contingency tables for Experiment 2, including numbers of pellet groups amplifying 8–10 microsatellite loci (Success) or amplifying at <8 loci (Fail) for two storage-time treatments of pellet groups stored in ATL: DNA extraction within 1 month or after 2 months.

	<1 month	>2 months
Success *n*	26	2
Fail *n*	19	22
Total *n*	45	24

Experiment 3

To determine whether rocking was helpful for pellets stored in ATL, we compared success of 46 samples rocked normally to 22 samples not rocked prior to extraction. Samples were stored for 9–36 days prior to extraction. We found that the performance of ATL was significantly greater for samples that were rocked than not ($G_1^2 = 7.55$, $P = 0.006$). Out of 46 samples rocked prior to extraction, 57% were successful, compared to only 27% of the samples not rocked prior to extraction (Table 3).

Table 3. Contingency tables for Experiment 3, including numbers of pellet groups amplifying 8–10 microsatellite loci (Success) or amplifying at <8 loci (Fail) for two rocking treatments of pellet groups stored in ATL: rocking for 1 hr vs. no rocking prior to DNA extraction.

	No rocking	Rocking
Success *n*	6	26
Fail *n*	16	20
Total *n*	22	46

Conclusions

Our findings indicate that EtOH is preferable to Buffer ATL for DNA preservation of deer pellet samples, especially if field logistics necessitate storage of samples for longer periods (i.e., >1 month). Storage time appears to have a large effect on DNA degradation for samples stored in ATL. If researchers are able to transfer samples from the field and extract DNA <1 day to 2 weeks from time of collection, it may become useful in the future to compare EtOH and ATL success rates for shorter storage time periods. We also stored samples at room temperature, but it is possible that DNA could be preserved in ATL more effectively if kept at colder temperatures, especially below -20 ˚C, as temperature conditions of sample storage have been shown to have a large effect on microsatellite genotyping success rate (Hajkova et al. 2006).

Literature cited

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Appendix B. UC Davis – *Mammalian Ecology and Conservation Unit*

Protocol for extracting DNA from deer pellets (update 7/18/2014)

I. Record in laboratory notebook:

Name of Person(s) Conducting Extraction:_____
Date Extraction was conducted on:_____
Start Time:_____
End Time:_____

II. Supplies needed:

Pipettes (P1000, P200) and tips (1000uL, 100 or 200uL)
proteinase K
Qiagen DNeasy blood & tissue kit (spin columns, several collection tubes and buffers ATL, AL, AW1, AW2, and AE)
Extra buffer AL (must be ordered in addition to that contained in the kit)
Molecular biology grade 96-100% ethanol
labeled 2-mL tubes, and one of the following for each sample: labeled weigh boat, labeled 1.5-mL tube, labeled/autoclaved 20-mL scintillation vial, spin column

III. Preparations before beginning:

___ CHECK STOCKS OF ALL NECESSARY BUFFERES BEFORE BEGINNING

___ Turn on incubators and allow to reach appropriate temperatures: dry bath at 56°C; incubator at 70°C.

___ Place buffer AE from kit in the 70°C incubator (extraction with heated buffer is more efficient).

___ clean the lab bench (10% bleach and a Clorox wipe)

___Write the LAB ID for samples you will be extracting and their locations in the appropriate notebook

___Label a 20-mL autoclaved scintillation vial and weigh boat for each sample (up to 8 samples plus 1 blank)

Note: If the pellets seem smaller than average, write "Fawn?" for that sample in the notebook

IV. Extraction procedures:

___ Use applicator stick to place 2 pellets from each sample into a weigh boat (one boat per 2 pellets). Put weight boats/pellets in
the 70°C incubator for 30 minutes to evaporate all ethanol

___ Add to the labeled 20-mL vial the 2 dried pellets and 1.5 mL buffer ATL; incubate in a rocker at room temp for 1 hour. *During this time, label the tubes to be used later (spin columns and final 1.5-mL tubes with sample ID, date, and extracting person(s) initials)*

___ After incubation, pipet 25 µl proteinase K into labeled 2-mL micro-centrifuge tubes

___ Add 600 µl of the lysate from each scintillation vial into its respective labeled 2-mL tube

___ Add 600 µL of Buffer AL (without EtOH) to each new lysate tube

___ Vortex for 10 seconds

___ Incubate at 56º C for ten minutes

___ Remove tube from incubator, vortex very briefly, and centrifuge briefly to get liquid off the cap

___ Turn off incubators

___ Add 600 µl 96-100% molecular biology-grade ethanol

___ Vortex for 10 seconds

___ Centrifuge briefly to pellet any remaining debris and get liquid off caps.

___ Place 600 µl of the lysate mixture into the spin columns from above (avoiding, if there are any, remaining solid particles). DO NOT discard the lysate! All of it is used.

___ Centrifuge spin columns for 1 minute at 8000 rpm

___ Remove spin columns from the centrifuge and place them in new collection tubes. Discard the previous collection tubes and filtrate into the appropriate waste receptacle.

