

RESEARCH NOTE

Testing a single-visit sampling approach for fecal DNA abundance estimation of tule elk in the Lake Pillsbury BasinBENJAMIN N SACKS^{1*}, JOSHUA P BUSH², AND THOMAS J BATTER^{1,3}¹ *University of California, Davis, One Shields Avenue, Davis, CA 95616, USA*² *California Department of Fish and Wildlife, North Central Region, 1701 Nimbus Road, Rancho Cordova, CA 95670, USA*³ *California Department of Fish and Wildlife, Northern Region, 660 S Main Street, Willets, CA 95490, USA**Corresponding Author: bensacks@ucdavis.edu**Key words:** abundance estimation, capture-recapture, *Cervus canadensis nannodes*, tule elk

Estimating abundance (N) in ungulate populations is fundamental to their management. Fecal DNA provides a noninvasive basis for estimating N in ungulate populations through capture-recapture-based methodologies (Brinkman et al. 2011; Lounsbury et al. 2015). Most recently, application of spatially explicit capture-recapture (SCR) methods have proven especially powerful, particularly in more solitary species such as deer (*Odocoileus* spp.; Brazeal et al. 2017; Furnas et al. 2020). These SCR methods also appear relatively robust to spatial clustering in more gregarious species such as elk (*Cervus canadensis*), particularly when both sexes are incorporated into models (Batter 2020; Bischoff et al. 2020). On the other hand, some populations of elk congregate at especially high densities, particularly females (i.e., cow groups). Elk aggregations can become even greater during the mating season (the “rut”), when bull elk, typically sexually segregated, join cow groups to compete for mates (Bowyer 2004; Weckerly 1998) providing an opportunity to estimate their group sizes with less time-intensive non-spatial approaches (Mena 2019). Large numbers of samples also can be collected in a single visit, which, if analyzed appropriately, may provide a more efficient means of estimating N than multi-sample non-spatial or SCR approaches.

In the present study, we tested such a non-spatially explicit single-visit approach (hereafter, single-visit) to estimating tule elk (*C. c. nannodes*) abundance at Lake Pillsbury, California, where females occur at very high density year-round, almost exclusively within a 5-km² basin (Batter 2020). The Lake Pillsbury Basin (hereafter, the Basin) is located in Lake County, California (39.450, -122.956) and encompasses a discrete patch of suitable tule elk habitat composed primarily of grassland, lacustrine, and mixed hardwood habitats at approximately 270 m above sea level surrounded by less hospitable (to tule elk), higher elevation coniferous forest (Batter 2020). Although the range for males of this population extends well beyond the Basin, high densities of bull elk are routinely

observed engaging in rut activity within the Basin during Sept–Oct yielding the greatest aggregate group sizes. We therefore conducted a single-visit survey in the Basin during the rut to determine if abundance estimates of congregated elk were comparable to those generated from a range-wide (i.e., encompassing both sexes year-round) SCR estimate prior to the rut (Batter 2020).

We compared abundance estimates derived from the present single-visit survey to an independent one from an SCR study conducted earlier the same year (Jun–Aug) over a much wider spatial extent (189 km²) that included the 5-km² Basin (Batter 2020). We also examined overlap between the two studies (i.e., single-visit and SCR) in detections of both female and male individuals to verify that females were comprehensively sampled within the Basin and to investigate the extent to which males may have been under-sampled by restricting our single visit survey to the Basin.

Five personnel conducted a single-visit survey on 14 October 2019 within the Basin, which involved collecting fecal pellets along transects through randomly selected plots (Fig. 1). Specifically, we divided the Basin into 150 250-m × 250-m sample plots and randomly selected 57 of them (approximately 40%) for sampling. We began sample transects at the mid-point of each plot's northern or southern boundary and traversed through the plot centroid, ending at the mid-point of the opposite edge (Brazeal and Sacks 2021). We stored fecal pellets in >95% ethanol prior to laboratory analysis. We analyzed fecal DNA using 20 microsatellites and a sex marker and assigned genotypes to individuals as described previously (Sacks et al. 2016; Batter et al. 2021). Because samples were collected in a single visit, we opted to use an urn model implemented in program Capwire to estimate N for capture-recapture data (Miller et al. 2005). We employed the likelihood ratio test in Capwire to select between the two innate rates model (TIRM), which allows some individual variation in detection probability, and the equal capture probability model (ECM), which does not. Methods used in the earlier SCR study were detailed elsewhere (Batter 2020). Briefly, we established 11 transects (4–6 km each) throughout the known range of the Lake Pillsbury population (189 km²) based on random selection of 4-km² plots stratified by habitat quality; sample processing and genetic identification were conducted the same as for the single-visit survey.