___ Add 600 µl of sample to the spin column and centrifuge as before. Transfer spin column to new collection tube and discard the previous collection tube and filtrate appropriately

___ Add the remaining 400 µl of sample to the spin column and centrifuge as before. Transfer spin column to new collection tube and discard the previous collection tube and filtrate appropriately

___ Add 500 µl of buffer AW1 (from the Qiagen kit) to the spin column and centrifuge for 1 min at 8000 rpm

___ After centrifugation, remove spin column and place it on a new collection tube. Discard the previous collection tube and
filtrate appropriately

___ Add 500 µl of buffer AW2 (from the Qiagen kit) to the micro-centrifuge tube and centrifuge the at MAX rpm for 3 min

___ Remove the spin column and place it in a new 1.5 ml micro-centrifuge tube. Discard the previous collection tube and filtrate

___ Add **50** µl of buffer AE directly onto the filter in spin column center (switching tips between samples) and incubate at room temperature for **5** minutes

___ After incubation, centrifuge the sample at 8000 rpm for 1 min

___ Remove the spin columns and discard them. Place the 1.5 ml tubes with the DNA sample into a labeled box in the refrigerator until ready for PCR.

___ Clean up the work area. Check to make sure there are enough extraction supplies for the next extraction and, if not, report
need to order more as necessary to the lab manager; record the time for extraction in the lab notebook

Appendix C. UC Davis – *Mammalian Ecology and Conservation Unit*
General guidelines for fecal capture-recapture studies

I. Perform a pilot study:

Genotyping success can vary substantially based on the study area and time of year due to any number of factors, such as climate, precipitation, herbivore diet, etc. Therefore, we recommend conducting pilot studies using ~100 pellet samples prior to finalizing plans for a project, as success can range from 30 to 80%, which makes a significant difference with respect to numbers of samples required to meet study objectives.

II. Sample collection and storage:

Currently, the best way we have found to preserve DNA in pellets is to immerse them in 95–100% ethanol (e.g., Sigma-Aldrich, Product No.: 459844). (*Because 100% ethanol is federally and state-regulated, ordering requirements may vary by institution and region; see below for shipping requirements*.) Can use polypropylene 15 ml conical tubes (e.g., Fisher Catalog No.: 14-959-49B). It is recommended to order tubes in sets of 50 that come with a polystyrene tube holder, which facilitates organization and keeping tubes upright, and test multiple brands of tubes before ordering in bulk to ensure the caps seal reliably! Although it is possible to obtain DNA by swabbing pellets in the field (e.g., with a Q-tip ®), we have performed side-by-side experiments and found this method to perform poorly relative to direct collection and preservation of pellets in ethanol. Protect samples from extreme heat and direct sunlight.
To reduce risk of contamination, pre-fill tubes with ethanol prior to collection of pellets. To the extent possible, collect only fresh pellets (preferably <1 week) that have not been rained on (see **Appendix D** for more specific criteria).

III. Shipping samples in 95–100% ethanol

If possible, transport samples directly to the laboratory. Because ethanol is flammable and hazardous, shipping of samples in ethanol is also regulated by the International Air Transport Association (IATA) and U.S. Department of Transportation (DOT) regulations. Individuals are legally required to have someone with IATA or DOT training package samples for shipping. Samples in 100% ethanol can be shipped by courier (Fed Ex, DHL, UPS) if the internal containers (i.e., conical tubes) are <30 mL and the total volume of the combined samples is <500 ml. Tubes must have space for liquid to expand (i.e., not be completely full), be sealed and secured into a polystyrene or other rack or otherwise bound together and inside of a ziploc bag containing sufficient absorbent material (e.g., newspaper) to absorb all ethanol were tubes to break, and the Ziploc bag put into a cardboard shipping box. The entire package must be able to withstand dropping from 6 feet and having similar sized boxes stacked upon it up to 10 feet high. Additionally, packages must be marked with a "Dangerous Goods in Excepted Quantities" label at least 100 mm on a side (e.g., http://www.cmarz.org/images/shiplabel_dangerousgoods.jpg). Ethanol is a Class 3 substance with UN No. 1170.

Appendix D. UC Davis – *Mammalian Ecology and Conservation Unit*

Noninvasive deer-pellet DNA capture-recapture transect sampling protocol

I. Design considerations

The sampling protocol described below was intended to support traditional non-spatial analyses for both closed and robust-design capture-recapture (CR) models, which require repeated sampling occasions. This design also has been used successfully for spatially explicit capture-recapture (SCR) analysis and, therefore, serves as a generic protocol for traditional and spatial noninvasive fecal abundance and survival estimation.