We collected 280 pellet groups during the single-visit survey, from which 151 samples (54%) were successfully genotyped. Although this overall success rate was relatively low, the figure masked a spatial heterogeneity that ranged from <20% in the northern portion of the Basin to >80% success in the southern portion of the Basin, closer to the lakeshore (Fig. 2). During the day of sampling, we only observed elk on the southern edge of the basin, where we also experienced the highest genotyping success, suggesting the fecal pellets collected there tended to be fresher than those collected to the north. Because genotyping success is highest in fresh samples, restricting sampling to locations where elk are observed immediately prior to sampling could increase the efficiency of future single-visit surveys. The success rate in a fecal DNA study of Roosevelt elk (*C. c. roosevelti*) in northern California that sampled pellets only from locations in current use by telemetered individuals was 82% (Mena 2019), which is comparable to ours in the southern portion of our study area.

The 151 genotypes obtained from our single-visit survey included 103 from females (67 individuals) and 48 from males (30 individuals). Using the recapture profiles of the 103 genotypes of 67 females, a likelihood ratio test implemented in Capwire indicated a better fit of the TIRM over the ECM, which resulted in an estimate of $N = 159$ females (95% CI: 106–189) in the Basin. This estimate was statistically indistinguishable from the spatially more extensive SCR estimate of female abundance for the Lake Pillsbury population (136 females, 95% CI: 100–172; Batter 2020). The concordance of these estimates, which were based on independent samples and methods of analysis with different assumptions, supports their general accuracy for estimating female elk abundance

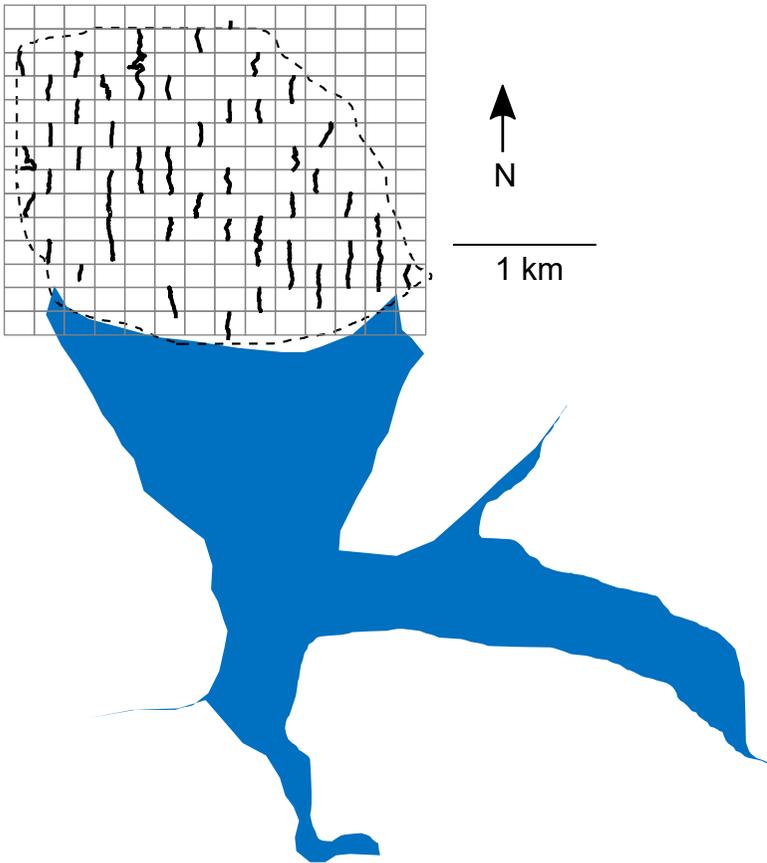


Figure 1. Sampling grid (grey) superimposed over the Lake Pillsbury Basin (black dashed polygon) in Lake County, California, USA, showing 57 transects in randomly selected plots sampled during a single-visit survey (14 Oct 2019).

in the Lake Pillsbury population. Using the recapture profiles of the 48 genotypes of 30 males, the likelihood ratio test similarly indicated a better fit of the TIRM over the ECM. In contrast to females, however, the abundance estimate for males in the Basin ($N = 71$, 95% CI: 41–96) was less than half that of the SCR estimate for the entire Lake Pillsbury population (148 males, 95% CI: 108–187; Batter 2020). Thus, our findings suggest that the single-visit survey was not appropriate for estimating the population abundance of males.

To further verify these conclusions, we investigated overlap in detections of individuals between the two studies (single-visit, SCR). The spatially broader SCR survey yielded a similar number of fecal pellet genotypes ($n = 155$), including 97 from females (49 individuals) and 58 from males (30 individuals) (Batter 2020). Based on 200 fecal genotypes from females sampled in the two surveys combined, all 85 individuals (100%) were sampled at least once in the Basin (Fig.3). Only two females were sampled outside the Basin during the SCR survey (one 4 times, the other 2 times), and both were additionally sampled within the Basin during the single-visit survey. Thus, the spatially broader SCR survey did not improve on the sampling of females over the single-visit survey restricted to the Basin, suggesting the latter approach was representative of the entire female population (i.e., sampling the Basin alone did not result in systematic under-detection of females). The single-visit survey restricted to the Basin also resulted in >70%

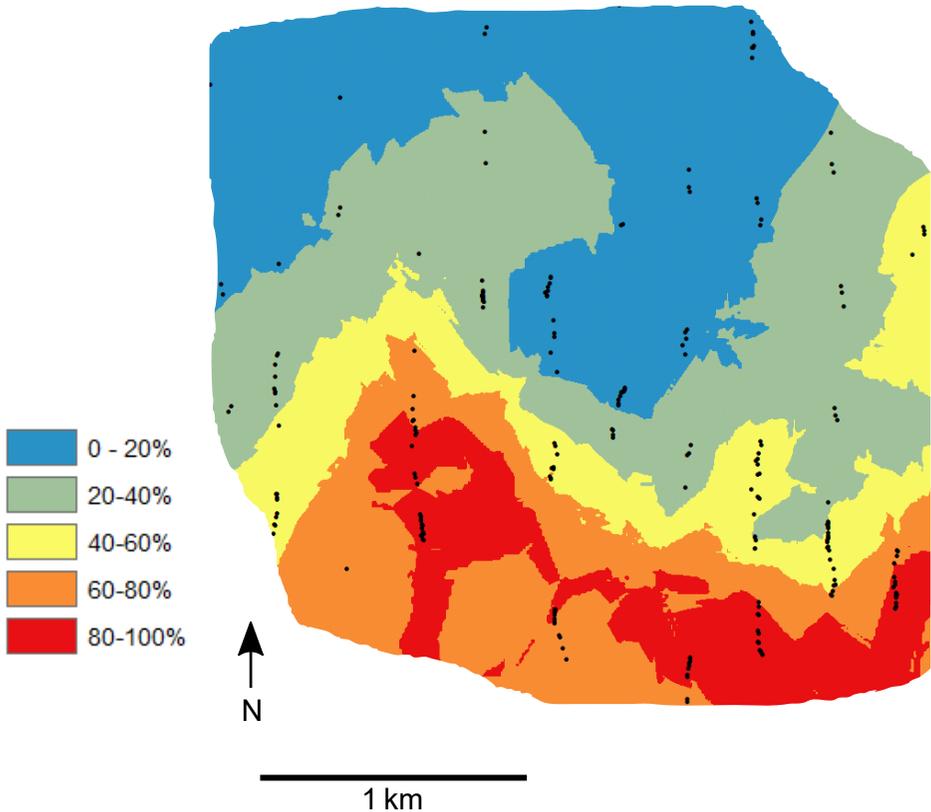


Figure 2. Locations of 280 elk fecal pellet groups (filled black circles) collected on 14 October 2019 from Lake Pillsbury Basin, Lake County, California, USA, superimposed upon an interpolated surface representing genotyping success. The high spatial heterogeneity in genotyping success rates presumably reflects heterogeneity of fecal pellets. The freshest elk pellets associated with highest genotyping success rates tended toward the south of the Basin, whereas older elk pellets associated with lowest genotyping success rates tended toward the north of the Basin. Interpolation was based on ordinary kriging using a variable search radius to include the 30 nearest fecal pellet groups.

more individuals sampled, suggesting it was more efficient than the broader SCR survey at sampling the female component of the population.

In contrast to females, however, many of the 106 fecal genotypes sampled from 51 males in the two 2019 surveys combined were of individuals sampled only outside the Basin during the SCR survey (Fig. 4). Of the 30 males sampled during the SCR survey, 15 were sampled only outside the Basin, 12 were sampled only within the Basin, and 3 were sampled both in and out of the Basin (1 male was sampled in both locations during SCR surveys). Of the 30 males sampled during the single-visit survey, 9 had been previously sampled during the SCR survey, and only 2 of these had been sampled outside the Basin. Thus, both abundance estimates and distributions of individual detections between the two studies indicate that for males the single-visit survey of the Basin was not representative of the broader population.

To the extent that monitoring of the female segment of the population is desirable, our results suggest that the single-visit survey protocol can substantially increase the efficiency of efforts to monitor the female component of the Lake Pillsbury Basin population. This approach was effective for this particular population because females

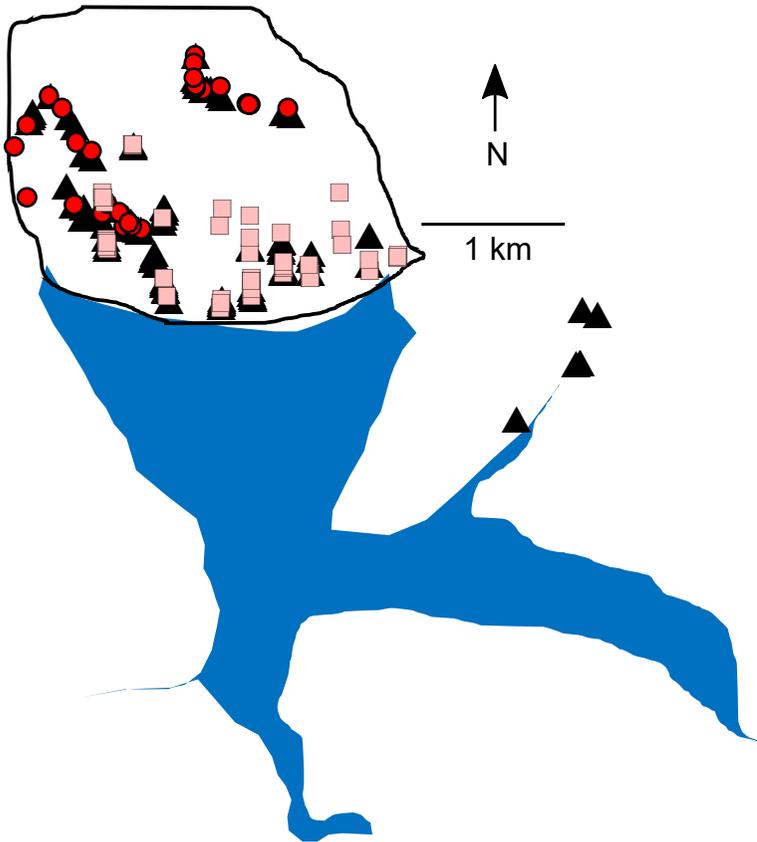


Figure 3. Successfully genotyped female tule elk pellets ($n = 200$) sampled from the Lake Pillsbury population, Lake County, California, USA, during the SCR survey (1 Jun–31 Aug 2019) throughout the range and the single-visit survey (14 Oct 2019) concentrated in the Lake Pillsbury Basin (circumscribed by black line), indicating samples from individuals that were sampled only during the SCR survey (red circles), only during the single-visit survey concentrated in the Lake Pillsbury Basin (light red squares), or during both surveys (black triangles), indicating that all females were sampled at least once in the Basin.

congregate in a single location, rather than being distributed across multiple spatially separated cow groups, as, for example occurs to the southeast in the Cache Creek population (Batter 2020). Thus, where efficiency is paramount and estimation of females alone is sufficient, this approach may be gainfully applied to other populations where females are concentrated in dense aggregations over relatively small spatial extents, such as the Potter Valley and Little Lake Valley tule elk populations in neighboring Mendocino County. We caution, however, that this approach would have been inappropriate for males in the Lake Pillsbury population and likewise would be inappropriate for other populations where the sampling site cannot be reasonably assumed to include all individuals during the sampling period.

In the present study, we opted to conduct a single-visit survey to estimate tule elk abundance in a high-use concentrated area necessitating a one-sample method of analysis, in our case use of Capwire. Although the approach worked well in the present case for the female population segment, protocols based on multiple surveys could also be used if desired to enable use of traditional multi-session methods such as the Huggins closed capture model (Huggins 1989; Mena 2019). Spatial capture-recapture models also

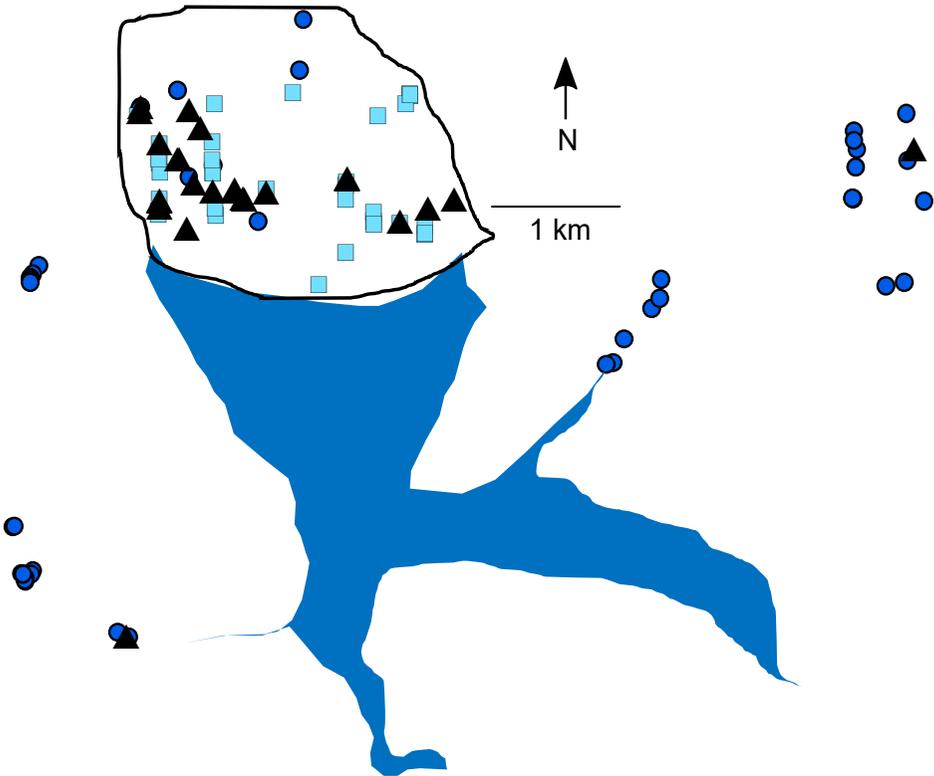


Figure 4. Successfully genotyped male tule elk pellets ($n = 106$) sampled from the Lake Pillsbury population, Lake County, California, USA, during the SCR survey (1 Jun–31 Aug 2019) throughout the range and the single-visit survey (14 Oct 2019) concentrated in the Lake Pillsbury Basin (circumscribed by black line), indicating samples from individuals that were sampled only during the SCR survey (blue circles), only during the single-visit survey (light blue squares), or during both surveys (black triangles), indicating that most males were sampled in the Basin or outside the Basin, but rarely ($n = 2$ individuals) both.

may perform well as long as the population is composed of multiple cow groups or both sexes are included. Because male space use accords more closely with SCR assumptions of independence, their inclusion in multisex SCR models helps offsets violations of this assumption by the female component of the population (Batter 2020). In cases such as the present one, however, where females essentially share a single home range and activity center, use of SCR to estimate female abundance in isolation from males entails severe violations to the assumption of independence, which could potentially bias point estimates and precision (Efford and Fewster 2013; Bischoff et al. 2020).

ACKNOWLEDGMENTS

We are grateful to C. White, A. Cheney, and C. Day of the California Department of Fish and Wildlife (CDFW) for field assistance, and to S. Vanderzwan of the University of California, Davis Mammalian Ecology and Conservation Unit (MECU) for laboratory assistance. We thank two anonymous reviewers for helpful comments on an earlier draft of this manuscript. This work was funded by CDFW and the University of California, Davis MECU.

LITERATURE CITED

- Batter, T. J. 2020. Development and implementation of DNA-based survey methods for population monitoring of tule elk (*Cervus canadensis nannodes*) in the Interior Coast Ranges of northern California. Dissertation, University of California, Davis, USA.
- Batter, T. J., J. P. Bush, and B. N. Sacks. 2021. Assessing genetic diversity and connectivity in a tule elk (*Cervus canadensis nannodes*) metapopulation in northern California. *Conservation Genetics* 22:889–901.
- Bischof, R., P. Dupont, C. Milleret, J. Chipperfield, and J. A. Royle. 2020. Consequences of ignoring group association in spatial capture–recapture analysis. *Wildlife Biology* 2020(1):1–13.
- Bowyer, R. T. 2004. Sexual segregation in ruminants: definitions, hypotheses, and implications for conservation and management. *Journal of Mammalogy* 85:1039–1052.
- Brazeal, J. L., and B. N. Sacks. 2021. Use of an enclosed elk population to assess two non-invasive methods for estimating population size. bioRxiv doi.org/10.1101/2021.05.21.445203.
- Brazeal, J. L., T. Weist, and B. N. Sacks. 2017. Noninvasive genetic spatial capture-recapture for estimating deer population abundance. *Journal of Wildlife Management* 81:629–640.
- Brinkman, T. J., D. K. Person, F. S. Chapin, III, W. Smith, and K. J. Hundertmark. 2011. Estimating abundance of Sitka black-tailed deer using DNA from fecal pellets. *Journal of Wildlife Management* 75:232–242.
- Efford, M. G., and R. M. Fewster. 2013. Estimating population size by spatially explicit capture-recapture. *Oikos* 122:918–928.
- Furnas, B. J., R. H. Landers, R. G. Paiste, and B. N. Sacks. 2020. Overabundance of black-tailed deer in urbanized coastal California. *Journal of Wildlife Management* 84:979–988.
- Huggins, R. M. 1989. On the statistical analysis of capture-recapture experiments. *Biometrika* 76:133–140.
- Lounsberry, Z. T., T. D. Forrester, M. T. Olegario, J. L. Brazeal, H. U. Wittmer, and B. N. Sacks. 2015. Estimating sex-specific abundance in fawning areas of a high-density Columbian black-tailed deer population using fecal DNA. *Journal of Wildlife Management* 79:39–49.
- Mena, R. 2019. Targeting social cohesion in female Roosevelt elk (*Cervus canadensis roosevelti*) groups minimizes survey efforts for fecal DNA capture-recapture estimates of abundance. Thesis, Humboldt State University, Arcata, CA, USA.
- Miller, C. R., P. Joyce, and L. P. Waits. 2005. A new method for estimating the size of small populations from genetic mark–recapture data. *Molecular Ecology* 14:1991–2005.
- Sacks, B. N., Z. T. Lounsberry, T. Kalani, E. Meredith, and C. Langner. 2016. Development and characterization of 15 polymorphic di-nucleotide microsatellite markers for tule elk using HiSeq3000. *Journal of Heredity* 107:666–669.
- Weckerly, F.W. 1998. Sexual Segregation and Competition in Roosevelt Elk. *Northwestern Naturalist* 79:113–118.

Submitted 18 Jun 2021

Accepted 19 July 2021

Associate Editor was K. Denryter