However, in future studies where the intent is solely to apply SCR analysis to density estimation, alternative designs tailored specifically to spatial analysis could be more efficient than the present one. For example, lengthening transects could be useful to increase the number of individuals for which the entire diameter of home range is sampled. More generally, sampling should emphasize spatial extent over repetition because SCR uses relative spatial positioning rather than temporal capture profiles as the basis for estimation. Potentially, a single search of a broader area, rather than multiple searches of a linear transect, could provide a more powerful data set for SCR. Another possible approach is to search plots sufficiently large to encompass many home ranges until a pre-determined number of pellet groups has been collected (which might require multiple visits, e.g., depending on deer density). A potentially important risk in need of assessment of single-search sampling is inadvertent inclusion of older samples, which could violate closure assumptions. It is possible that this potential risk would not be a problem in practice if (a) collectors could reliably distinguish pellets greater than 2–3 weeks old or (b) genotyping success were sufficiently low for the older pellets to prevent them significantly influencing results. Regardless, such assumptions should be tested empirically.

II. Sampling protocol

The overall design includes 24 transects (1.2 km) that are established randomly within 4 sites (30 km^2) that represent the habitat of the entire study area. Each transect is sampled 3 to 6 times (see main text for optimizing number of runs).

Step 1: establish 4 sample plots

- In GIS or other map, overlay study area with grid composed of 30-km^2 grid cells
- Determine habitat composition of the entire study area and each grid cell
- Select 4 grid cells for which their combined habitat composition approximates that of the entire study area with the following constraint: no two grid cells can share an edge (diagonal is ok)

Step 2: randomly establish 6 transects within each plot

- In GIS, generate 6 random points within each grid cell to be the initial transect starting points

- Generate alternate random starting points with pre-assigned (random) priority to be used in the event that one or more of the initial 6 random starting points is unusable due accessibility problems (cliffs, lakes, private property and no permission granted) or if the area is unsafe to sample

Step 3: field establishment of transects and sampling

- Use 2 people for the first run of a transect: one to establish transect and the other to collect pellets

Establishing transects

- Navigate to the predetermined start point for each transect
- From that point, walk in the randomly assigned compass bearing until a game trail is encountered
- Mark the GPS locations as the starting point of the transect (write this on transect establishment data sheet, **Appendix E**, in addition to saving), triple flag and write name, date, and point letter "A" (e.g., UV2-3Jun13-A), TURN ON the track log, and tape as necessary with single flagging.
- Double-flag every 100 m (based on pacing) and mark flagging in consecutive letters (e.g., UV2-B), mark waypoint in GPS unit to facilitate future veg sampling
- Follow the deer trail in *whichever direction is closest to the bearing*, and at each fork in the trail take the turn closest to the bearing. If both forks are equidistant from the bearing, use a pre-determined rule of taking the left or right path. If the trail disappears entirely, go in a straight line along the compass bearing, continuing to flag, until you reach another trail.
- Check track log distance regularly, and when you have covered 1–1.2 km flag (transect ID, date, last letter, e.g., UV2-3Jun2013-N) and mark the end point on the GPS and additionally record on data sheet. TURN OFF the track log and save it, naming the transect after the pre-determined starting point (e.g., UV2).

Collecting pellets

- For every pile of pellets within a **2 m strip** (1m on either side of the transect), determine the pellets condition and whether or not to collect:
    - Eroded: outer coating has been weathered. NEVER COLLECT
    - Dull: Whole but no longer shiny. COLLECT DURING RESAMPLING (not first run)
    - Shiny: Not wet, but still has a mucus coating. ALWAYS COLLECT
    - Wet: Some of the pellets (inner) are wet, outer still dry. ALWAYS COLLECT
    - Slimy: Every pellet is wet. ALWAYS COLLECT

- Remove all pellet groups, whether collected or not, within a **4 m strip** (2m on either side of trail), by brushing away with your boot/hands/sticks.
- For pellets to be collected, collect 4–6 pellets (or for very small pellets try to equal mass of 4 normal pellets) into a tube WITHOUT TOUCHING them (i.e., use a stick or new gloves for each pellet group to transfer pellets into tube) and seal the lid.
- Record data for each pellet group on a separate line of the datasheet **(Appendix F)** according to naming convention below**.**
- Mark the GPS location for each pellet group and name as labeled on the tube (see naming convention below). If two pellet groups are in the same location, save one GPS point and note that on datasheet.
- End of day: always download waypoints onto computer as backup; backup once per week if possible

Pellet tube naming convention Pellet Transect # (UV2) – "Y" and 2 digit Year – "R" (run number) – "PG" (Pellet group #); Example: UV2-Y13-R1-PG3 (Union Valley transect number 2 – year = 2013 – 3rd time running – 3rd pellet group collected on that transect run)

Appendix E. UC Davis – *Mammalian Ecology and Conservation Unit*

Transect establishment datasheet

Collector(s):		**Easy road access?**	
Description of habitat and changes (systematic veg sampling later):			
Site:		**Transect ID:**	
Date (mo/day/yr):		**Compass Bearing:**	
Start Coord:		**End Coord:**	
Elevation:		**Last point letter:**	
Collector(s):		**Easy road access?**	
Description of habitat and changes (systematic veg sampling later):			

Appendix F. UC Davis – *Mammalian Ecology and Conservation Unit*

Deer Pellet Collection Datasheet (6/7/13)

Date	Transect ID	Run #	Pellet Grp #	Near-est pt(s)	Latitude	Longitude	Elev (m)	Way pt #	Fresh (Y/N)	initials

Notes: