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Monitoring Desert Ungulates via Fecal DNA-Based Capture Recapture

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MONITORING DESERT UNGULATES VIA FECAL DNA-BASED CAPTURE RECAPTURE

by

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ABSTRACT

Monitoring Desert Ungulates Using Fecal DNA-Based Capture-Recapture

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Informed management of wildlife populations requires reliable estimation of abundance, survival, and other demographic parameters. Obtaining estimates of this kind can be difficult, especially for species that are wide-ranging and exist in low densities. Over the past few decades non-invasive sampling methods such as remote camera traps and collection of DNA samples (i.e. feces and hair) has become quite common. My study examined the feasibility and effectiveness of non-invasive genetic sampling (NGS) of fecal DNA samples combined with traditional capture-recapture models to estimate abundance and survival of two species of desert-dwelling ungulates in the Mojave and Sonoran Deserts of southeastern California.

In Chapter 2, using artificial water sources as focal sampling sites I estimated abundance and annual survival of desert mule deer (*Odocoileus hemionus eremicus*) using fecal DNA-based capture-recapture methods in the Little Chuckwalla Mountains located within the Sonoran Desert, California, USA from 2015 to 2017. Abundance

estimates were 386 (95% CI = 264-509), 351 (95% CI = 281-420) and 301 (95% CI = 260-342) in 2015, 2016, and 2017 respectively. Between year apparent survival (2015-2016 and 2016-2017) was approximately 22% higher for females (0.90, 95% CI = 0.59-0.98) than males (0.71, 95% CI = 0.51 – 0.85). My results provide the first abundance and survival estimates of desert mule deer in California in over 13 years.

In Chapter 3, I compare two different methods for estimating abundance of desert bighorn sheep (*Ovis canadensis nelsoni*) in the Marble Mountains located within the Mojave Desert, California, USA in 2016 and 2017. By implementing traditional ground mark-resight and fecal DNA-based capture-recapture techniques during the same time frame, I compared the two methods by evaluating cost and precision (coefficient of variation) through field-observed results and simulation data. My results showed that fecal DNA-based capture-recapture methods can achieve much higher precision at a fraction of the cost when compared to traditional ground mark-resight surveys.

(146 pages)

PUBLIC ABSTRACT

Monitoring Desert Ungulates Using Fecal DNA-Based Capture-Recapture

Stephen S. Pfeiler

Estimates of population abundance and survival are critical for effective wildlife management. Obtaining estimates of these kind using traditional wildlife monitoring techniques (i.e. ground and aerial surveys) has proven to be difficult, especially for species that are wide ranging and exist in small, patchily distributed populations.

My objective was to implement fecal DNA-based capture-recapture surveys to estimate abundance and survival of two different ungulate populations that inhabit the deserts of southeastern California. I also compared fecal DNA-based capture-recapture techniques to traditional methods by evaluating the costs and precision associated with both methods. Using artificial water sources as focal sampling sites, I performed sampling during the summers of 2015, 2016, and 2017 in the Mojave and Sonoran Deserts of California. I was able to obtain reasonably precise estimates of abundance and survival for both species.

To my knowledge, my study provides the first abundance and survival estimates of desert mule deer in California in over 13 years. Additionally, my study shows that when compared to traditional methods, fecal DNA-based capture recapture techniques can achieve much higher precision at a fraction of the cost.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

As the magnitude and severity of human-related impacts to the environment continue to increase, the integrity of healthy, self-regulating wildlife populations is put at risk. The diverse interactions between wildlife populations, the environment, and anthropogenic activities pose a constant and exponentially-increasing challenge to wildlife management agencies to manage these populations (Apollonia et al. 2017). Wildlife managers are faced with the difficult task of monitoring and managing wildlife populations that are subject to hunting, disease, climate change, habitat fragmentation/loss, and much more. Often the first piece of information needed to make informed wildlife management decisions for any species is reliable abundance estimates (Shaffer 1981). When applied to small, spatially clustered and/or wide-ranging populations with low densities, standard wildlife sampling designs such as ground and aerial surveys are neither cost nor labor efficient (Thompson et al. 1998) because they have low detection probabilities and yield poor estimates of population abundance (Marshal et al. 2006). Because patchily distributed populations are often difficult to observe using traditional methods (Thompson and Bleich 1993), non-invasive approaches such as remote camera traps and collection of genetic samples through feces or hair has become quite common (Waits 1997, Waits and Paetkeu 2005, Marshal et al. 2006, Kendall et al. 2008, Brinkman et al. 2011, Furnas et al. 2018). Subsequently, the identification of unique individuals using DNA microsatellites has become very common in the field of wildlife management (Lukacs and Burnham 2005).

Desert ungulate populations are typically monitored using traditional techniques that include ground mark-resight surveys, helicopter surveys, radio-telemetry, and GPS-collared individuals (Koenen et al. 2002, Krausman et al. 2006, Marshal et al. 2006, Woodruff et al. 2016). These techniques are often used to monitor desert bighorn sheep (*Ovis canadensis nelsoni*) and desert mule deer (*Odocoileus hemionus eremicus*) populations (Marshal et al. 2006, Cain et al. 2008, Bleich et al. 2010, Conroy et al. 2018). These traditional techniques have proven to be an effective way to estimate abundance, but may have drawbacks in terms of cost, efficiency and precision. Aerial counts can lead to statistical uncertainty due to incomplete spatial and temporal coverage and low or varying sightability during the survey (Celantano and Garcia 1984, Douglas and Longshore 1995, Conroy et al. 2014; 2018). Even though varying levels of sightability during aerial counts can be accounted for, there is often statistical uncertainty in sightability estimates, which in turn leads to considerable statistical uncertainty in abundance estimates (Conroy et al. 2014). Given desert ungulates typically have low population densities and large survey areas, ground surveys often yield low return per unit effort (Thompson and Bleich 1993). In addition, the need to capture and collar animals for aerial and ground mark-resight surveys can cause unintended stress or injury to the animals (Jacques et al. 2009). To overcome this issue, a sampling design that allows for sampling efforts to be concentrated at or near a variable of interest for wildlife (i.e. water, scent stations) can be used (Thompson and Seber 1996).

DNA obtained through non-invasive sources can be an effective tool for monitoring secretive and sparse wildlife populations when combined with traditional

capture-recapture techniques to estimate abundance and demographic vital rates such as survival (Taberlet et al. 1999, Waits and Paetkau 2005). Over the past 2 decades, advances in non-invasive genetic sampling has allowed wildlife managers to successfully estimate abundance using DNA obtained from fecal pellets. The use of non-invasive genetic sampling is appealing because the animals do not need to be captured, handled, or even seen (Taberlet et al. 1999), thus eliminating the risk of unintended stress and injury to the animal (Jacques et al. 2009). The increasing use of non-invasive genetic sampling and capture-recapture for wildlife populations has led to advances in field study design for DNA-based mark-recapture studies (Boulanger et al. 2004, 2008) and the ability of analytical models to account for uncertainties from both field and laboratory procedures (Lukacs and Burnham 2005, Knapp et al. 2009) continue to develop. Furthermore, advances in DNA sequencing technologies has led to lowered costs (Fredlake et al. 2008) allowing biologists to address traditional management issues in an efficient and cost-effective way (Latch et al. 2015).

In chapter 2, I evaluated the effectiveness of using fecal DNA-based capture-recapture to estimate abundance and survival of desert mule deer in the Sonoran Desert of southeastern California. In chapter 3, I compared the precision and cost effectiveness of two methods used to estimate abundance of desert bighorn sheep populations in the Mohave Desert of southeast California: traditional ground-based mark-resight, and newer fecal DNA-based capture-recapture. The first objective of my study was to design and implement a sampling design focused at artificial water sources that could be used in conjunction with traditional capture-recapture methods to estimate survival and

abundance of desert-dwelling ungulates. The second objective was to compare the costs and precisions of estimating abundance using both fecal DNA-based capture-recapture and traditional ground mark-resight methods. The third objective was to create a simulation-based approach for determining the efficacy for each method.

THESIS FORMAT

Chapters 2 and 3 were written and formatted as individual manuscripts ready for publication in specific peer-reviewed journals. Both chapters will be submitted to *The Journal of Wildlife Management.* Because my work was a collaboration among several other people and entities, co-authors are listed at the start of each chapter; thus I shifted from the singular (e.g., "I") to the plural (e.g., "we") throughout chapters 2 and 3.

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CHAPTER 2

ESTIMATING ABUNDANCE OF DESERT MULE DEER USING FECAL DNA-BASED CAPTURE-RECAPTURE [1]

ABSTRACT

Wildlife conservationists and managers often need to estimate abundance and demographic parameters to monitor the status of populations, as well as to ensure they are meeting management goals. Recently, DNA capture-recapture surveys have become increasingly common in situations where physical surveys are consistently difficult or counts are small or variable. Due to the rugged environment they inhabit, low population densities, and cryptic behaviors, it has been difficult to monitor desert mule deer and assess population status. In attempts to overcome these monitoring difficulties, our goal was to assess the effectiveness of estimating abundance and survival of desert mule deer (*Odocoileus hemionus eremicus*) using fecal DNA-based capture-recapture. We designed and implemented a fecal DNA sampling design focused at water sources during the dry seasons (June-July) of 2015-2017 in the Little Chuckwalla Mountains located in the Sonoran Desert of southeastern California. We used the capture-recapture data in the POPAN open-population model to estimate abundance, and the Cormack-Jolly-Seber model to estimate annual survival. Over the 3-year study, population abundance estimates ranged from 386 in 2015 to 301 in 2017. Precision (coefficient of variation; CV) of male and female abundance estimates ranged 7.6-15.1%. Annual apparent survival for females and males was 0.91 (CV= 9%) and 0.71 (CV=13%) respectively.

[1] Coauthored by Mary Conner, Jane McKeever, Tom Stephenson, Rachel Crowhurst, and Clinton Epps.

This study demonstrates that fecal DNA-based capture-recapture is an effective method for estimating abundance and survival of desert mule deer. If expanded to encompass additional mountain ranges adjacent to our study area, this technique shows the potential to monitor the larger meta-population of desert mule deer in the Sonoran desert of California.

INTRODUCTION

For most wildlife populations, management efforts are often aimed at increasing, reducing, or maintaining a desired population goal (Williams et al. 2001). Effective management of wildlife populations requires an understanding of the factors that influence demographic parameters such as survival, reproduction rates, and movement, because ultimately, these factors drive fluctuations in population abundance (Williams et al. 2001). However, the first and most important piece of information needed to understand population dynamics is an estimation of abundance (Shaffer 1981). For wide-ranging species that exist in low densities, gathering data needed for dependable population estimates is often difficult (Woodruff et al. 2016). When applied to small, spatially clustered populations with wide distributions, standard wildlife sampling designs such as ground and aerial surveys are neither cost nor labor efficient (Thompson et al. 1998) because they have low detection probabilities often yield poor estimates of population abundance (Marshal et al. 2006). To overcome this issue, a sampling design that allows for sampling efforts to be concentrated at or near a variable of interest for wildlife (i.e. water, scent stations) can be used (Thompson and Seber 1996). Furthermore, since patchily distributed populations are often difficult to survey using traditional

methods (Thompson and Bleich 1993), non-invasive approaches such as remote camera traps and collection of genetic samples through feces or hair has become quite common (Waits 1997, Waits and Paetkeu 2005, Marshal et al. 2006, Kendall et al. 2008, Brinkman et al. 2011, Furnas et al. 2018). Subsequently, the identification of unique individuals using DNA microsatellites has become very common in the field of wildlife management (Lukacs and Burnham 2005).

DNA obtained through non-invasive sources (e.g., feces and hair) can be an effective tool for monitoring secretive and sparse wildlife populations when combined with traditional capture-recapture techniques to estimate abundance and demographic vital rates such as survival (Taberlet et al. 1999, Waits and Paetkau 2005). Non-invasive genetic sampling is appealing because the animals do not need to be captured, handled, or even seen (Taberlet et al. 1999), thus reducing the chance of unintended stress to the animal (Jacques et al. 2009). The increasing use of non-invasive genetic sampling and capture-recapture for wildlife populations has led to advances in field study design for DNA-based mark-recapture studies (Boulanger et al. 2004, 2008) and the ability of analytical models to account for uncertainties from both field and laboratory procedures (Lukacs and Burnham 2005, Knapp et al. 2009) continue to develop. Furthermore, advances in DNA sequencing technologies has led to lowered costs (Fredlake et al. 2008) allowing biologists to address traditional management issues in an efficient and cost-effective way (Latch et al. 2015).

Non-invasive genetic sampling has most commonly been used to study bear (*Ursus* spp.) populations using DNA obtained from hair samples (Taberlet and Bouvet

1992, Boulanger et al. 2004, 2008, Kendall et al. 2008, Fusaro et al. 2017). Wehausen et al. (2004) presented an improved method for sampling fecal pellets, which used sloughed intestinal epithelial cells that are present on the surface of fecal pellets. This technique has now been applied to estimate population abundance of many species including mountain hares (*Lepus timidus*) (Rehnus and Bollmann 2016), otters (*Lutra lutra*) (Vergara et al. 2014), and mule deer (*Odocoileus hemionus*) (Brazeal et al. 2017, Furnas et al. 2018). Collection of DNA through fecal pellets is attractive not only because of its non-invasive nature, but also the ease of collection and potential for obtaining large sample sizes (Poole et al. 2011).

In attempts to overcome the challenge of monitoring populations that inhabit densely-vegetated forests where direct observation from ground and/or air is difficult, Brinkman et al. developed (2010) and field tested (2011) a protocol for extracting DNA from fecal pellets for Sitka black-tailed deer (*Odocoileus hemionus sitkensis*). This study successfully estimated abundance with error rates similar to those for black and grizzly bears (Paetkau 2003, Kendall et al. 2008). Since the advent of the Brinkman et al. (2010) study, fecal DNA has been successfully used to estimate population size and vital rates for similar ungulate species that also inhabit moist mountainous and woodland ecoregions (Poole et al. 2011, Hettinga et al. 2012, Goode et al. 2014). This technique has also been successfully used in the dry, mountainous desert region of Afghanistan for Argali (*Ovis ammon*; Harris et al. 2010). This study suggested that the use of capture-mark-recapture models and genetic data derived from fecal samples is often the only reliable option available to monitor such isolated, wide-ranging ungulates that inhabit

rugged environments. More recently Woodruff et al. (2016) used NGS-CR techniques to successfully estimate abundance and survival of the endangered Sonoran Pronghorn (*Antilocapra americana sonoriensis*) in the Sonoran desert of southern Arizona. If continued, this study also has the potential for determining the population's trajectory (i.e. λ - population growth rate) (Woodruff et al. 2016). Thus, since the advent of Wehausen et al.'s (2004) fecal DNA extraction technique, DNA-based methods for identifying individual ungulates have become increasingly refined over the past 2 decades.

Having 11 distinct subspecies (Latch et al. 2009), *Odocoileus hemionus* are large ungulates that occupy a range extending over most of western North America in all biomes excluding the arctic tundra. Desert mule deer (*Odocoileus hemionus eremicus*) are known to occur at low densities (Thompson and Bleich 1993, McLean 1930) and are patchily distributed (Celentano and Garcia 1984) in the Sonoran Desert of southeastern California. Even though mule deer in this area are exposed to a variety of anthropogenic impacts that are potentially detrimental to their populations, such as harvest, habitat degradation, recreation use (Marshal et al. 2006), and more recently, solar energy development, few studies have evaluated how these impacts influence their abundance and distribution. Due to the rugged environment they inhabit, low population densities, and cryptic behaviors, monitoring these populations has been difficult. However past studies on these mule deer populations have successfully estimated survival and sex and age ratios using aerial and telemetry surveys (Celentano and Garcia 1984), as well as with hunter surveys (Thompson and Bleich 1993). Marshal et al. (2006) estimated

abundance, sex ratios, deer densities, and home range size using remote photography of radio-collared deer at artificial water catchments, although precision was relatively low (average CV=32%).

To expand the application of non-invasive genetic sampling capture-recapture techniques to desert ecosystems and to contribute to the knowledge and assessment of elusive desert mule deer populations, our objective was to develop methodology to estimate population parameters, including abundance, survival, sex ratios, and possibly rate of population change (λ) for desert mule deer using fecal DNA-based capture-recapture models. Because deer congregate around water sources during the dry season (June and July), we implemented a sampling strategy using all known water sources (CDFW, unpublished data) as fecal DNA sampling sites during the dry seasons of 2015, 2016, and 2017. We also randomly selected sites without water from mule deer habitat within the study area in 2017. These sites allowed us to estimate the proportion of the population that used water sources and whether or not there is a population of deer that does not visit water.

STUDY AREA

The study area is located in the northwest part of the Sonoran Desert in southeastern California, approximately 40 km southwest of Blythe in the Little Chuckwalla Mountains of Riverside County (Fig. 2.1). The study area ranged from approximately 700 km^2 (in 2015 and 2017) to 970 km^2 (in 2016) in size and elevations range from 120 m to 1370 m above sea level. Over the duration of the 3-year study, annual average precipitation in Rice Valley (approximately 50 km north of the study

area) averaged 195 mm with 2015 receiving the most rainfall throughout the dry season (Fig. 2.2). Further, temperatures in 2015 were slightly lower than temperatures in 2016 and 2017 (WRCC 2019). During the study period, in June and July, daytime temperatures regularly exceeded 45º C, and nighttime lows rarely dipped below 33º C. Land ownership is primarily public with >90% of the study area managed by the U.S. Bureau of Land Management.

Andrew et al. (1999) defined the three primary landforms within this study area: mountains, piedmont (rolling hills), and flats. The vegetation community of mountainous areas are mostly dominated by creosote bush (*Larrea tridentate*), brittle-bush (*Encelia farinosa*), and ocotillo (*Fouquieria splendens*) (Marshal et al. 2005). Most of the flat piedmont landscape consists of crypto-biotic soils and desert pavement that primarily supports creosote bush. Xeroriparian zones, located both within and along the banks of episodic flood channels supports most of the plant biomass within the study area (Marshal et al. 2005). Consequently, desert deer range coincides primarily with the distribution of these desert washes (Heffelfinger 2006, Heffelfinger et al. 2006). The vegetation that inhabits these xeroriparian zones make up the microphyll woodland habitat in which catclaw (*Acacia greggi*), mesquite (*Prosopis glandulosa*), and palo verde (*Cericidium floridum*) are most abundant. Desert mule deer do not traditionally migrate in predictable patterns but move nomadically across long-distances based on seasonal variation in water and food availability (Heffelfinger et al. 2006, deVos 2003, Marshal et al. 2002).

Literature suggests that dry season home ranges of desert mule deer are limited to

within 5 km of water sources (Marshal et al. 2006). Using this distance, study area boundaries shown in Fig. 2.1 were determined by creating a polygon consisting of all sampled water sources, with each water source having a buffer of 5-km. Further research based on animal movements or spatial capture mark-recapture models is needed to create an accurate study area boundary. Since we were only estimating population size and not density, we did not attempt to accurately identify the study area boundary.

METHODS

Field Methods

Samples were collected during the summers of 2015, 2016 and 2017. Sampling occurred during June and July; which is the hottest and driest time of year when desert ungulates occupy areas in close proximity to water sources (Bleich et al. 2010, Ordway and Krausman 1986). At each water source, 2-4 transects were delineated along established game trails, which are most commonly found in episodic flood channels (Fig. 2.1). During a preliminary site visit, each 250 meter-long transect was marked with fluorescent tape. All existing pellets were removed to prevent collection of old samples during the first sampling session which began 4-6 days after the preliminary site visit.

Sampling was performed by collecting every pile of fecal pellets that were visible from the transect center line (~ 15m-20m). Pellets that appeared to be degraded or from more than one individual were discarded. Each sample consisted of 15 to 20 fecal pellets and were placed in small paper bags. Remaining pellets from each pile were covered with sand to prevent resampling during future occasions. Intervals between sampling

occasions ranged from 3 to 6-days for each field season. Each sample bag was labeled with the GPS location, date, sample identification number, collectors initials, and sample quality. Sample quality was recorded as either “good”, “fair”, or “poor”. We recorded sample quality solely for experimental purposes to determine if we could identify in the field sample quality as it related to genotyping success rate. Pellets of good quality appeared to be moist, soft, and/or deposited within 24 hours of sampling. Fair quality pellets appeared dry but retained an outer sheen with minimal cracks. Poor quality samples, most likely deposited shortly after the previous sampling session, were dry and had a diminished sheen with excessive surface cracking.

We performed 6, 5, and 4 sampling sessions in 2015, 2016, and 2017 respectively. During all three years, we sampled at the same 10 artificial water sites (original sites; Fig. 2.1). In 2016, we located an additional 7 artificial water sites (new sites) and performed a single sampling occasion at these sites to determine if we were missing water sites used by the population. In attempts to capture the population of deer that did not visit water sites in 2017, we selected 10 sampling sites (non-water sites) located away from water sources, but within delineated deer habitat (e.g., xeroriparian/microphyll woodland habitat; Fig. 2.1), and sampled these locations during all 4 sessions. For logistical and safety reasons, these sites were located ≤1,000 m from roads.

Due to funding limitations, and because we collected an extremely high number of samples (> 1,000 for 2015 and 2016) we used a sub-sampling process to determine which samples to submit for DNA analysis. Sub-samples were selected based on simulation work that estimated sample size that attempted to maintain a constant CV($\hat{N}$)

among sampling sites (i.e., water sources), as well as based on sample quality and logistical considerations. Simulations used a range of the likely number of deer using the low, medium, high, and very-high use water sources, a wide range of probabilities of capture (p), and 5 sampling occasions, which was the number good sampling occasions we had in 2015 and 2016 (we had one session in 2015 with a high proportion of ruined samples due to rain). We ran 100 iterations for each simulation scenario (i.e., combination of likely population size and p). For each simulation scenario, an estimate of N and its SE was output, from which we calculated CV($\hat{N}$). To determine sample size, we determined the p required for a CV($\hat{N}$) = 0.05 ($p_{subsamp}$). We then estimated the number of samples needed as the likely population size* $p_{subsamp}$. For the likely population size, we used the middle value for the range of likely population sizes for each water source classification. For example, based on field observations we estimated that 10-30 deer used the low-use water sources; we used 20 as the likely population size. For a population size of 20, p = 0.47 was needed to get CV($\hat{N}$) = 0.05; thus 20 × 0.47 = 10 (we always rounded up) subsamples were required. An additional sub-sampling rule was added in which a minimum of 1 sample per transect per session was submitted, which could increase the number of subsamples required. We only used this sub-sampling process for years 2015 and 2016. Because we collected fewer samples in 2017 (fewer sampling occasions and we only collected high quality samples), we were able to submit all samples collected for DNA analysis. DNA analysis for all selected samples was conducted at Oregon State University.

Genetic Analyses

DNA was obtained using the pellet-scraping method detailed in Wehausen et al. (2004) to collect epithelial cells from the exterior surface of pellets. DNA was extracted from pellet scrapings using a modified AquaGenomic Stool and Soil protocol (MultiTarget Pharmaceuticals LLC, Colorado Springs, CO). Modifications included the addition of 450 µL of AquaGenomic solution to pellet scrapings, the use of 1.0 mm silica/zirconium beads (BioSpec Products Inc., Bartlesville, OK) for cell lysis, and the addition of 12 mAU proteinase K (Qiagen Inc., Valencia, CA) for recovery of mitochondrial DNA. Lastly, 150 µL of AquaPrecipi solution (MultiTarget Pharmaceuticals) was added to cell lysate to remove PCR inhibitors present in fecal samples.

DNA was amplified at seventeen markers (14 microsatellites and three markers for sex identification) for each sample using the polymerase chain reaction (PCR, Appendix A). This suite of microsatellite markers has been used to study mule deer across much of the state of California (Pease et al. 2009, Bohonak and Mitelberg 2014) (Table 2.1). Amplification products were visualized on a 2% agarose gel prestained with GelRed. Products were diluted accordingly, ethanol-precipitated to remove salts, and submitted for fragment size analysis on the ABI 3730 DNA analyzer (Applied Biosystems) at the Oregon State University Center for Genome Research and Biocomputing (Corvallis, OR). We used GeneScan 500 LIZ dye size standard and called allele sizes in GeneMapper v.4.1 (Applied Biosystems).

Samples were initially amplified in three separate PCR reactions each; those that

produced data at fewer than 50% of the loci in the first three replicates were considered poor quality and were not rerun. Samples that produced partial genotypes at ≥ 50% of microsatellite loci were rerun 3-6 more times depending on the completeness of initial replicates, while samples that produced complete genotypes in the first three replicates were considered finalized. For a genotype to be accepted for a particular locus, each allele in a heterozygote genotype had to be observed twice, while the single allele in a homozygote genotype had to be observed three times. Any sample that consistently showed more than 2 alleles at a single locus was considered contaminated and removed. One locus ("B") was monomorphic across all years and was not included in identification analyses. Samples that produced data at 10 or more of the remaining 14 microsatellite loci were used in the individual identification analyses.

Using the online individual-identification program CERVUS version 3.0.3 (Kalinowski et al. 2007) and the population-specific allele frequencies tabulated for this population, we estimated the cumulative probability of identity for unrelated deer (PID) and for siblings (PIDsibs) (Waits et al. 2001) for all 14 microsatellite loci. We analyzed all samples as one herd and searched for recaptures across all 10 water sources. When identifying recaptures we used a maximum PID of $1x10^{-4}$ and PIDsibs of $1x10^{-2}$.

Abundance Estimation

We created capture histories for each uniquely identified mule deer encountered during the study. Each individual was either detected (1) or not detected (0) in each sampling occasion. Individuals that were detected >1 time during the same sampling occasion were counted as duplicate samples; only a single detection per individual per

sampling occasion was counted. We evaluated population closure each year using program Close Test (Stanley and Burnham 1999). Each year, Close Test indicated the population was open ($p = 0.056$ in 2015 and $p < 0.005$ in 2016 and 2017), so we used an open population model for estimating population size. We used a POPAN formulation a Jolly-Seber model in Program MARK (Arnason and Schward 1995, Link and Barker 2005) to estimate abundance of an open population for each year of the study. For open capture-recapture modeling, POPAN also provides within-year estimates of apparent survival (φ; the probability that a captured animal would survive and remain in the study area between sampling occasions), detection probability (p), and probability of entry (p_{ent}; probability a new animal entered the population between sampling occasions). Because the closed capture sampling period was relatively short (≤6 weeks), we consider φ to represent the probability a mule deer remained on the study area. Since an open population may have new individuals entering or leaving the population between capture sessions, the abundance estimate derived from POPAN is an estimate of the population size of all animals using the study area during the study period. We constructed a series of models and fit them to the capture data for each year. We used every possible combination of models in which p, p_{ent}, and ϕ were held constant (.) or were allowed to vary by sex (g) and sampling occasion (t); N was always estimated separately by sex (g). To estimate the population of deer that did not visit water, we removed the individuals captured at the non-water sources from the capture histories and re-ran our models for 2017. We then compared the model-averaged estimates with and without the non-water detections.

For each year, we estimated overdispersion ($\hat{c}$) to determine goodness–of–fit of the global POPAN model that converged well $\{\phi(g*t)\ p(g*t)\ p_{ent}(.)\}$ by using Program RELEASE. Program RELEASE performs a series of Chi-square tests to evaluate model fit, with $\hat{c}$ estimated as $\frac{\chi^2}{df}$, where χ^2 is the sum over all chi-squared tests and df is the sum of the degrees of freedom. When $\hat{c}$ was >1, we used $QAIC_c$ for model selection and $\hat{c}$ to inflate variances of parameter estimates (Burnham and Anderson 2002).

In addition to estimating population size, we estimated annual ϕ between years using the live recaptures formulation of the Cormack-Jolly-Seber (CJS) model in Program MARK. The CJS model estimates recapture probabilities (*p*) for each sampling occasion. For the CJS model, ϕ is the probability that an animal survived the time-period and was available for capture, which in this case is stayed on the study area. We merged the capture histories from the POPAN model for each deer. For each year, we binned the POPAN encounter histories; if a deer was detected ≥1 time a 1 was recorded, but if a deer was not detected for that year, a 0 was recorded. We constructed a series of models and fit them to the capture data. Because there were only 3 encounters, we could only construct very simple models. We created a total of 19 *a priori* models in which *p* and ϕ were held constant (.) or were allowed to vary by gender (*g*) and time (*t*). Program RELEASE would not work for the 3-encounter CJS data set. For the CJS model, we estimated overdispersion using the most global CJS model that converged well by using the median $\hat{c}$ simulation procedure in Program MARK.

We used likelihood-based model selection criteria (i.e., AICc; Burnham and Anderson 2002) to evaluate the candidate models for the POPAN and CJS models. To

account for model uncertainty, the annual estimates of N and ϕ were obtained by model averaging, in which each model contributed to the final estimate according to its AIC_c weight.

RESULTS

Field Sampling

We collected 548-1,232 fecal samples each year for the 3 years of the study. We subsampled in 2015 and 2016; we attempted genotyping 548-591 samples each year (Table 2.2; additional genetic results are in Appendix A). Genotyping success was high in 2015 and 2017 (87%), and low in 2016 (52%) (Table 2.2). In 2015 and 2017, sample quality was directly related to genotyping success; in 2016, sample quality was inversely related to genotyping success (Table 2.2). In total, there were 1,281 detections and 447 unique deer were sampled across the 3 years of this study (Tables 2.3 and 2.4). Over the course of this 3-year study, we collected and analyzed 346 (27%) duplicate samples. The number of detections per individual ranged from 1 to 8 in 2015, 1 to 6 in 2016, and 1 to 16 in 2017. We detected 33% of the deer in >1 year (Table 2.3). In 2016, 35 individuals were identified at the 5 new water sources. Of these, 33 (94%) were not detected at an original water site during all three years. In 2017, 15 individuals were identified at non-water sites. Of these, 7 (47%) were not detected at a water site in 2015, 2016, or 2017. Based on 2017 captures, approximately 2.8% (6/211) of deer in the study area were not detected at water sites.

Across all 447 unique deer for the three sampling seasons, the number of alleles

per locus ranged from 2 (Table 2.5; loci F, H, J, and L) to 7 (locus N). Marker failure was most commonly seen at microsatellite loci C and H, and the sexing markers, ZFX and SRY-WFL. Three of these four markers have fragment sizes of 300bp or larger (excluding SRY-WFL, which is 220bp but is located on the Y chromosome; Table 2.5), thus it is not unexpected that they would not work as well on fecal DNA, which is often degraded compared to DNA derived from tissue. No loci deviated from expectations of Hardy-Weinberg equilibrium (HWE) when data from all three years were pooled.

Population Abundance

For the most global model that would converge each year, estimates of $\hat{c}$ were <1.0 for all years, suggesting no overdispersion and no lack of fit (range 0.79-0.97). Therefore, we used AIC_c model selection for POPAN models. Each year, some POPAN models did not converge, with the convergence issues focusing p_{ent} and all estimates for the group of unknown sex. That is, models in which p_{ent} was estimated separately by sex often did not converge, and models in which ϕ, p, and p_{ent} was estimated separately for the unknown sex group did not converge. All non-converging models were deleted, and we performed model averaging on the converging models to obtain abundance estimates. The top POPAN models were different for each of the three years (Tables 2.6). Top models include combinations of variation by sex (g), sampling occasion (t), both, or was constant by sex and time for each of the model parameters (ϕ, p, and p_{ent}). None of the top POPAN models for 2015, 2016 or 2017 had time variation among sampling sessions. Although there were differences in the structure of ϕ and p_{ent} for top models (Table 2.6), estimates of movement in and out of the study area was similar for females and males,

but varied by year (Table 2.7). During the closed-capture sampling period, the probability of moving on or off the study area was lowest in 2015, highest in 2017, and intermediate in 2016 (Table 2.7). The *p*-values from Close Test also supported these results ($p = 0.056$ in 2015 and $p < 0.005$ in 2016 and 2017).

Our model averaged estimate of abundance (number of animals that ever entered the study area) for females ranged from a high of 185 in 2015 to a low of 138 in 2017 (Table 2.8). Male estimates were 11-18% lower than female estimates, but had a similar annual pattern. Males ranged from a high of 152 in 2015 to a low of 120 in 2017 (Table 2.8). Due to the small number of individuals with unknown sex, both the abundance estimates and recapture rates for that group were much lower, more variable, and had wide confidence intervals (Table 2.8 and Fig. 2.2).

The detection probability was equal for males and females for the top models in 2015 and 2016; for these years detection for the sampling sessions ranged from 0.12 to 0.42, with an overall average of 0.36 (Fig. 2.3). Detection probabilities were slightly different between the sexes and higher for 2017, ranging from 0.42 to 0.56 (Fig. 2.3). Within years, 4-10% of deer were detected at >1 water source during the 3 years of the study. Distances between water sources where deer were recaptured ranged from 3.7 to 12.9 km, with most (88%) distances <10 km (Tables 2.9-2.11). In 2016, there were also 2 individuals that were recaptured at new water sites, which were 7.9 km and 12.9 km from the water sources they were originally detected (Table 2.10). In 2017, 8 deer were detected at both a non-water site and an original water site, with distances between these recaptures of ~4 km for 7 of the recaptures and 24 km for 1 of the recaptures (Table

2.11).

Survival

Median $\hat{c}$ was 2.27 on model $\phi(t)\ p(.)$ suggesting some overdispersion. However, with only 3 encounters, overdispersion was difficult to estimate because we could not run a global model due to parameter confounding. Therefore, we did not adjust models for overdispersion. In the top CJS models (within 2 ΔACI_c units), ϕ varied by sex but there was no clear model for detection probability (Table 2.12). Apparent survival was higher for females (0.90-0.91) than for males (0.71), although the difference was not significant as the confidence intervals widely overlapped (Table 13). Precision was higher for females (CV = 8.8%) than for males (CV = 12.3-14.3%).

DISCUSSION

Our methods of using fecal DNA-based capture-recapture proved to be an effective technique for estimating abundance and survival of desert mule deer. Not only did this study provide the first abundance estimates for part of this population in over 12 years (see Marshal et al. 2006), but our estimates achieved relatively high precision and our data provided insight to movement patterns. Further, our results showed that only a small proportion of deer did not visit water, indicating that only sampling at water sources is adequate for monitoring the study area population. Survival was estimable from among year recaptures at water sources and, given the survival data, fecal DNA capture-recapture has potential for estimating recruitment and the rate of population change (λ) using the Pradel temporal symmetry model (Pradel 1996, Nichols and Hines

2002, Pradel et al. 2009). The fact fecal DNA-based capture-recapture can provide estimates of demographic vital rates in addition to abundance makes this method especially useful, because survival and recruitment provide insights to population change that are important for informing management actions.

Many studies of mule deer populations are focused on estimating deer density (i.e., estimating deer/km^2 based on the home range size of the study population [see McLean 1940, Longhurst et al. 1952, Albert and Krausman 1993, Sanchez-Rojas and Gallina 2000, Koenen et al. 2002, Martinez-Munoz et al. 2003, and Marshal et al. 2006, Furnas et al.2018]). In these cases, home range size is estimated based on movement data obtained through VHF or GPS-collared individuals. We did not have VHF or GPS collared individuals in the study area, and thus were unable to estimate the home range size of the study population for comparison with other studies. However, when estimated based on our approximated study area size (704 km^2 to 966 km^2), deer density ranged from 0.36-0.55 deer/km^2. Assuming our study area reflects the seasonal (dry season) home range of desert mule deer when they are concentrated around water, our approximate densities may be biased high compared to other studies that have reported densities based on true (yearly) home range sizes (Albert and Krausman 1993, Sanchez-Rojas and Gallina 2000, Koenen et al. 2002). These findings, biased or not, are still consistent with other studies that have shown that this is a low-density mule deer population (Celetano and Garcia 1987, Thompson and Bleich 1993, Marshal et al. 2006).

When compared to other studies that have estimated *N* of ungulates using traditional methods, our estimates from fecal DNA mark-recapture had higher precision

(CV = 7-16%). Bartmann et al. (1986) achieved CV values ranging from 22-47% for mule deer abundance using distance sampling and counts, while Conroy et al. (2014) achieved a CV of 33% for bighorn sheep abundance using aerial counts in conjunction with sightability models. Similarly, using remote camera traps and VHF-collared individuals in a nearby Mojave Desert study area, Marshal et al. (2006) produced abundance estimates of desert mule deer that achieved CV values of 32%. However, when compared to other studies that have estimated abundance using fecal DNA-based capture-recapture, our estimates achieved similar precision values (CV range = 7.4%-15% [Poole et al. 2011; Brazeal et al. 2017]). Although fecal DNA mark-recapture can yield precise estimates of abundance, the cost per precision unit, such as %CV, needs to be calculated and compared to other methods to understand its cost efficiency.

Our annual estimates of ϕ for females and males (0.90 and 0.71 respectively) were similar to Marshal et al. (2006) estimates of Sonoran desert mule deer survival (0.96 for females and 0.79 for males), which were obtained via remote camera trapping with radio-collared individuals (Marshal et al. 2006). Our estimates of ϕ for females and males achieved moderate precision (CV = 9% and 13% respectively) and were comparable to similar studies for desert mule deer using VHF collars or camera mark-resight. Other studies that have used fecal DNA-based capture-recapture to estimate survival of ungulates have achieved higher precision than our study. For instance, Hettinga et al. (2012) used fecal DNA-based capture-recapture to estimate survival of the North Interlake Woodland Caribou (*Rangifer tarandus caribou*) in Canada. This study achieved CV values as low as 6.3% for females and 8.5% for males. Similarly, Woodruff et al.

(2016) used fecal DNA-based capture-recapture methods to estimate survival of Sonoran pronghorn that achieved approximate CV values ranging from 4.2% to 6.8%. It is likely that our low precision of ϕ observed in both years (2015-2016 and 2016-2017) is attributed to the low genotyping success in 2016 (Table 2.3). We speculate that the low genotype success of 2016 caused some individuals to remain undetected throughout the sampling season, ultimately effecting both annual estimates of ϕ.

Although not part of the study design or intent, the detection of individuals at >1 water source provided insight to movement and water use patterns. Marshal et al. (2006) found that desert mule deer remained within 5 km of water sources during the dry season. Similarly, we found the maximum inter-drinker distance in which we detected an individual at >1 water source within a year was 12.9 km, and 88% of the movement distances were <10 km. Among years we detected individuals at multiple water sources up to 23.9 km apart suggesting higher dispersal rates during the hot-rainy (July-September) and cool-dry seasons (October-December) (Marshal et al. 2006). Hervert and Krausman (1986) found that when desert mule deer are denied access to traditional water sites, they traveled outside their established home range in search of water. Further, 94% of the individuals detected at the new water sources in 2016 were not detected at any of the original water sources in 2015, 2016, or 2017. Together, these findings suggest that desert mule deer tend to habitually visit the same water source, and that each water source sustains its own local sub-population of mule deer during the hot-dry season.

Rautenstrauch and Krausman (1989) documented that desert mule deer in southwest Arizona generally use the same seasonal home ranges near water sources from

year to year during the hot-dry season. However, we found that approximately 9% of the individuals detected throughout the study were detected in 2015 and 2017, but not in 2016. Since desert mule deer are known to detect and react to distant rainfall (McLean 1930, Rautenstrauch and Krausman 1989), some individuals may have changed their seasonal home range locations from year to year. We speculate that the individuals absent in 2016 likely left the study area following the monsoon season in 2015 where they established a new seasonal home range throughout the hot-dry season of 2016. It is also possible that these individuals were not detected due to the low genotyping success observed in 2016.

When compared to 2016 and 2017, there was much more precipitation throughout the dry season in 2015 (Fig. 2.2). We would have expected mule deer to be less reliant on water sources in 2015, however our abundance estimates do not reflect this assumption. Abundance estimates in 2015 were much higher than 2016 and 2017 (Table 2.8). Rautenstrauch and Krausman (1989) suggested that the concentration of deer near water sources during the hot-dry season may lead to decreased forage availability. It is possible that the rainfall throughout the dry season in 2015 may have enhanced forage quality and quantity near water sites allowing more animals to remain congregated around water sources without leaving in search of food.

Our closure tests indicated this is an open population with mule deer moving into and out of the study area between sampling occasions. We made an effort to sample all known water sources within the study area, however it is possible that there are additional water sources that we did not sample. If this is true, it is likely that individuals moving to

and from unsampled water sources are the cause of the open population and our abundance estimates may be biased low. Further, there are several water sources outside of the study area surrounding adjacent mountain ranges. It is possible that the scale of our study was too small and did not account for individuals using water sources adjacent to our study area. Expanding our study area to include water sources in nearby mountain rages could result in population closure.

Our genotyping success rates in 2015 and 2017 (87%) were higher than other studies involving ungulates (range = 33%-79% [Harris et al. 2010, Brinkman et al. 2011, Poole et al. 2011, Goode et al. 2014, Lounsberry et al. 2015, Woodruff et al. 2016, Brazeal et al. 2017, Furnas et al. 2018]). However, Hettinga et al. (2012) achieved genotype success rates of caribou as high as 93%. This is likely due to their ability to collect fresh samples (≤ 2 days old) that were preserved by freezing temperatures. It may be that hot summer conditions preserve DNA by quick drying, with a different mechanism but with similar results as DNA collected under freezing temperatures. We used identical sampling protocols from year to year, so we are unsure why we observed a particularly low genotype success rate in 2016 (52%; Table 2.2). We speculate it is likely due to DNA degradation from improper sample storage (~6 months) at a location with fluctuating temperature and humidity. This low success rate in combination with a high number of duplicate samples directly reflects the lower precision observed in our estimates from 2016 (Table 2.8). Consistent with similar studies, sample quality at the time of collection was a good indicator of genotype success rate (Goode et al. 2014, Woodruff et al. 2016). In 2015 and 2017, samples that were rated as “good” had much

higher success rate (up to 73% higher) than “fair” or “poor” samples (Table 2.2). Because results from 2015 and 2016 confirmed we could visually discern samples that had a higher probability of successfully genotyping than other samples, we focused on collecting high quality samples in 2017. This change in sampling strategy reduced the number of samples we collected in the field and reduced out field time and time spent subsampling. Moreover, precision for 2017 was the highest among our 3 study years. Based on these results, when there are a large number of samples available for collection at water sources in the desert, we recommend collecting only “good” quality samples for more cost-efficient sampling.

Duplicate samples (multiple samples from the same individual for a sampling occasion) made up a large portion of our successfully-genotyped samples. Collecting a high number of duplicate samples is inefficient because only 1 detection per individual per sampling session can be used in a capture history. The subsampling process we used in 2015 and 2016 did not reduce the proportion of duplicate samples compared to 2017, wherein we only collected visually high-quality samples and analyzed all samples collected. The ratio of duplicates to non-duplicates was similar for the 3 years of the study. Because there is no evident way to prevent or reduce the number of duplicate samples collected, we strongly recommend that future sampling designs take this into account by assuming approximately 25% of all samples collected will be duplicates.

While the number of sampling occasions, sampling intervals, and the quality of collected samples all influence precision of abundance estimates, sampling design influences their accuracy. To determine the accuracy of abundance estimates, based on

sampling solely around water sources, for the entire population of desert mule deer in an area, we recommend implementing a sampling design that includes random and representative sites throughout the study area in addition to sampling around water sources. Further, we recommend collecting only the visually highest quality fecal samples to improve sampling efficiency. Finally, we recommend collecting data or using sampling designs that allow for estimation of density. We did not have appropriate data to use spatial capture-mark-recapture (see Brazeal et al. 2017, Furnas et al. 2018) to estimate density, but we recommend evaluating this design for future use. Alternatively, the addition of GPS and/or VHF collared individuals would provide insight to movement patterns and seasonal home range size, while also allowing deer density to be estimated at a larger scale (see Rautenstrauch and Krausman 1989, Marshal et al. 2006).

MANAGEMENT IMPLICATIONS

Fecal DNA-based capture-recapture is an effective technique for monitoring abundance and survival of desert mule deer, and had high precision compared to traditional techniques. Concentrating sampling efforts at all known water sources during the summer provided an efficient way to sample nearly the entire population within our study area. If expanded to mountain ranges adjacent to our study area, fecal DNA mark-recapture can potentially provide demographic estimates and movement data at the metapopulation level. It is important to note that this method may not be the best alternative for other populations in different systems where DNA genotyping does not have as high a success rate or samples are sparse or cryptic and more time consuming to collect. We recommend using a simulation-based approach for determining which

method(s) is most cost effective (i.e., $ per/%CV) in other systems. Even though our fecal DNA-based capture-recapture population estimates were precise, it is important to note that this method does not yet provide data on disease status or the age structure of the population; these data may need to be obtained through other methods.

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TABLES AND FIGURES

Table 2.1. Microsatellite loci used for individual analysis of desert mule deer (*Odocoileus hemionus eremicus*) fecal samples from the Little Chuckwalla Mountains, California, USA, 2015-2017, with fluorescent dye labels, primer concentrations and references for the original primer publication.

Marker Name	**Reference**	**OSU Dye label**	**OSU primer concentration (uM)**
Locus M	Jones et al. 2000	6-Fam	0.05
Locus P	Jones et al. 2000	6-Fam	0.2
Locus K	Jones et al. 2000	Vic	0.125
Locus N	Jones et al. 2000	Vic	0.075
Locus D	Jones et al. 2000	Ned	0.15
Locus R	Jones et al. 2000	Pet	0.5
Locus B	Jones et al. 2000, in Pease et al 2009	Pet	0.25
Locus C	Jones et al. 2000, in Pease et al 2009	Ned	0.1
Locus F	Jones et al. 2000, in Pease et al 2009	Vic	0.1
Locus G	Jones et al. 2000, in Pease et al 2009	6-Fam	0.1
Locus H	Jones et al. 2000, in Pease et al 2009	Pet	0.2
Locus J	Jones et al. 2000, in Pease et al 2009	Ned	0.15
Locus L	Jones et al. 2000, in Pease et al 2009	6-Fam	0.25
Locus S	Jones et al. 2000, in Pease et al 2009	Pet	0.3
Locus V	Jones et al. 2000, in Pease et al 2009	6-Fam	0.05
ZFX-F+R	Aasen and Medrano 1990	6-FAM	0.2
SRY-F+R CDFW	Fain and Lemay 1995; Gilson et al. 1998	NED	0.2
SRY-F+R OSU	Fain and Lemay 1995; Gilson et al. 1999	NED	0.1

Table 2.2. Summary sample sizes and genotype success rate of desert mule deer fecal DNA study, Little Chuckwalla Mountains, California, USA 2015-2017.

Year	Sample quality	No. samples collected	No. samples analyzed	Success (%)[a]
2015	Total	1,232	591	87.0
	Good	471	290	92.9
	Fair	616	200	85.1
	Poor	145	25	58.1
2016[b]	Total	1,044	550	52.2
	Good	601	396	50.3
	Fair	385	140	56.4
	Poor	58	14	64.3
2017[c]	Total	548	548	87.2
	Good	413	413	93.0
	Fair	109	109	72.5
	Poor	10	10	20.0

[a] Genotype success rate, defined as producing a genotype at 10 or more of the 14 microsatellite loci.

[b] Includes samples from 5 new water sites.

[c] Includes samples from 6 non-water sites and does not include 16 samples with unlabeled sample quality.

Table 2.3. Number of detections and number of unique desert mule deer identified through fecal DNA analysis in the Little Chuckwalla Mountains, California, USA, 2015-2017.

Year	No. of detections	No. of individuals	Female	Male	Unknown
2015[a]	516	234	127	101	6
2016[b]	287	193	85	100	8
2017[c]	478	211	103	89	19

[a] Detections from 10 original water sites.

[b] Detections from 10 original water sites and 5 new water sites.

[c] Detections from 10 original water sites and 6 non-water sites.

Table 2.4**.** Number and sex of unique desert mule deer sampled within and across three years of fecal DNA sampling efforts in the Little Chuckwalla Mountains, California, USA, 2015-2017.

Year(s) captured	**n**[a]	**Female**	**Male**	**Unknown**
2015 only	113	52	56	5
2016 only	84	27	50	7
2017 only	102	38	45	19
2015 and 2016	39	22	17	0
2015 and 2017	40	28	12	0
2016 and 2017	27	12	15	0
2015, 2016, and 2017	42	25	17	0
Total	447	204	212	31

[a] Total number of individuals.

Table 2.5. Number of unique desert mule deer genotyped (n), allelic richness (Na), allele size range, and observed (H_O) and expected (H_E) heterozygosity values for fourteen microsatellite loci and one sexing marker analyzed in desert mule deer (*Odocoileus hemionus eremicus*) fecal samples from the Chuckwalla Mountains, California, USA, 2015-2017.

Locus	n	Na	Ho	He	Range (bp)
C	381	3	0.54	0.61	318-338
D	446	3	0.54	0.54	162-194
F	447	2	0.08	0.08	151-172
G	421	3	0.57	0.61	324-365
H	373	2	0.43	0.47	356-360
J	427	2	0.46	0.5	235-255
K	442	4	0.67	0.7	200-216
L	418	2	0.27	0.29	263-303
M	444	5	0.34	0.34	148-176
N	428	7	0.73	0.74	258-294
P	441	6	0.72	0.72	221-240
R	408	4	0.45	0.48	266-303
S	435	6	0.63	0.64	203-219
V	445	3	0.41	0.38	91-99
SRY-WFL/SRY-OSU/ZFX*	416	n/a			222 (Y chrom) 120 (Y chrom) 445 (X chrom)

* Sex identification markers.

Table 2.6. Top 10 model selection results from an open population POPAN design analysis for desert mule deer in the Little Chuckwalla Mountains, California, USA, 2015-2017. For each year, different models did not converge thus there are different sets of models for each year.

Model[a]	**K**	**AIC_c**	**Δ AIC_c**	**w_i**	**Deviance**
2015					
φ(f, m=unk(.)) p(f=m(t), unk(.)) p_{ent}(.)	13	814.804	0	0.23	-619.2
φ(m=f, unk(.)) p(t) p_{ent}(.)	12	815.305	0.5	0.18	-616.56
φ(f=m, unk(.)) p(f=m(t), unk(.)) p_{ent}(.)	13	815.639	0.8	0.15	-618.36
φ(f, m=unk(.)) p(f=m(t), unk(.)) p_{ent}(f, m=unk(.))	14	816.199	1.4	0.11	-619.95
φ(g) p(t) p_{ent}(.)	13	816.65	1.8	0.09	-617.35
φ(f=m(t), unk(.)) p(f=m(t), unk(.)) p_{ent}(.)	16	817.124	2.3	0.07	-623.35
φ(f=m(t), unk(.)) p(t) p_{ent}(.)	16	818.638	3.8	0.03	-621.84
φ(g) p(t) p_{ent}(m=f, unk) p_{ent}(fix male, unk)	13	818.719	3.9	0.03	-615.28
φ(g) p(f=m(t), unk(.)) p_{ent}(.)	13	819.062	4.3	0.03	-614.94
φ(g) p(t) p_{ent}(m=f, unk) p_{ent}(unk fix)	12	820.165	5.4	0.02	-611.7
2016					
φ(.) p(f(t)=m(t),unk(.)) p_{ent}(m, f=unk)	12	360.834	0	0.16	-370.382
φ(.) p(t) p_{ent}(m, f=unk.)	11	361.13	0.3	0.14	-367.835
φ(.) p(t) p_{ent}(f, m=unk)	11	361.377	0.5	0.12	-367.588
φ(.) p(t) p_{ent}(.)	10	361.74	0.9	0.10	-364.997
φ(f=unk, m(.)) p(t) p_{ent}(.)	11	361.897	1.1	0.10	-367.068
φ(m=unk, f(.)) p(t) p_{ent}(.)	11	362.142	1.3	0.09	-366.823
φ(.) p(t) p_{ent}(g)	12	363.356	2.5	0.05	-367.859
φ(.) p(g*t) p_{ent}(m, f=unk)	16	363.739	2.9	0.04	-376.711
φ(m=f, unk(.)) p(t) p_{ent}(.)	11	363.927	3.1	0.04	-365.038
φ(.) p(m=f, unk+t) p_{ent}(.)	11	363.954	3.1	0.03	-365.011
2017					
φ(g) p(.) p_{ent}(.)	8	508.883	0	0.22	-470.935
φ(.) p(g*t) p_{ent}(.)	17	509.173	0.3	0.19	-490.092
φ(g) p(t) p_{ent}(.)	11	509.329	0.4	0.18	-476.854
φ(g) p(.) p_{ent}(f,m=unk)	9	510.182	1.3	0.12	-471.744
φ(g) p(.) p_{ent}(f=m, unk)	9	510.947	2.1	0.08	-470.979
φ(g) p(m=f(t), unk(.)) p_{ent}(.)	12	511.128	2.2	0.07	-477.202
φ(.) p(g) p_{ent}(.)	8	512.023	3.1	0.05	-467.794
φ(g) p(.) p_{ent}(g)	10	512.271	3.4	0.04	-471.777
φ(t) p(g*t) p_{ent}(.)	19	513.487	4.6	0.02	-490.246
φ(.) p(.) p_{ent}(.)	6	514.327	5.4	0.02	-461.31

[a] Key to model notation: K = No. of parameters; AIC_c = Akaike Information Criterion corrected ; Δ AIC = difference between the model listed and the AIC_c of the best model; W_i = model weights based on model AIC_c compared to all other model AIC_c values; φ =

apparent survival; p = capture probability; pent = probability of entry; t = encounter occasion as a categorical variable; g = sex as a categorical variable; m, f, and , u = male, female and unknown sex categories respectively; "." = constant across year, encounter occasion, and sex.

Table 2.7. Within year open population model parameter of probability of remaining on the study area (φ) and probability of new individuals entering the study area (p_{ent}) during each sampling session in the Little Chuckwalla Mountains, California, USA, 2015-2017.

	2015		2016		2017	
	ϕ*	p_{ent}	ϕ*	p_{ent}	ϕ*	p_{ent}
Female	0.91	0.04	0.60	0.12	0.88	0.18
Male	0.89	0.04	0.63	0.14	0.84	0.17
Unknown	0.16	0.05	0.61	0.13	0.56	0.17

Table 2.8. Estimated population abundance of desert mule deer by sex for fecal DNA capture-recapture in the Little Chuckwalla Mountains, California, USA, 2015-2017.

		$\hat{N}$*	se	LCI	UCI	CV (%)
2015	Female	185	18.2	149	220	9.9
	Male	152	15.8	121	183	10.4
	Unknown	49	57.7	0	162	116.9
	Total	386	62.5	264	509	16.2
2016	Female	177	26.7	125	229	15.1
	Male	158	22.4	115	202	14.1
	Unknown	15	6.7	2	29	43.1
	Total	351	35.5	281	420	10.1
2017	Female	138	10.5	118	159	7.6
	Male	120	9.3	102	138	7.8
	Unknown	43	15.2	13	73	35.5
	Total	301	20.7	260	342	6.9

* Abundance estimates using open population formulation (POPAN) of capture-recapture models in Program MARK (Arnason and Schward 1995, Link and Barker 2005); N* = population of animals that entered the study area throughout the duration of the study period; se = standard error; LCI = lower 95% confidence interval; UCI = upper 95% confidence interval.

Table 2.9. Within year movement as indicated by across-drinker recaptures for desert mule deer sampled in the Little Chuckwalla mountains, California, USA, 2015. Inter-drinker distances (km) are shown above the diagonal, with pairs of drinkers that showed recaptures highlighted in grey. Number of deer sampled at each pair of drinkers is shown below the diagonal.

	BGT	**BKH**	**CKS**	**CRW**	**DDM**	**LBN**	**MYW**	**PRW**	**RNY**	**YDR**
BGT	-	12.7	25.9	14.1	20	19.2	26.8	7	23.9	34.1
BKH	2	-	15.3	7.3	7.9	12.5	21.7	15.2	12.5	27.7
CKS			-	11.9	8.2	8.9	12.7	24.5	3.7	15.2
CRW		1		-	8.3	5.8	14.7	12.8	10.8	21.3
DDM		3			-	10	18	20.7	4.8	22.5
LBN				1	1	-	9.1	16.3	9.7	15.5
MYW							-	22.2	15.6	7.5
PRW								-	23.6	29.7
RNY			5		4				-	18.9
YDR							2			-

Table 2.10. Within year movement as indicated by across-drinker recaptures for desert mule deer sampled in the Little Chuckwalla mountains, California, USA, 2016. Inter-drinker distances (km) are shown above the diagonal, with pairs of drinkers that showed recaptures highlighted in grey. Number of deer sampled at each pair of drinkers is shown below the diagonal.

	KNB	MED	BEN	BHW	BGT	BKH	CKS	CRW	DDM	LBN	MYW	PRW	RNY	YDR
KNB		10.4	18.5	26.7	28.8	16.3	8.7	16.8	8.9	16.3	21.5	29.5	6.6	23.2
MED			8.7	17.7	29.9	19.7	4.3	15.9	12.4	11.9	12.6	28.1	7.7	12.9
BEN				9.2	36.5	27.6	12.7	22.6	20.8	17.4	12.8	33.4	16.3	7.9
BHW					45.3	36.8	21.8	31.6	30	26.1	19.7	41.6	25.4	12.6
BGT						12.7	25.9	14.1	20	19.2	26.8	7	23.9	34.1
BKH							15.3	7.3	7.9	12.5	21.7	15.2	12.5	27.7
CKS								11.9	8.2	8.9	12.7	24.5	3.7	15.2
CRW									8.3	5.8	14.7	12.8	10.8	21.3
DDM										10	18	20.7	4.8	22.5
LBN							2				9.1	16.3	9.7	15.5
MYW										1		22.2	15.6	7.5
PRW													23.6	29.7
RNY														18.9
YDR		1	1								2			
CNH														

Table 2.11. Within year movement as indicated by across-drinker recaptures for desert mule deer sampled in the Little Chuckwalla mountains, California, USA, 2017. Inter-drinker distances (km) are shown above the diagonal, with pairs of drinkers that showed recaptures highlighted in grey. Number of deer sampled at each pair of drinkers is shown below the diagonal.

	CKW	**BGT**	**BKH**	**CKS**	**CRW**	**DDM**	**LBN**	**MYW**	**PRW**	**RNY**	**YDR**	**GGG**	**HHH**	**DDD**
CKW		24.9	15.8	3.6	10.8	9.9	6.4	9.2	22.6	6.5	12.7	13	9	13.2
BGT			12.7	25.9	14.1	20	19.2	26.8	7	23.9	34.1	24.5	30.4	16.6
BKH		1		15.3	7.3	7.9	12.5	21.7	15.2	12.5	27.7	22	23.8	4
CKS					11.9	8.2	8.9	12.7	24.5	3.7	15.2	16.6	12	12
CRW						8.3	5.8	14.7	12.8	10.8	21.3	14.7	17.4	7.6
DDM							10	18	20.7	4.8	22.5	20.2	18.8	4.1
LBN					2			9.1	16.3	9.7	15.5	10.2	11.6	11.5
MYW							2		22.2	15.6	7.5	5.2	4.4	20.3
PRW										23.6	29.7	19	26.2	18.3
RNY				2		1	1				18.9	18.8	15.4	8.8
YDR								5				11.7	3.9	25.6
GGG													9.4	21.6
HHH			1								5			21.8
DDD			2											
EEE														

Table 2.12**.** Model selection results from a Cormack-Jolly-Seber design analysis for desert mule deer in the Little Chuckwalla Mountains, California, USA, 2015-2017.

Model	K	AIC_c	ΔAIC_c	w_i	Deviance
φ(g) *p*(.)	4	723.302	0	0.418	16.094
φ(g) *p*(t)	5	724.838	1.5	0.194	15.583
φ(g) *p*(m=f,u)	5	725.112	1.8	0.169	15.856
φ(g) *p*(g)	6	726.667	3.4	0.078	15.354
φ(g*t) *p*(.)	7	728.437	5.1	0.032	15.057
φ(m=f,u) *p*(g)	5	728.597	5.3	0.03	19.341
φ(t) *p*(g)	5	729.154	5.9	0.022	19.898
φ(m=f,u) *p*(.)	3	729.82	6.5	0.016	24.65
φ(f=u,m) *p*(f=u,m)	4	731.232	7.9	0.008	24.024
φ(m=f,u) *p*(m=f,u)	4	731.61	8.3	0.007	24.402
φ(m=f,u) *p*(t)	4	731.62	8.3	0.007	24.412
φ(g) *p*(g*t)	9	731.773	8.5	0.006	14.229
φ(g*t) *p*(g)	9	731.92	8.6	0.006	14.377
φ(m=f, u(t)) *p*(.)	5	733.562	10.3	0.002	24.307
φ(.) *p*(.)	2	734.263	11	0.002	31.121
φ(m=f, u(t)) *p*(m=f, u)	6	735.352	12	0.001	24.039
φ(m=f,u) *p*(m=f,u)	6	735.352	12	0.001	24.039
φ(t) *p*(.)	3	736.155	12.9	0.001	30.985
φ(.) *p*(t)	3	736.155	12.9	0.001	30.985

[a] Key to model notation: K = No. of parameters; AIC_c = Akaike Information Criterion corrected ; Δ AIC = difference between the model listed and the AIC_c of the best model; W_i = model weights based on model AIC_c compared to all other model AIC_c values; φ = apparent survival; p = capture probability; t = encounter occasion as a categorical variable; g = sex as a categorical variable; m, f, u = male, female, and unknown sex categories respectively; "." = constant across year, encounter occasion, and sex.

Table 2.13. Annual apparent survival probability (ϕ) of desert mule deer in the Little Chuckwalla Mountains, California, USA, from 2015 to 2016 and 2016 to 2017.

Sex	Year	ϕ	SE	LCL	UCL	CV (%)
Female	2015-2016	0.90	0.08	0.59	0.98	9
Male	2015-2016	0.71	0.09	0.51	0.85	13
Unknown	2015-2016	0.25	0.20	0.04	0.74	81
Female	2016-2017	0.91	0.08	0.58	0.99	9
Male	2016-2017	0.71	0.10	0.50	0.86	13
Unknown	2016-2017	0.25	0.21	0.04	0.74	82

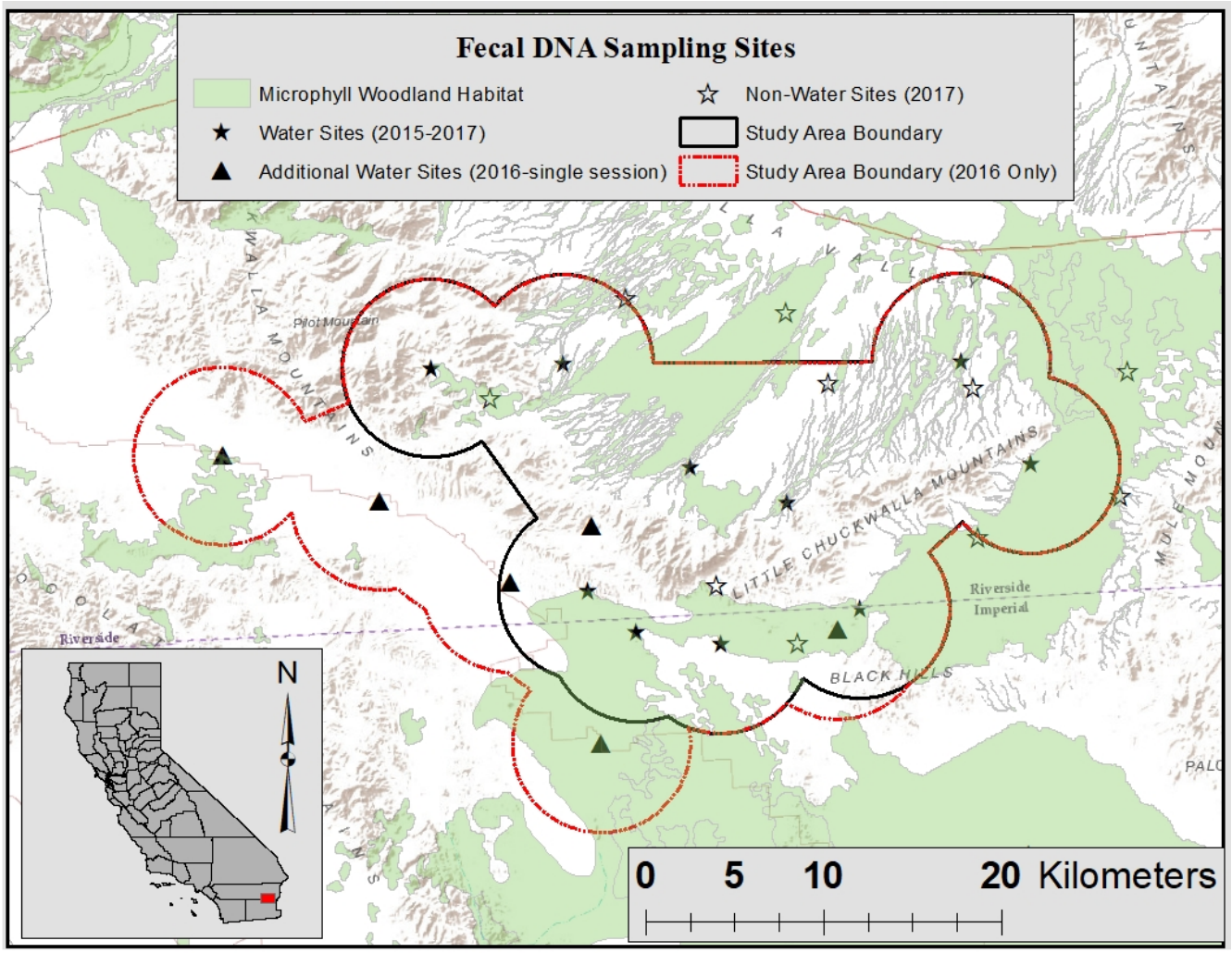

Fig. 2.1. Desert mule deer fecal sampling locations in 2015 -2017 (stars), in 2016 at new sites (triangles), and in 2017 at non-water sites (hollow stars) in the Little Chuckwalla Mountains, California, USA.

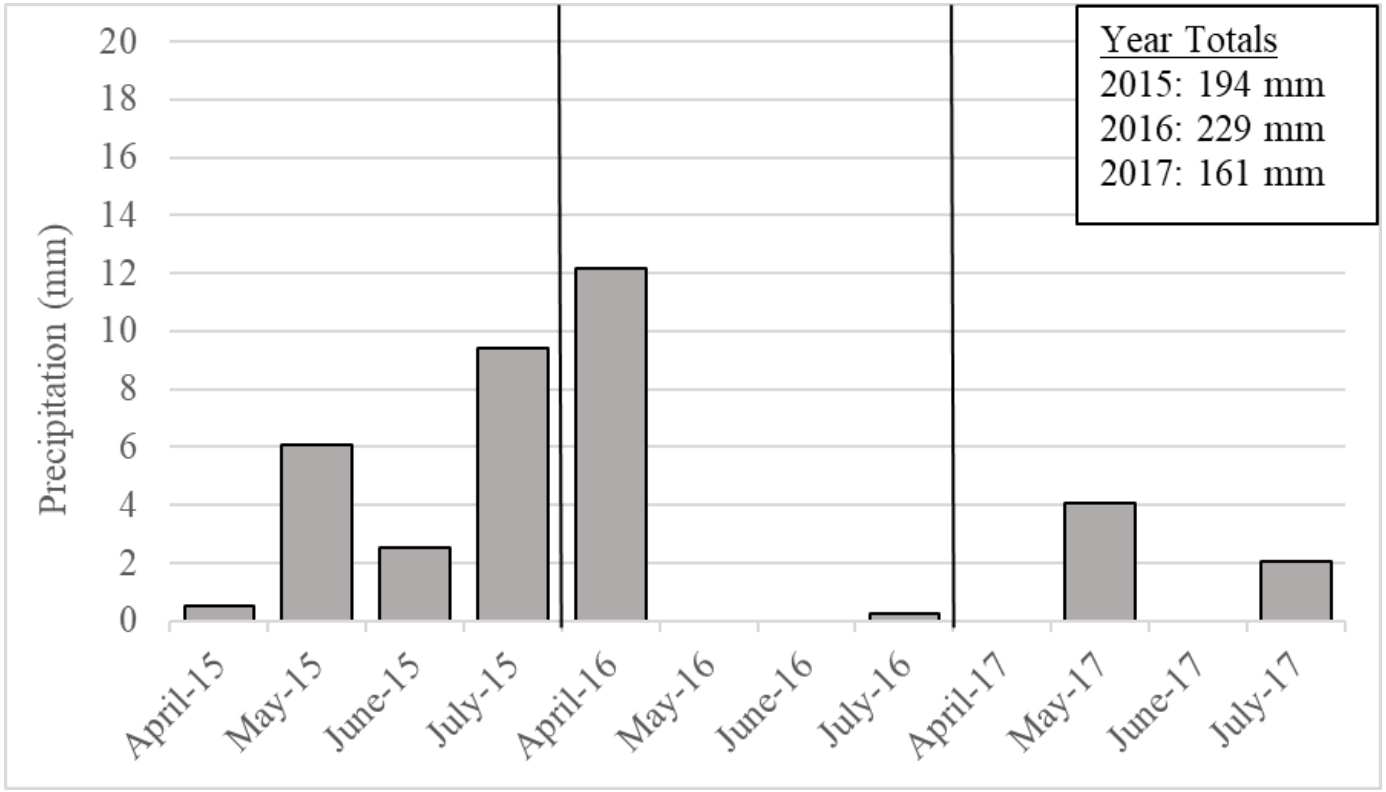

Fig. 2.2. Dry season (April through July) and yearly precipitation (mm) in Rice Valley, CA, USA, 2015-2017 (WRCC 2019).

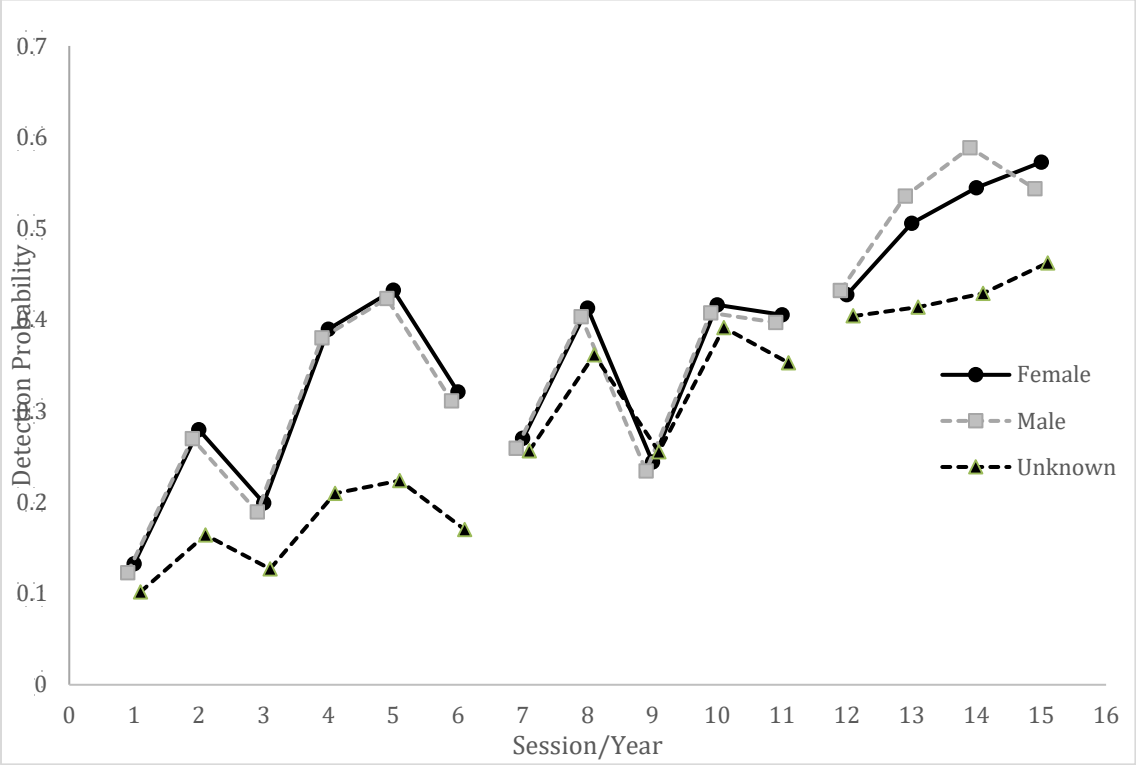

Fig. 2.3. Model-averaged detection probability (p) by sex and session of desert mule deer in the Little Chuckwalla Mountains, CA, USA, 2015-2017. Confidence intervals not shown for clarity.

CHAPTER 3

COMPARING TECHNIQUES FOR MONITORING DESERT BIGHORN SHEEP[21]

ABSTRACT

Wildlife management is often driven by the need to estimate population abundance because it provides crucial information needed to make well-informed management decisions. However, obtaining these estimates can be difficult and costly, particularly for small, spatially clustered populations with wide distributions. For this reason, DNA surveys and capture-recapture modeling has become increasingly common in areas where direct observation is consistently difficult or counts are small or variable. We compared the precision and cost effectiveness of two methods used to estimate abundance of desert bighorn sheep populations: traditional ground-based mark-resight, and newer fecal DNA-based capture-recapture. During the dry seasons (June-July) of 2016 and 2017, we collected fecal samples to estimate abundance of bighorn sheep visiting water sources in the Marble Mountains located in the Mojave Desert of southeastern California. Concurrently, we also conducted annual ground-based mark-resight surveys to estimate abundance. Our population abundance estimates from fecal DNA-based capture recapture achieved much higher precision (CV=5.1%-6.5%) than our estimates derived from ground-based mark-resight (CV= 20.5%-55.6%). We compared costs between the 2 methods for our study and using simulations for a variety of sampling scenarios that were possible for our study system. Our simulations indicated that, for a

[1] Coauthored by Mary Conner, Jane McKeever, Tom Stephenson, Dave German, Rachel Crowhurst, and Clinton Epps.

population of similar size (100), 2 visits, and resight probability (0.20), which represents field-based estimates from our study on bighorn sheep in the Marble Mountains, a CV of 12% was as low as could be reasonably obtained for mark-resight. Based on our simulations, we predict the cost of abundance estimates for this level of precision (CV = 12%) based on fecal DNA- capture-recapture would be 28% that of ground-based mark-resight (i.e., a 72% reduction in cost). The application of fecal DNA-based capture-recapture is a highly cost-effective alternative for estimating abundance of relatively small populations of desert bighorn sheep. The integration of simulated study designs with cost analyses provides wildlife management with a tool to identify the most effective method for estimating abundance over a wide variety of potential sampling scenarios.

INTRODUCTION

Wildlife management is often driven by the need to estimate population abundance (*N*) because it provides crucial information needed to make well-informed management decisions. However, obtaining these estimates can be difficult and costly, particularly for small, spatially clustered populations with wide distributions. In these situations, standard wildlife sampling designs are neither cost nor labor efficient because they often yield low detection probabilities, which in turn, yield poor estimates of *N* (Marshal et al. 2006).

Desert ungulate populations are typically monitored using traditional techniques that include helicopter surveys, ground mark-resight surveys, radio-telemetry, and GPS-collared individuals (Koenen et al. 2002, Krausman et al. 2006, Marshal et al. 2006,

Woodruff et al. 2016). These techniques are often used to monitor desert bighorn sheep populations (Cain et al. 2008, Bleich et al. 2010, Conroy et al. 2018). These traditional techniques have proven to be an effective way to estimate abundance, but may have drawbacks in terms of cost, efficiency and precision. Aerial counts can lead to statistical uncertainty due to incomplete spatial and temporal coverage and low or varying sightability during the survey (Conroy et al. 2014; 2018). Even though varying levels of sightability during aerial counts can be accounted for, there is often statistical uncertainty in sightability estimates, which in turn leads to considerable statistical uncertainty in $\hat{N}$ (Conroy et al. 2014). Given low population densities and large survey areas of desert ungulates, ground surveys often yield low return per unit effort (Thompson and Bleich 1993). Aerial and ground mark-resight surveys require animals be captured and collared which can cause unintended stress the animals (Jacques et al. 2009) and is often associated with high costs. However, captures also provide opportunities to collect additional data (i.e., disease and body condition) that are not easily obtainable though other methods.

Over the past 20 years, non-invasive approaches such as remote camera traps and collection of genetic samples through feces or hair have become quite common (Waits 1997, Waits and Paetkau 2005, Marshal et al. 2006, Kendall et al. 2008, Brinkman et al. 2011, Brazeal et al. 2017). Identification of unique individuals from non-invasive samples using DNA microsatellites has become common in the field of wildlife management (Lukacs and Burnham 2005) and can be used in mark-recapture models for estimating N. The use of non-invasive genetic sampling (NGS) is appealing because the

animals do not need to be captured, handled, or even seen (Taberlet et al. 1999). Further, non-invasive approaches typically have higher capture probabilities compared to conventional mark-recapture techniques and eliminate the effects of marking individuals in a population (Mills et al. 2000). The increasing use of non-invasive genetic sampling for wildlife populations has led to advances in field study design for DNA-based mark-recapture studies (Boulanger et al. 2004, 2008, Rutledge et al. 2009) and the ability of analytical models to account for uncertainties from both field and laboratory procedures (Lukacs and Burnham 2005, Knapp et al. 2009) continue to develop. These non-invasive methods have been successfully used to estimate *N* of desert ungulates (Woodruff et al. 2016). It is unknown how these DNA-based techniques compare to traditional monitoring strategies in desert ecosystems in terms of efficiency (i.e. cost per individual monitored) and precision of abundance estimates (*N*).

Desert bighorn sheep (*Ovis canadensis nelsoni*) are desert-dwelling ungulates that are patchily distributed throughout desert "sky island" mountain ranges with small and isolated populations throughout the Sonoran, Mojave, and Great Basin deserts of the southwest United States (Epps et al. 2004). The development of an effective tool that provides precise estimates of *N* would allow wildlife managers to monitor population trends, set practical harvest quotas, and potentially evaluate impacts of disease and solar development in the desert (Lovich and Ennen 2011, Lutz et al. 2011). Currently, California Department of Fish and Wildlife (CDFW) monitors desert bighorn sheep populations in 13 ranges within the Mojave and Sonoran deserts of southern California, primarily in hunted populations to ensure harvest quotas are allocated sustainably.

Beginning in 2013 in the Marble Mountains, which is one of the historically monitored ranges, ewes and rams were collared with marked VHF and GPS collars to monitor adult survival rates and serve as marks for mark-resight surveys. Beginning in 2015, annual ground counts in May and June were conducted to estimate *N* and monitor lamb survival (Prentice et al. 2018). The consistent monitoring of this herd over multiple years created a unique opportunity to compare mark-resight and DNA-based techniques for monitoring *N*.

The main objective of this study was to compare the precision and cost effectiveness of traditional ground-based capture-recapture methods with fecal DNA-based capture-recapture methods to estimate *N* for desert bighorn sheep populations. To this end, we designed and conducted a fecal DNA-based capture-recapture study to estimate abundance for the bighorn sheep population in the Marble Mountains and compare to ground-based mark-resight estimates that were conducted during the same time period. Our second objective was to use our empirical data and simulations to compare cost per percent precision between the 2 methods for a range of potential study designs. This information allows wildlife conservation biologists and managers to determine which method is the most cost efficient for achieving monitoring goals and provide a general framework for cost/precision comparisons for similar study designs.

STUDY AREA

The study area is located within the northern portion of the Marble Mountains, just outside the southern border of the Mojave National Preserve of southeastern California (Fig. 3.1). The range is bordered by Interstate Highway 40 to the north,

Kelbaker Road to the west, and U.S. Route 66 to the south. Valleys composed of expansive alluvial fans isolate the study area from other ranges (Epps et al. 2005, Cain et al. 2008). The area is composed of steep, rocky mountains, rolling foothills, and washes. Elevations range from 350 m to 1150 m above sea level. Daily maximum temperatures during the dry seasons (June-July) of 2016 and 2017 ranged from approximately 38° C to 40° C (WRCC 2018). The Mojave Desert is characterized by a bimodal pattern of average monthly precipitation (Wehausen 1992) with most precipitation occurring in January and during the monsoon season of July and August. Rainfall at the Granite Mountains weather station (approximately 8 km north of the study area) during the dry season (April through July) was 69 mm and 2 mm in 2016 and 2017 respectively (Fig. 3.2) (WRCC 2018). The vegetation community is dominated by shrub species including creosote bush (*Larrea tridentata*), brittlebrush (*Encilia farinosa*), burro weed (*Ambrosia dumosa*), white ratany (*Krameria grayi*), eastern Mojave buckwheat (*Eriogonum fasciculatum*), Mormon tea (*Ephedra viridis*), cat claw (*Acacia greggi*), desert lavender (*Hyptis emoryi*), burrobush (*Hymenoclea salsola*), and bush groundsel (*Senecio douglassi*) (Wehausen 1992). Barrel cactus (*Ferocactus cylindraceus*) is locally abundant in some areas as well.

Past management efforts in desert ecosystems have focused on the establishment of artificial water sources (guzzlers) to sustain big game populations during summer months when rainfall is scarce and daily temperatures are high. Past observational studies have found that bighorn sheep limit their distribution to <4 km from water sources during the summer, especially for females that are subject to gestation and lactation (Cain et al.

2008). Because bighorn sheep congregate around available water sources during the dry season (Turner and Weaver 1980, Ordway and Krausman 1986, Andrew et al. 1997, Andrew and Bleich 1999, Bleich et al. 2010), we used the 3 functioning water sources in the study area as focal sampling sites for fecal DNA.

METHODS

Field Methods

During the summers of 2016 and 2017 we implemented ground mark-resight surveys and fecal DNA-based capture-recapture surveys to estimate abundance of bighorn sheep in the Marble Mountains. Closure tests via program CloseTest (Stanley and Burnham 1999) failed to reject the null hypothesis, indicating this population is closed to immigration and emigration between sampling sessions. Because ground mark-resight surveys and fecal DNA sampling sessions were not concurrent, the finding that this population is closed allowed us to assume that the same population was surveyed for each method. Since lambs are not marked (i.e. GPS/VHF collar, ear tags) only adults and yearlings (i.e. animals ≥1 year old) were used for ground mark-resight estimates. Similarly, we made an effort to sample only fecal pellets deposited by adults. Pellets deposited by lambs were determined in the field based on size. In general, lambs deposit very small pellets compared to those of adults. However, pellet size can vary by individual, so it is possible that some lambs were included in our fecal DNA-based capture-recapture estimates.

We conducted ground mark-resight surveys once in May and once in June of

2016 and 2017 when most lambs are approximately 3 months old and easy to identify. The number of live marks with functioning collars in the population was verified before each survey via telemetry using either GPS or VHF collars. Surveys were conducted along 4 routes (Fig. 3.1), which together encompassed the entire survey area. We surveyed each route with groups of 1-3 people. All bighorn sheep were identified in the field by age category and sex: adult ewe, adult ram, yearling ewe, yearling ram, lamb, or unclassified. Marked individuals were identified by verifying GPS/VHF collar make and color and ear tag color combinations.

We collected fecal DNA samples during the dry seasons of 2016 and 2017. Sampling occurred during June and July, which is the hottest and driest time of year when bighorn sheep are found closest to water sources (Ordway and Krausman 1986, Bleich et al. 2010). At each water source, sampling was focused on 3-4 transects delineated along established game trails (Fig. 3.1). During a preliminary site visit, each 250 meter-long transect was marked with fluorescent tape. All existing pellets were removed during a preliminary site visit to prevent collection of old samples during the first sampling session; the first sampling session began 4-6 days after the preliminary site visit. There were 6 sampling occasions in 2016 and 4 sampling occasions in 2017. Intervals between sampling occasions ranged from 3 to 6 days for each field season. In addition to samples collected at water sites, we also collected samples during ground mark-resight surveys which occurred in both May and June of each year. These samples were grouped into a single sampling occasion as described below. Based on survival and movement data collected in the past (CDFW, unpublished data), as well as closure test

results from POPAN, we assumed demographic and geographic closure (i.e., no births, deaths, immigration, or emigration) throughout the duration of our field sampling for each year.

Sampling was performed by collecting every pile of fecal pellets visible from the transect center line. Pellets that appeared to be degraded or from more than one individual were discarded. Each sample consisted of 15 to 20 fecal pellets and were placed in small paper bags. Remaining pellets from each pile were covered with sand to prevent resampling during future occasions. Each sample bag was labeled with the GPS location, date, sample identification number and collectors initials.

Genetic Analyses

All samples underwent DNA extraction and analysis in the Epps Population Genetics Laboratory at Oregon State University (OSU). DNA was obtained using the pellet-scraping method (Wehausen et al. 2004) to collect epithelial cells from the exterior surface of pellets. DNA was extracted from pellet scrapings using a modified AquaGenomic Stool and Soil protocol (MultiTarget Pharmaceuticals LLC, Colorado Springs, CO). Modifications included the addition of 450 µL of AquaGenomic solution to pellet scrapings, the use of 1.0 mm silica/zirconium beads (BioSpec Products Inc., Bartlesville, OK) for cell lysis, and the addition of 12 mAU proteinase K (Qiagen Inc., Valencia, CA) for recovery of mitochondrial DNA. Lastly, 150 µL of AquaPrecipi solution (MultiTarget Pharmaceuticals) was added to cell lysate to remove PCR inhibitors present in fecal samples.

We attempted to amplify ten markers (9 microsatellites plus one marker for sex

identification) for each sample using the polymerase chain reaction (PCR). The primer pair used for sex identification amplifies the amelogenin gene located on both the X and Y chromosomes (Yamamoto et al. 2002); in bighorn, the Y chromosomal fragment is characterized by a 44bp deletion relative to the X chromosome (214bp versus 258bp). These 10 markers have been used to characterize genetic structure and identify individuals in populations of desert bighorn sheep (Epps et al. 2018) and were expected to have sufficient power to resolve individuals within the Marble Mountains (Table 3.1). Samples were initially amplified in three separate PCR reactions each; those that produced ≥3 alleles at any locus were considered contaminated (e.g., by accidently collecting pellets from more than one individual in a single sample) and not rerun (n=13). Samples that produced partial genotypes at ≥50% of the microsatellite loci were rerun three more times. For a genotype to be accepted for a particular locus, each allele in a heterozygous genotype had to be observed twice, while the single allele in a homozygous genotype had to be observed three times.

Using the online individual-identification program CERVUS version 3.0.3. (Kalinowski et al. 2007) and the population-specific allele frequencies tabulated for this population, we estimated the cumulative probability of identity for unrelated bighorn sheep (P_{ID}) and for siblings or parent-offspring pairs (P_{IDsibs}) for all 9 microsatellites. We then recalculated P_{ID} and P_{IDsibs} with successively reduced numbers of loci, to determine the minimum number of loci required to identify individuals. When identifying unique individuals, we used a maximum P_{ID} of $1x10^{-4}$ and P_{IDsibs} of $1x10^{-2}$, necessitating that all samples be genotyped at ≥7 of the 9 microsatellite loci.. We analyzed all samples as one

population and searched for recaptures across all three water sources and the survey transect. After identifying recaptures within the 2017 samples, we compared those unique individuals against genotypes from bighorn sampled in 2016. GenAlEx (Peakall and Smouse 2006, 2012) was used to calculate number of alleles, expected and observed heterozygosities for each locus, and to test whether loci were in Hardy-Weinberg equilibrium.

Abundance Estimation

Using count data on marked and unmarked bighorn obtained through ground mark-resight surveys, we used the two-sample Lincoln-Peterson bias-corrected estimator to estimate $\hat{N}$ and its variance (Williams et al. 2001). All adult and yearling ewes were grouped into the total female populations and all adult and yearling rams were grouped into the total male population.

Using data derived from our fecal DNA samples, we estimated $\hat{N}$ using a robust Huggins formulation of closed capture-recapture models in Program MARK (White and Burnham 1999, White et al. 1999). DNA samples that successfully amplified were considered detections. We created capture histories for each uniquely identified bighorn sheep encountered during the study. Each individual was either detected (1) or not detected (0) in each sampling occasion. Only a single detection per individual per sampling occasion was counted. All samples collected during ground surveys were counted as a single additional sampling occasion at the end of each year. Because the order of sessions only affects estimates if a behavioral response is present in the data and we expect no such effect, this approach is permissible for our application (Boulanger et

al. 2008, Kendall et al. 2008). Water site detections in 2016 and 2017 were entered as sessions 1-6 and 8-11 respectively. Detections from samples that were collected during ground surveys (opportunistically) were entered as sessions 7 and 12 for 2016 and 2017 respectively. The sex of each bighorn sheep was entered as a group covariate. We used likelihood-based model selection criteria (i.e., AICc; Burnham and Anderson 2002) to evaluate a suite of candidate models. Models were chosen to account for potential variability of survival and encounter probability by sex, sampling occasion, and year. In particular, to account for differences in capture probabilities due to environmental changes during the study period, such as temperature differences among sampling occasions, we constructed a model that allowed capture probabilities to vary (categorically) by sampling occasion. Because of the non-invasive nature of collecting fecal samples, we assumed a behavioral response (i.e. trap happy, trap shy) was unlikely. However, a different recapture from initial capture probability (modeled by the behavioral response models) can occur through shifts in space use during the study period; we constructed models to test this assumption. Because there was model variation, we used model averaging (Lukacs and Burnham 2005, Doherty et al. 2012) to estimate *N* by sex.

We recognize the different water and space use patterns among individuals can induce capture heterogeneity, and capture heterogeneity based on differences in individuals is a concern when estimating *N* with closed-capture mark-recapture models (Pollock et al. 1990, Boulanger et al. 2004, Pederson et al. 2012). We tested the importance of heterogeneity by reconstructing the top model(s) (models within 2 ΔAICc

units from top model) with heterogeneity included using the Huggins-Pledger closed-capture full heterogeneity model with a mixture of 2 capture probabilities (Pledger 2000).

Cost Comparison

Using simulations together with empirical cost and mark-resight data, we compared the efficacy of traditional ground mark-resight and fecal DNA capture-recapture techniques by estimating the cost and coefficient of variation (CV) of $\hat{N}$ for each method and sampling scenario. Costs for each method were broken down into 2 categories: overhead costs, and costs per visit (Tables 3.2 and 3.3). The cost per visit for each method included all field work-based costs for performing a ground survey or sampling occasion. This includes all wages and travel expenses associated with one visit. Overhead cost for ground mark-resight included all costs associated with capturing and marking individuals in the population. The number of marked individuals available for resighting needs to be known for ground mark-resight methods (McClintock et al. 2009), so we calculated costs based on marking animals with VHF collars, even though many of the animals were wearing GPS collars. Here, we only consider the case where collars are used for estimating abundance. However, if the collars are used for other reasons such as survival and movement, the additional costs for the marks could be greatly reduced.

We used a constant price per animal captured and marked amortized over the life of each collar. Overhead costs for fecal DNA capture-recapture included all costs associated with lab work and DNA extraction, using a constant price per sample. Specifically, this cost includes DNA extraction and genotyping at 9 microsatellite loci and one sexing marker with a minimum of 3 replicate PCRs per sample. Once all costs

associated with each method were determined, we used the simulation feature in Program MARK (White and Burnham 1999, White et al. 1999) to determine CV($\hat{N}$) for different sampling scenarios by method. The total cost of each scenario was calculated by adding the total visit cost (visits * cost/visit) to the overhead cost of marking animals in the population (marks * cost/animal marked). The cost/animal marked was calculated using the 5-year factory-provided life expectancy of VHF collars (Telonics MOD-315 and ATS M2230), set at 60 bpm.

Using costs and simulation outputs from Program MARK, we compared the efficacy of both fecal DNA capture-recapture and traditional ground mark-resight by evaluating cost and precision (CV) of each method for various sampling scenarios. We chose the sampling scenarios to bracket the range of sampling intensities commonly used for estimating abundance for small populations. For the ground mark-resight simulations, we used a logit normal mark-resight model (McClintock et al. 2009). Simulation scenarios (n=720) were constructed with similar inputs as for the fecal DNA. That is, we used varying values of sampling occasions (3-6), population size (100-300), and resight probability (0.15-0.30), with an additional input variable that included the number of marks in the population (10-50). Using cost and simulation data we compared the efficacy of both fecal DNA capture-recapture and traditional ground mark-resight by estimating cost and precision (CV) for each scenario and method.

For the fecal DNA simulations, we used the full likelihood formulation of a closed-capture model. We constructed simulation sampling scenarios (n=144) with varying sampling occasions (3-6), population sizes (100-300), and detection and

redetection probabilities (0.15-0.30), with detection probability set equal to redetection probability. We estimated the CV($\widehat{N}$) for different sampling scenarios that represent sampling designs commonly used for estimating abundance for small populations. The theoretical number of samples required for each simulation was estimated as the product of the number of sampling occasions, populations size, and detection probability. For example, for a simulation with 4 visits, a population size of 200, and detection probability of 0.20, approximately 160 samples would be needed to produce the estimated standard error (SE) for that specific simulation scenario. To correct for sample genotype failure, we divided the theoretical sample number by our observed genotype success rate in the lab (98%). The total cost of each simulation scenario was then calculated by adding total visit costs (visits * cost/visit) to total sample costs (samples * cost/sample).

RESULTS

Abundance Estimation

Ground mark-resight surveys produced minimum counts of 147 individuals in 2016 and 133 individuals in 2017. In 2016, 17 females and 6 males in the population were marked; in 2017, 9 females and 6 males were marked. Mark-resight estimates varied widely between sexes and years; estimates of *N* were 20-40% higher for males than females, and $\widehat{N}$ dropped by 27% and 45% for females and males respectively (Table 3.4). However, the patterns were not statistically significant because precision was low for estimates for both sexes. The CV was lower for females (21-23%) than for males (29-56%) during the study (Table 3.4).

From fecal DNA collections, we submitted 356 and 277 samples for genotyping in 2016 and 2017 respectively; genotype success rates were ≥97% for both years (Table 3.5). A total of 141 and 107 unique individuals were identified in 2016 and 2017 (Table 3.5). In total, 171 unique bighorn sheep were detected by fecal DNA across the two years (104 females, 67 males), with 77 individuals detected in both years (50 females, 27 males). In 2017, 30 new individuals were detected that had not previously been identified in 2016 (16 females, 14 males). The number of alleles per locus ranged from 3 (loci OarFCB304 and MAF33; Table 3.6) to 7 (locus OarHH62). Amplification failure was low, but most commonly seen at locus AE129 (Table 3.6). After removing all recaptures from the data set, no locus showed significant departure from Hardy-Weinberg equilibrium.

In 2016, 90 individuals were redetected (detected 2-8 times each) and 51 were detected only once. Seventy-three unique individuals were identified from samples collected at water sources and 17 individuals were identified from samples collected during ground surveys. However, most of the unique individuals (98%) detected from samples collected during ground surveys were also detected at water sources surveys; only 4 animals were detected solely during ground surveys.

In 2017, 72 individuals were redetected (detected 1-9 times each) and 35 were detected only once. Seventy-seven of these individuals were also detected in the 2016 sampling period. One-hundred-two unique individuals were identified from samples collected at water sources and 20 individuals were identified from samples collected during ground mark-resight surveys. However, most of the individuals (95%) detected

from samples collected during ground surveys were also detected at water sources; only 5 individuals were detected solely during ground surveys.

Our top model (w_i = 0.515) indicated survival (*S*) varied by sex, and detection probability (*p*) varied by year and sampling occasion and was equal to redetection probability (*c*) (Table 3.7). However, the second-best model (w_i = 0.397) included sex as constant, indicating little difference in survival between the sexes. We constructed models that included heterogeneity for the top 2 models, which had ≥95% of model weight (Table 3.7). The models with heterogeneity did not perform well compared to their counterparts without heterogeneity; the 2 models with heterogeneity were ≥16.4 ΔAICc units down from the top model.

Detection probabilities from samples at water sources ranged from 0.16 to 0.33 (mean = 0.24, 95% CI 0.18-0.31) for 2016 and from 0.18 to 0.52 (mean = 0.37, 95% CI 0.28-0.46) in 2017. Detection probabilities from samples collected during ground surveys were lower than those collected from water sources (mean = 0.10, 95% CI 0.06-0.16 in 2016 and mean = 0.16, 95% CI 0.11-0.25 in 2017). Because we only had 2 years of capture data, we were not able to produce reliable estimates of *S*, immigration, or emigration.

Our model-averaged estimates from the fecal DNA capture-recapture indicated that there was an approximately 30% decline in abundance from 2016 to 2017 (Table 3.4). For both years, there were 1.7 times more females than males in the population (Table 3.4). Precision values (CV) for both sexes were high for both years; the CV ranged between 5.1%-6.5% (Table 3.4).

Costs

The total cost for capturing and marking 10 bighorn sheep was $23,211 (Table 3.2). This cost includes all costs associated with capture crew expenses (i.e. flight time, daily rate, fuel truck), collars, travel, accommodations, an on-site veterinarian, as well as the costs associated with a 15-person basecamp for animal processing. With 10 marks in the population each lasting 5 years, the calculated cost/animal captured and marked was $464/animal (Table 3.2). The cost for a single ground mark-resight survey was estimated to be $3,108/visit (Table 3.2). This includes all travel expenses, per diem, and wages for 6 technicians to perform an 8-hour survey.

The total cost of obtaining $\hat{N}$ using fecal DNA capture-recapture in 2016 and 2017 was $21,872 and $16,406 respectively (Table 3.3). The costs varied between years because there were more visits (6 versus 4) and samples analyzed (356 versus 277) in 2016 compared to 2017. The cost for a single visit was $916/visit for both years. This cost includes technician wages, per diem, and round-trip travel expenses for two technicians traveling from the CDFW Bishop field office (600 miles round trip). Processing and genotyping of any feces collected during these surveys was an additional cost at $46 per sample.

Simulations

Simulated outputs for each scenario included $\hat{N}$ and its SE, from which we estimated CV. Overall, simulations indicated that cost is highly related to the CV for both methods, with the cost per percent CV increasing non-linearly as the CV decreased (Tables 3.9 and 3.10). For example, for ground-based mark-resight surveys, the cost

increases by 3.2x to reduce the CV from 26 to 13%, a 50% reduction. For ground mark-resight, our simulations indicated that the number of marked individuals and number of visits had a large impact on precision (Table 3.8, Fig. 3.3). However, it was relatively less expensive to reduce the CV by doubling the number of collars rather than doubling the number of visits. For example, it was 40% less expensive to improve the precision from a CV of 42% to 26% by doubling the number of collars (10 to 20) compared to doubling the number of visits (3 to 6). Additionally, simulations showed that a low CV (i.e. CV < 20%) is not achievable with a low number of marks in the population, regardless of population size, detection probability, or number of visits (Fig. 3.3). In circumstances in which the number of marked individuals is low, precision is highly dependent upon both detection probability and number of visits. For small populations (i.e., n = 100), a relatively high detection probability ($p \geq 0.25$), number of visits ($n \geq 6$), and number of marks in the population ($n \geq 35$) are required to achieve high precision (CV ≤10%) with ground mark-resight surveys. The scenario that achieved the highest precision (CV=6%) had a simulated population size of 100, a detection probability of 0.30, 6 visits, and 50 marked individuals, which yielded an estimated cost of approximately $42,000.

For fecal DNA-based capture-recapture, precision was higher for larger populations for all simulated scenarios (i.e., combinations of detection probability and number of visits) (Fig. 3.4). Precision can be greatly increased by adding additional visits; in a simulation with population size of 100, CV decreased from 27% to 15% with the addition of a single visit (Table 3.9). The estimated cost for this 11.7% increase in precision was $1,855.

Simulations for fecal DNA-based capture-recapture indicated that costs were lower for a given precision level (CV%) compared to the ground mark-resight methods (Fig. 3.5). For example, to achieve a CV of approximately 12% under the scenarios in Tables 3.9 and 3.10, which were similar to our observed field sampling conditions, it would cost $32,535 or $2,722/CV% for mark-resight and $9,274 or $792/CV% for fecal DNA. That is, for what we consider a desirable and attainable sampling design and precision level, the overall cost would be ~$23,000 less (72% lower) for fecal DNA compared to mark-resight.

DISCUSSION

Our method of using DNA-based capture-recapture with desert bighorn sheep fecal DNA provided abundance estimates with higher precision and at a lower cost than traditional ground mark-resight surveys. Previous studies have demonstrated the ability of fecal DNA-based capture-recapture to provide abundance and density estimates when applied to wild ungulate populations (Brinkman et al. 2011, Goode et al. 2014, Lounsberry et al. 2015, Woodruff et al. 2016, Furnas et al. 2017). However, few studies have evaluated the cost effectiveness (i.e. cost/level of precision) of this method when compared to traditional methods of estimating abundance (Poole et al. 2011, DeMay et al. 2015, Janecka et al. 2011). In this study, we had the opportunity to compare both methods and their associated costs and precision.

The application of fecal DNA-based capture-recapture to estimate *N* of ungulates that inhabit desert environments is likely to be a cost-effective alternative to traditional approaches. However, in cooler climates where artificial water sources are not in use, and

highly concentrated sampling sites are not available, fecal DNA-based capture-recapture may be more costly (Poole et al. 2011). In addition, if collars are deployed for other reasons, such as monitoring survival or spatial movements or habitat use, then the cost per collar could be reduced for ground based mark-resight, which may make it cost-competitive or less expensive than fecal DNA mark-recapture. Regardless, it is important that wildlife managers understand the benefits, costs, and limitations associated with alternative techniques that may achieve similar results (Kilpatrick et al. 2013), especially when management is hindered by budgetary constraints commonly found in wildlife studies (McClintock et al. 2009). Accordingly, our simulation-based cost comparison methods can be broadly applied to other systems as a tool for determining the most effective approach. Furthermore, our methods provide a framework for selecting the most efficient study design when comparing within a technique (e.g., Table 3.10).

Collection of fecal samples minimizes the likelihood of observing "trap-happy" or "trap-shy" behavioral responses sometimes seen with other genetic sampling methods (e.g., Boulanger et al. 2006) and because no lures are used, may minimize individual capture heterogeneity (Marucco et al. 2011). However, because we concentrated our sampling efforts at water sources, our study had the potential to violate the assumption of equal capture probability (detection probability in this case) across all individuals in a population (Lukacs and Burnham 2005). That is, there may be individuals that did not use the water sources and thus were not sampled. Abundance estimates can be biased when capture heterogeneity is present (Lukacs and Burnham 2005). However, models with heterogeneity did not perform well and had very low model weight ($wi<0.001$),

indicating there was little heterogeneity in our detection (i.e., capture) probabilities. For other studies where heterogeneity may be an issue, study designs that have high detection (capture) probabilities and a high number of sampling occasions (Ebert et al. 2010) help mitigate the resulting bias caused by heterogeneity in *p*. Increasing either detection probability or the number of sampling occasions can be difficult and costly (e.g., Harris et al. 2010, Poole et al. 2011), particularly when the species of interest inhabits remote locations, exists in low densities, or has large home ranges. Our strategy to sample around water sources was based on the expectation of a high detection probability, and we had a sufficient number of sampling occasions, which resulted in a high probability of capturing an animal at least once (p^*); p^* was 0.83 and 0.87 for 2016 and 2017. This, combined with the fact that only 2% of the uniquely identified animals were detected away from water sources likely explains why there was no detectable individual heterogeneity in our detection probabilities.

Although it is unlikely capture heterogeneity biased our estimates of abundance for fecal DNA, the estimates may include a small proportion of lambs. Although we made an effort to exclude samples deposited by lambs, lambs that are ≥ 6 months old before the summer sampling period may produce pellets that are difficult to differentiate from adults. We likely detected some lambs in the 30 new individuals in 2017 that were not detected during 2016 surveys. This may account for the higher female abundance estimates for fecal DNA compared to mark-resight estimates (Table 3.4), although it is difficult to assess differences because the CIs are so wide for the mark-resight estimates (Table 3.4). If management goals require an estimate of the adult abundance, additional

studies should be done to determine the proportion of lambs included in the fecal DNA samples.

The precision of our estimates (CV=5.1-6.5%) compare well with those of Brinkman et al. (2011) (CV=6.1-9.5%) who used similar sampling methods along game trails to collect fecal samples of Sitka black-tailed deer in Alaska. The slightly higher precision observed in our estimates likely stems from the high genotype success rate (98% vs. 51%) resulting in higher detection probabilities. A recent study by Brazeal et al. (2017) applied spatially explicit capture-recapture (SCR) techniques using similar sampling methods as Brinkman et al. (2011) for estimating mule deer (*Odocoileus hemionus*) abundance in the central Sierra Nevada mountains of California. Despite having a low genotyping success rate (47%), the annual abundance estimates from this study still achieved reasonable precision (CV=20%-28%; Brazeal et al. 2017).

The high CV for $\hat{N}$ from ground mark-resight surveys likely stems from various sampling issues with our ground mark-resight surveys. That is, we had a low number of marks in the population and conducted a limited number of surveys (n=2), both of which often result in low sighting probabilities, which in turn results in abundance estimates with low precision (McClintock et al. 2009). For instance, we saw 0 of 6 marked males during the first survey of 2016 and we saw 9 of 9 marked females during the first survey of 2017. Seeing all or no marks during mark-resight studies results in high uncertainty of population estimates. In an extensive aerial mark-resight study of mule deer in large pastures where population sizes were known, Bartmann et al. (1986) found that for small populations (25-50 in the study) a large proportion (>45%) of the population should be

marked to obtain reliable estimates and confidence intervals. As noted by Bartmann et al. (1986), "this requirement that may nullify usefulness of the approach in many situations". Alternatively, a high number of surveys can also yield estimates with much higher precision, a condition that was reinforced by our simulations. Bartmann et al. (1986) found that any increase in the number of surveys beyond 2 improved the reliability of abundance estimates. Because of the logistical difficulty of ground surveys for desert bighorn sheep in the Marble Mountains, increasing the number marked individuals is more cost-efficient than increasing the number of sampling occasions (Table 3.10). However, this may not be true with other populations. We strongly recommend using our approach of combining a cost analysis with simulated study designs for determining the most efficient technique of estimating *N* in other systems.

The costs of obtaining $\hat{N}$ through fecal DNA-based capture-recapture is also dependent on genotyping success rates (Taberlet et al. 1996), which suggests that sampling designs should aim to collect fresh samples that contain high-quality DNA (Ruibal et al. 2009). Both the age of fecal pellets and season of collection have significant effects on amplification rates of fecal samples (Piggott 2014). Genotype success rates are typically highest for samples collected during the summer or during dry periods (Piggott 2014). Furthermore, genotyping error rates may significantly increase one week after deposition (Piggott 2014). Seven-day intervals between fecal DNA sampling occasions worked well for our study. Our DNA genotyping success rate was nearly 100% meaning samples were not overexposed to elements that degrade DNA (i.e. ultra violet rays and moisture). By clearing transects after each sampling occasion, we were able to target

fresher pellets and reduce the chance of collecting samples that were contaminated by multiple individuals. Sampling intervals longer than one week could potentially lead to the accumulation of too many pellet piles and make it easy to contaminate samples by collecting pellets deposited by multiple individuals, especially at water sources in the desert. However, if proper protocols are applied fecal DNA-based capture-recapture can work well in dry desert systems.

Desert bighorn sheep are a valued natural resource that are perpetually threatened by natural and anthropogenic impacts (Dolan 2006) including disease (Wehausen et al. 2011), habitat fragmentation (Epps et al. 2005, Epps et al. 2018), and climate change (Epps et al. 2004). The bacterial respiratory pathogen *Mycoplasma ovipneumoniae* (*M. ovi*) is known to be associated with an all-age die-off in one population and poor lamb recruitment across affected populations (Epps et al. 2016). Therefore, a primary management goal for the conservation of desert bighorn sheep is identifying and monitoring populations that carry *M. ovi.*, which currently requires capturing animals to test for infection and exposure. The use of fecal DNA-based capture-recapture alone does not provide data that allows management to monitor the prevalence of disease. However, the capability of fecal DNA-based capture-recapture to produce precise estimates of abundance can greatly assist conservation goals by detecting population declines over a short period of time, thus making management actions timelier and more effective.

The difference in dry season precipitation (Fig. 3.2) unlikely influenced our abundance estimates, however due to higher demand for water, it may explain the increased detection probabilities observed at water sources in 2017. Both methods

detected a decline in abundance of approximately 30% from 2016 to 2017. There was no change in our sampling strategies from 2016 to 2017 that would result in a decline in abundance, and survival of radio-collared bighorn in the study area showed a 31% mortality rate for that time period (n=29). We speculate that this decline is disease-related. The disease outbreak of *M. ovi.* was first documented in the Marble Mountains in 2013 and has since resulted in a high rate of lamb mortality and low recruitment (CDFW, unpublished data). This low recruitment has resulted in a disproportionate number of old to new individuals within the population (CDFW, unpublished data). Helicopter surveys conducted during the spring of 2018 documented a relatively large abundance of older rams (e.g., class III and IV) and absence of young rams (e.g., class I and II) in nearby populations where *M. ovi.* was present (CDFW, unpublished data). Further, the 2018 helicopter surveys conducted in the Newberry-Ord Mountains (~65 miles west of the Marble Mountains), where *M. ovi.* is not present, documented an even distribution of ram age classifications suggesting higher lamb recruitment into the population (CDFW, unpublished data).

MANAGEMENT IMPLICATIONS

For small populations of desert ungulates, fecal DNA-based capture-recapture can provide a cost-effective alternative to traditional ground mark-resight methods for estimating *N* with high precision. The high costs associated with capturing and marking bighorn sheep and the high cost of ground surveys in the isolated and rugged terrain of our study area resulted in costly ground-based mark-resight surveys. In contrast, our fecal DNA collection sites were close together and easily accessible which minimized

field-sampling costs in this system. It is important to consider that capture and survey costs may be less expensive for ungulates that inhabit different landscapes. For example, the costs of capturing and marking a population of mule deer in a concentrated winter range may be sufficiently less expensive such that mark-resight is a cost-effective alternative, relative to fecal DNA, for estimating abundance. In addition, if collars are deployed for other reasons, such as monitoring survival or spatial movements or habitat use, then the cost per collar could be reduced for ground-based mark-resight, which may make it cost-competitive or less expensive than fecal DNA mark-recapture. Further, we assumed each collar would last 5 years. Different makes/models with different settings (i.e., 30 BPM vs. 60 BPM) may last longer, which would also greatly reduce collaring costs. Fecal DNA-based capture-recapture methods lack the ability to determine the age of individuals within a population. Therefore, the use of fecal DNA-based capture-recapture to estimate recruitment is only possible with a long enough time series (i.e., ≥3 years). However, if yearlings can be reliably identified during ground mark-resight surveys, recruitment can be estimated within a single survey. We recommend using a simulation-based approach for determining what method(s) is most cost effective (i.e., $ per/%CV) in other systems. In general, we recommend a simulation approach to provide a cost comparison of different methods, as well as for a cost comparison within methods for a wide variety of potential sampling scenarios. Even though our fecal DNA-based capture-recapture population estimates were very precise, it is important to note that this method does not provide data on the age structure of the population which need to be obtained through other methods and may require additional ground-based field work and

some capture. Currently, there is no known method to detect disease from fecal pellets. However, the ability of trained scent dogs to detect cervical and lung cancer in humans (Guerrero-Flores et al. 2017, Fischer-Tenhagen et al. 2018) as well as detect fecal samples from low density moose populations (Kretser et al. 2016) may be applied to detect disease from fecal samples in future research.

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TABLES AND FIGURES

Table 3.1. Microsatellite loci used for individual analysis of desert bighorn sheep (*Ovis canadensis nelsoni*) fecal samples from the Marble Mountains, California, USA, 2016-2017, with fluorescent dye labels, primer concentrations and references for the original primer publication.

Locus	Reference	Dye Label	Primer Concentration (uM)
AE129	Penty *et al.* 1993	Vic	0.25
AE16	Penty *et al.* 1993	Ned	0.2
OarFCB193	Buchanan & Crawford 1993	Pet	0.25
OarFCB304	Buchanan & Crawford 1993	Pet	0.2
OarHH62	Ede *et al.* 1994	6-Fam	0.15
MAF33	Buchanan & Crawford 1992	Vic	0.175
MAF36	Swarbrick *et al.* 1991	Vic	0.1
MAF48	Buchanan *et al.* 1991	Ned	0.2
TCRBV62	Crawford *et al.* 1995	6-Fam	0.25
SE47/48*	Yamamoto *et al*. 2002	Ned	0.2

*Sex identification marker

Table 3.2**.** Costs of capturing and marking (via helicopter and net gun) 10 desert bighorn sheep, and performing one ground mark-resight survey in the Marble Mountains, California, USA, 2016.

Captures	Item	Cost per unit	Cost
	Helicopter flight time	$1,650/hour	$11550 (7 hours of flight)
	Helicopter daily rate	$1,472/day	$1,472
	Helicopter fuel truck[a]	$2.75/mile	$468 (170 miles)
	Per-animal bonus	$100/animal	$1000 (10 animals)
	Technicians[b]	$20/hour	$2,700 (135 man-hours)
	Perdiem	$46/day	$920 (20 person-days)
	Hotel	$110/night	$2,200 (20 people, 1 night)
	Travel[c]	$0.52/mile	$530 (1,020 miles)
	Veterinarian[d]	$371/day	$371
	VHF collars	$200/collar	$2000 (10 collars)
	Total		$23,211
Surveys	Item	Cost per unit	Cost[*]
	Technicians[e]	$15/hour	$1,620 (108 man-hours)
	Perdiem	$46/day	$552 (12 person-days)
	Travel[f]	$0.52/mile	$936 (1,800 miles)
	Total		$3,108

[a] 170-mile round trip from Barstow, CA.

[b] 9 hours * 15 people.

[c] 6 vehicles * 170-mile round trip from Barstow, CA.

[d] $215/day + $110 (hotel) + $46 per diem.

[e] 6 people * 18 hours/person (8-hour survey, 10 hours of travel).

[f] 3 vehicles * 600-mile round trip from field office in Bishop, CA.

[*] Ground survey crew camp in field; no hotel costs included.

Table 3.3. Costs (U.S. dollars) for collecting and genotyping fecal samples from desert bighorn sheep in the Marble Mountains, California, USA, 2016-2017.

Item	Cost per unit	2016[a]	2017[b]
Technician[c]	$15/hour	$2,520 (168 man-hours)	$1,680 (112 man-hours)
Per diem[d]	$46/day	$1,104 (24 person-days)	$736 (16 person-days)
Travel[e]	$0.52/mile	$1,872 (3,600 miles)	$1,248 (2,400 miles)
DNA Analysis[f]	$46/sample	$16,376 (356 samples)	$12,742 (277 samples)
Total Cost		$21,872	$16,406

[a] Cost for 6 visits.

[b] Cost for 4 visits.

[c] 2 people * 14 hours (4 hours sampling + 10 hours of travel).

[d] 2 people * 2 days.

[e] 1 vehicle * 600-miles (round trip from field office in Bishop, CA).

[F] 9 microsatellite loci plus 1 sexing marker; 3 replicates per sample.

Table 3.4**.** Estimated population abundance ($\hat{N}$) of desert bighorn sheep by sex for fecal DNA capture-recapture and ground mark-resight methods in the Marble Mountains, California, USA, 2016-2017.

		2016					2017				
		$\hat{N}$	n*	SE ($\hat{N}$)	CI	CV ($\hat{N}$)	$\hat{N}$	n*	SE ($\hat{N}$)	CI	CV ($\hat{N}$)
Fecal DNA[a]	Females	105	87	5.6	94-116	5.3	75	66	3.8	68-83	5.1
	Males	63	52	4.1	55-71	6.5	44	41	2.8	39-50	6.2
Ground MR[b]	Females	88	17	18	66-131	20.5	64	9	14.8	41-100	23.1
	Males	149	6	43.6	44-506	29.3	81	6	45	29-225	55.6

[a] Abundance estimates using robust Huggins formulation of closed capture-recapture models in Program MARK (White and Burnham 1999, White et al. 1999).

[b] Abundance estimates using the two-sample bias-adjusted Lincoln-Peterson estimator (Williams et al. 2001).

* Fecal DNA: total number of unique individuals identified; Ground MR: Total number of known marks in population.

Table 3.5**.** Desert bighorn sheep fecal samples collected and analyzed with genotyping success* rates by year in the Marble Mountains, California, USA, 2016-2017.

	2016	2017
No. samples collected	356	277
No. samples analyzed [a]	349	271
Success [b]	97%	99%
No. of detections	338	269
No. of individuals	141	107
Females	87	66
Males	52	41
Unknown	2	0

[a] Samples remaining after contaminated samples were removed.

[b] Percent success (excluding contaminated samples).

* Number of samples that amplified at enough loci (≥7) to have a PID <0.01 and PID for sibling of <0.05.

Table 3.6. Number of unique bighorn sheep genotyped (n), allelic richness (Na), allele size range, and observed (H_O) and expected (H_E) heterozygosity values for nine microsatellite loci and one sexing marker analyzed in desert bighorn sheep (*Ovis canadensis nelsoni*) fecal samples from the Marble Mountains, California, USA, 2017.

Locus	N	Na	H_O	H_E	Range (bp)
AE129	107	6	0.85	0.801	167-187
AE16	107	5	0.72	0.724	84-94
OarFCB304	107	3	0.673	0.642	144-150
OarHH62	106	7	0.783	0.815	104-130
MAF33	107	3	0.533	0.613	122-126
MAF36	85	4	0.506	0.439	87-99
MAF48	107	5	0.701	0.666	120-128
OarFCB193	107	5	0.654	0.666	105-117
TCRBV62	107	5	0.738	0.692	169-179
SE47/48*	107	n/a			214 (Y chrom) 258 (X chrom)

*Sex identification marker

Table 3.7**.** Model selection results from a Huggins closed-capture robust-design analysis for desert bighorn sheep in the Marble Mountains, California, USA, 2016-2017.

Model[a]	K	AIC_c	ΔAIC_c	w_i	Deviance
S(g) *p*(yr*t)=*c*(yr*t)	14	1877.791	0.0	0.515	2049.555
S(.) *p*(yr*t)=*c*(yr*t)	14	1878.314	0.5	0.397	2050.078
S(.) *p*(yr*t)=*c*(yr*t) (Diff. Opp. Yr.)	16	1882.602	4.8	0.046	2050.078
S(.) *p*(yr*t)=*c*(yr*t) (Diff. Opp.)	15	1883.021	5.2	0.038	2052.646
S(.) *p*(yr*t*g)=*c*(yr*t*g)	26	1887.347	9.6	0.004	2032.781
S(.) *p*(yr*t)=*c*(yr*t) (Diff. Opp.)	12	1906.244	28.5	0.000	2082.258
S(.) *p*(yr)=*c*(yr) (Diff. Opp. Yr.)	6	1916.836	39.0	0.000	2105.369
S(.) *p*(yr)=*c*(yr) (Diff. Opp.)	6	1919.222	41.4	0.000	2107.755
S(.) *p*(yr*t*g) *c*(yr*t*g)	46	1923.451	45.7	0.000	2021.548
S(.) *p*(.)=*c*(.) (Diff. Opp. Yr.)	5	1929.210	51.4	0.000	2119.797
S(.) *p*(t*g)=*c*(t*g)	16	1933.960	56.2	0.000	2101.436
S(.) *p*(yr)=*c*(yr)	4	1950.204	72.4	0.000	2142.836
S(.) *p*(yr*g)=*c*(yr*g)	6	1953.842	76.1	0.000	2142.375
S(.) *p*(g*y) *c*(g*y)	10	1959.622	81.8	0.000	2139.847
S(.) *p*(.)=*c*(.)	3	1960.586	82.8	0.000	2155.254
S(.) *p*(.) *c*(.)	4	1961.854	84.1	0.000	2154.486
S(.) *p*(g)=*c*(g)	4	1962.295	84.5	0.000	2154.927
S(.) *p*(g) *c*(g)	6	1965.524	87.7	0.000	2154.057

[a] Key to model notation: K = No. of parameters; AIC_c = Akaike Information Criterion corrected; Δ AIC = difference between the model listed and the AIC_c of the best model; W_i = model weights based on model AIC_c compared to all other model AIC_c values; S= survival rate; p = detection probability; c = redetection probability; yr = year as a categorical variable; t = encounter occasion as a categorical variable; g = sex as a categorical variable; "." = constant across year, encounter occasion, and sex; Diff. Opp. = there was a difference in capture rate for opportunistically-collected samples.

Table 3.8. Cost of simulated ground mark-resight survey scenarios when true population size is 100, resight probability (p) is 0.2, and number of marked animals range from 10-50 with 3 visits.

True population size	No. of visits	p	No. of marks	$\hat{N}$[a]	se($\hat{N}$)	CV	Total Cost[b]
100	3	0.2	10	108	45.1	42%	$13,966
100	3	0.2	15	110	36.0	33%	$16,287
100	3	0.2	20	102	26.1	26%	$18,608
100	3	0.2	25	100	21.4	21%	$20,929
100	3	0.2	30	104	20.1	19%	$23,251
100	3	0.2	35	100	16.6	17%	$25,572
100	3	0.2	40	106	17.6	17%	$27,893
100	3	0.2	45	102	13.6	13%	$30,214
100	3	0.2	50	101	12.1	12%	$32,535

[a] Simulated population estimate.

[b] (No. of marks * $464/animal) + (No. of visits * $3,108/visit).

Table 3.9. Costs of simulated fecal DNA sampling scenarios when true population size is 100, encounter probability (p) is 0.20, and number of visits range from 3 to 6.

True population size	No. of visits	p	$\hat{N}$ [a]	SE($\hat{N}$)	CV	Corrected sample count [b]	Total Cost [c]
100	3	0.2	111	29.8	26.9%	61	$5564
100	4	0.2	101	15.3	15.2%	82	$7419
100	5	0.2	100	11.7	11.7%	102	$9274
100	6	0.2	101	9.0	8.9%	122	$11129

[a] Simulated population estimate.

[b] (Theoretical sample count [population size* no. visits* p] / 98% genotyping success rate).

[c] (Corrected sample count * $46/sample)+(No. visits * $916/visit)

Table 3.10. Value of simulations paired with cost and precision for designing studies on desert bighorn sheep in Marble Mountains, California, USA.

Method	Case	CV	Total cost	CV reduction	Cost increase	Cost/CV% reduction
Mark resight	Base case [a]	42%	$13,966	-	-	-
	Double p [*]	22%	-	20%	-	-
	Double visits	27%	$23,348	15%	$9,382	$625
	Double marks	26%	$18,608	16%	$4,642	$290
Fecal DNA	Base case [b]	27%	$5,564	-	-	-
	Double p	7%	$8,158	20%	$2,594	$130
	Double visits	9%	$11,129	33%	$5,565	$169

[a] Base case scenario for mark resight: visits = 3, population size = 100, p = 0.20, and no. of marks = 10.

[b] Base case scenario for fecal DNA capture-recapture; visits = 3, population size = 100, and p = 0.20. [*] p = detection probability.

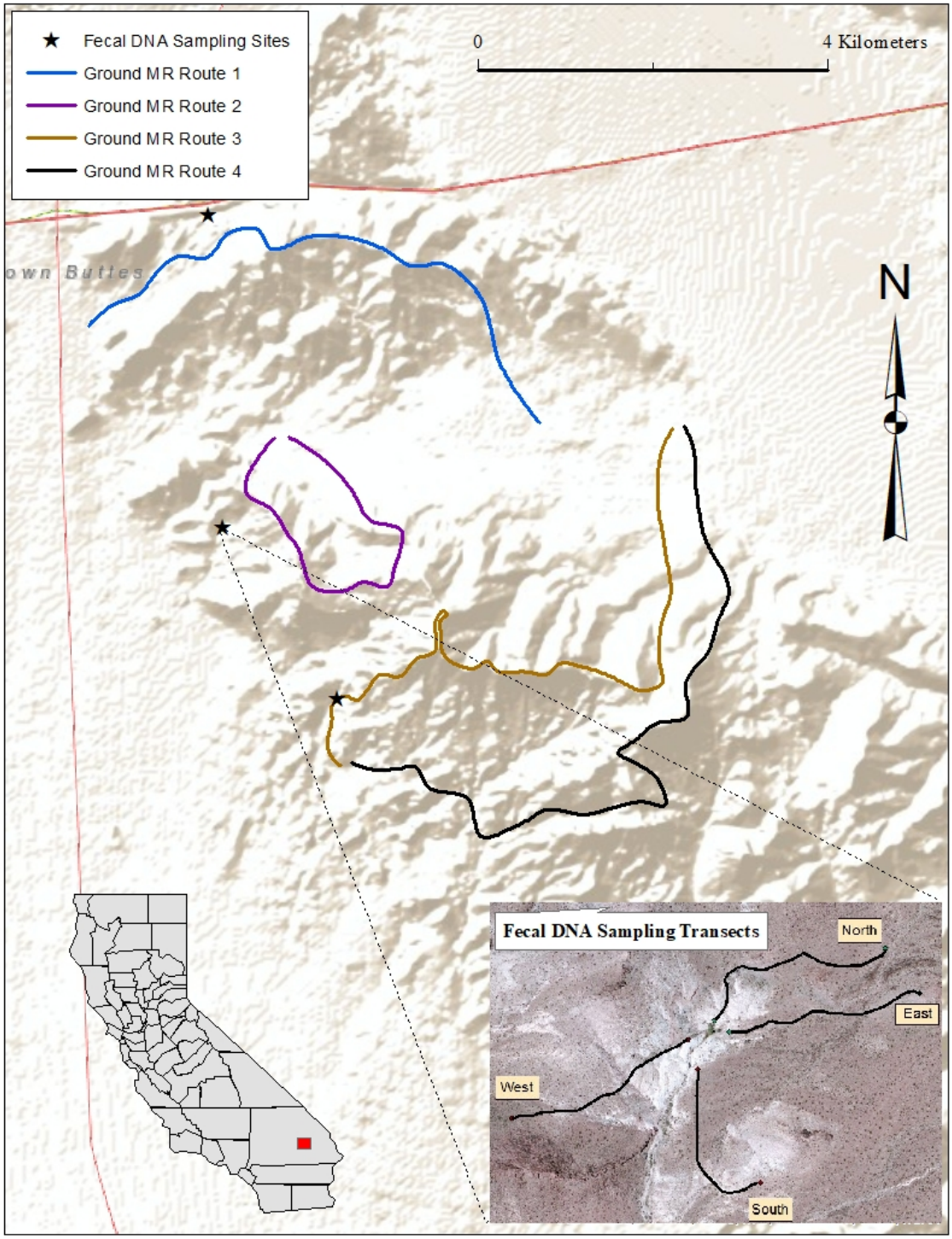

Fig. 3.1. Location of study area and fecal DNA collection sites in the Marble Mountains, California, USA, 2016-2017.

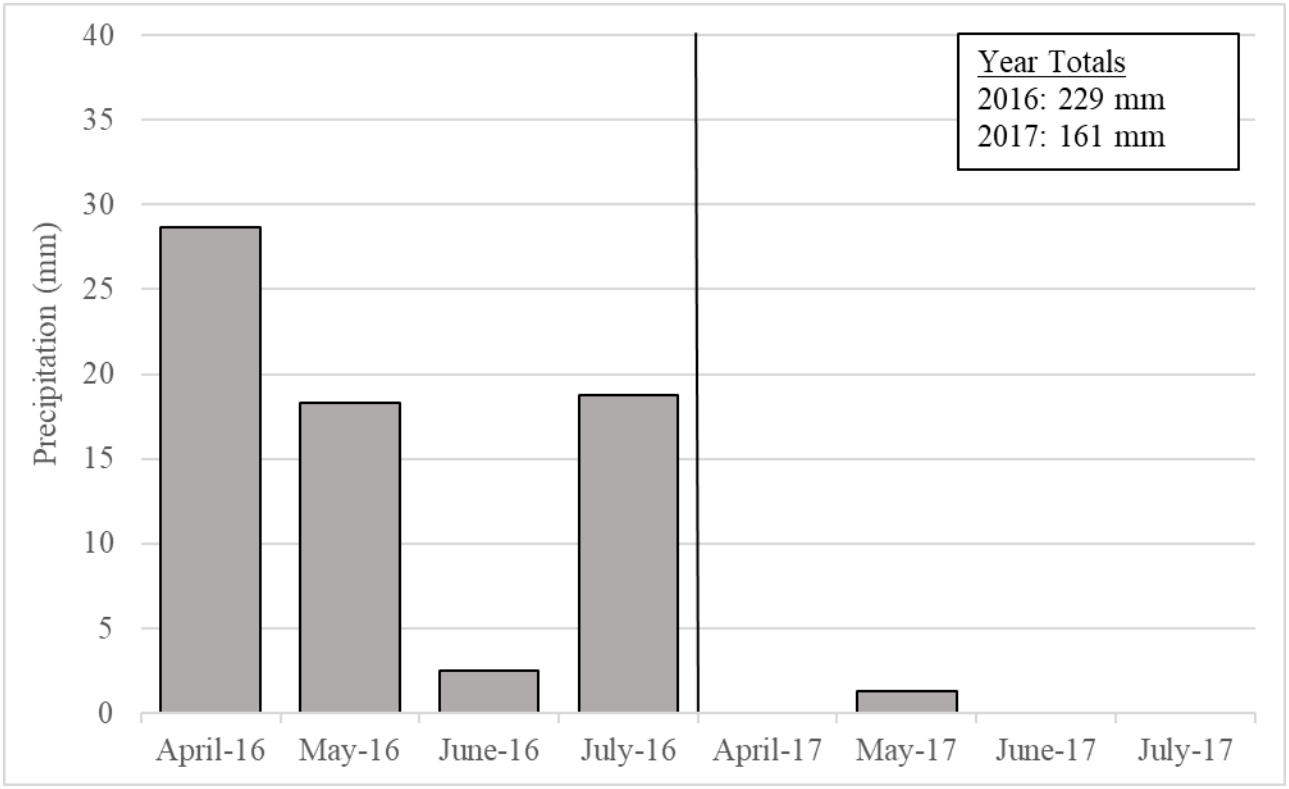

Fig. 3.2. Dry season (April through July) and yearly precipitation (mm) in the Granite Mountains, CA, USA, 2016-2017 (WRCC 2019).

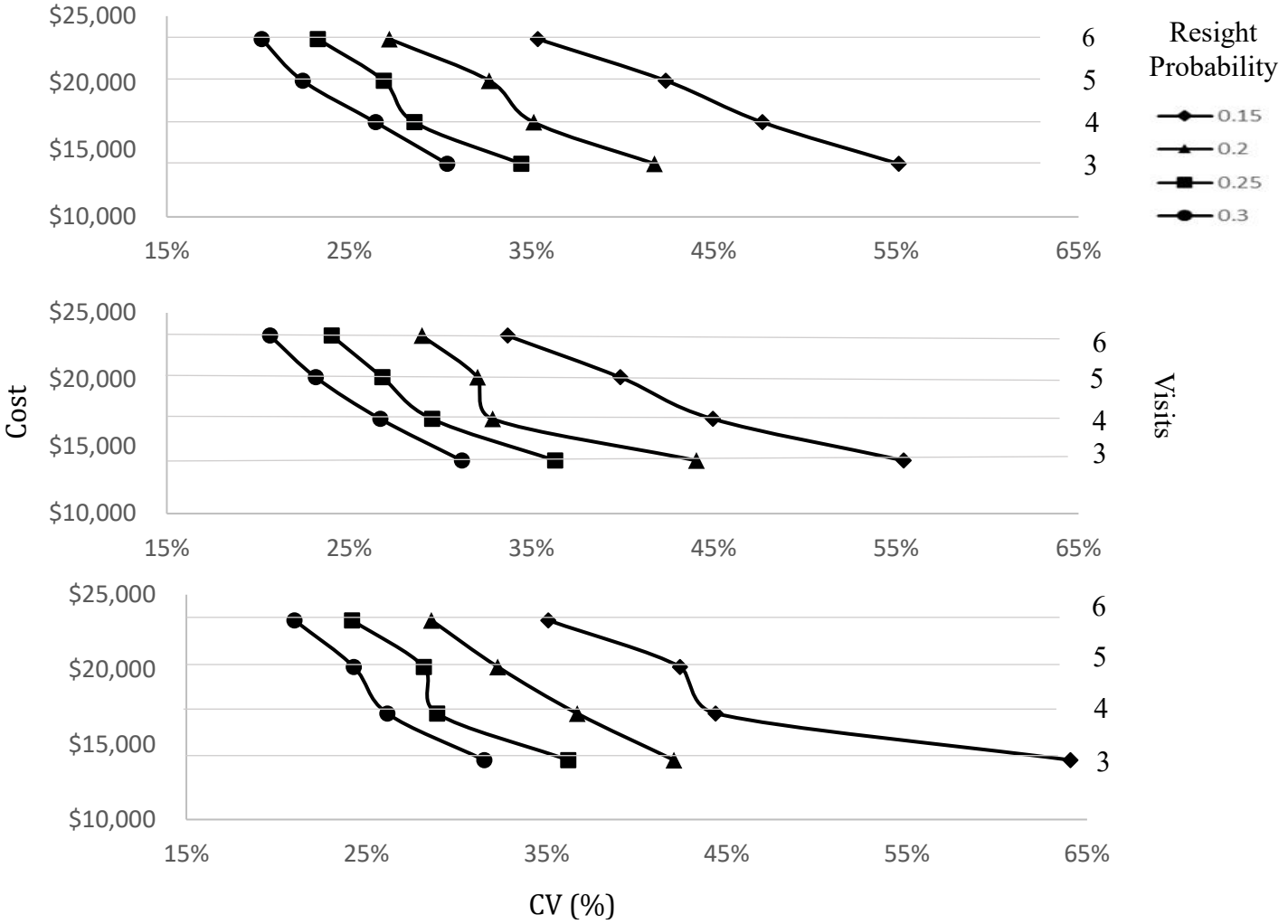

Fig. 3.3. Simulated costs and precision (CV) of ground-based mark-resight scenarios when number of marks in population is n=10 and for varying values of population size, resight probability, and number of visits.

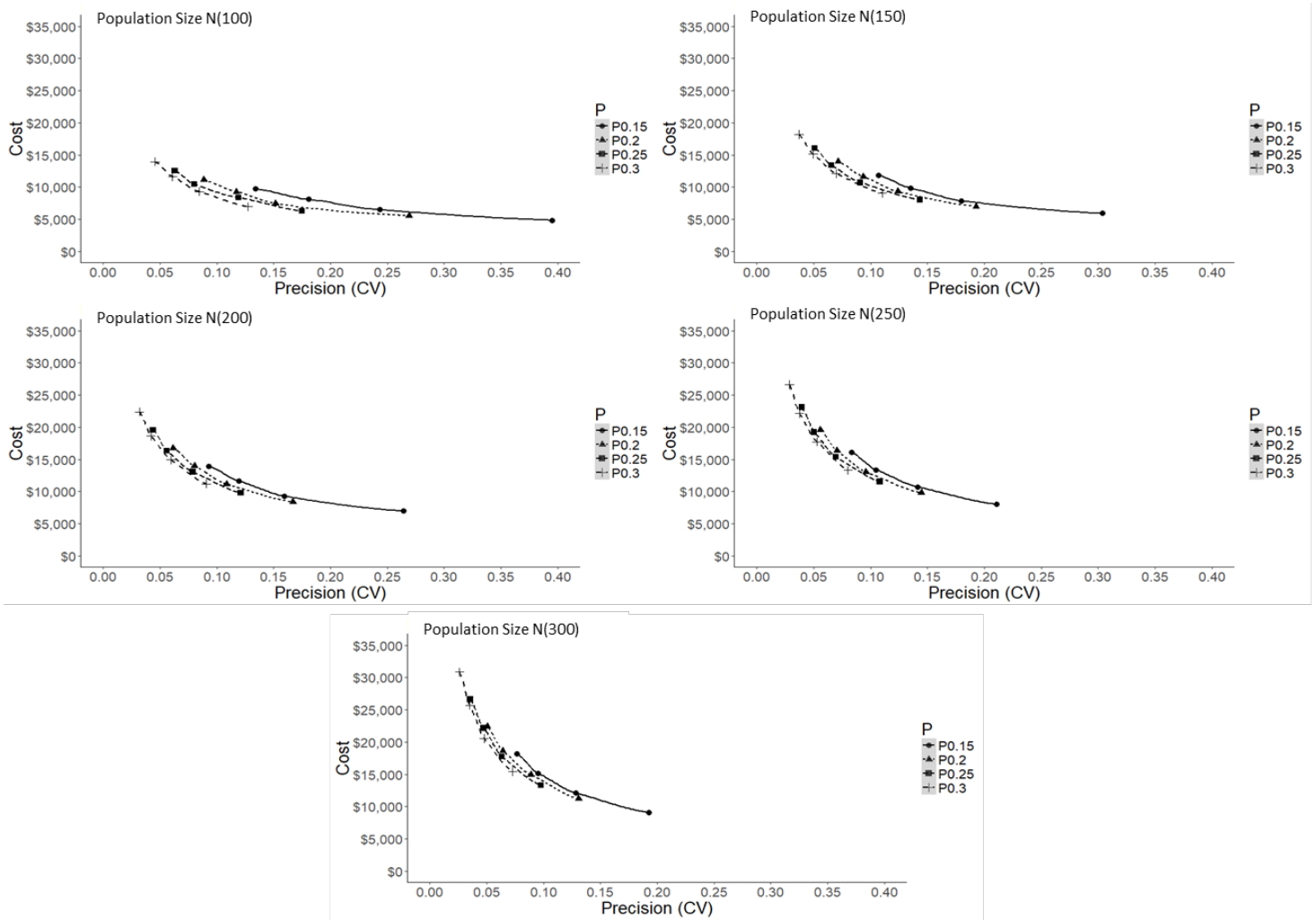

Fig. 3.4**.** Simulated cost and precision (CV) of fecal DNA capture-recapture scenarios with varying values of population size, encounter probability, and number of visits[a].

[a] Points on each line represent 6, 5, 4, and 3 visits (from left to right) respectively.

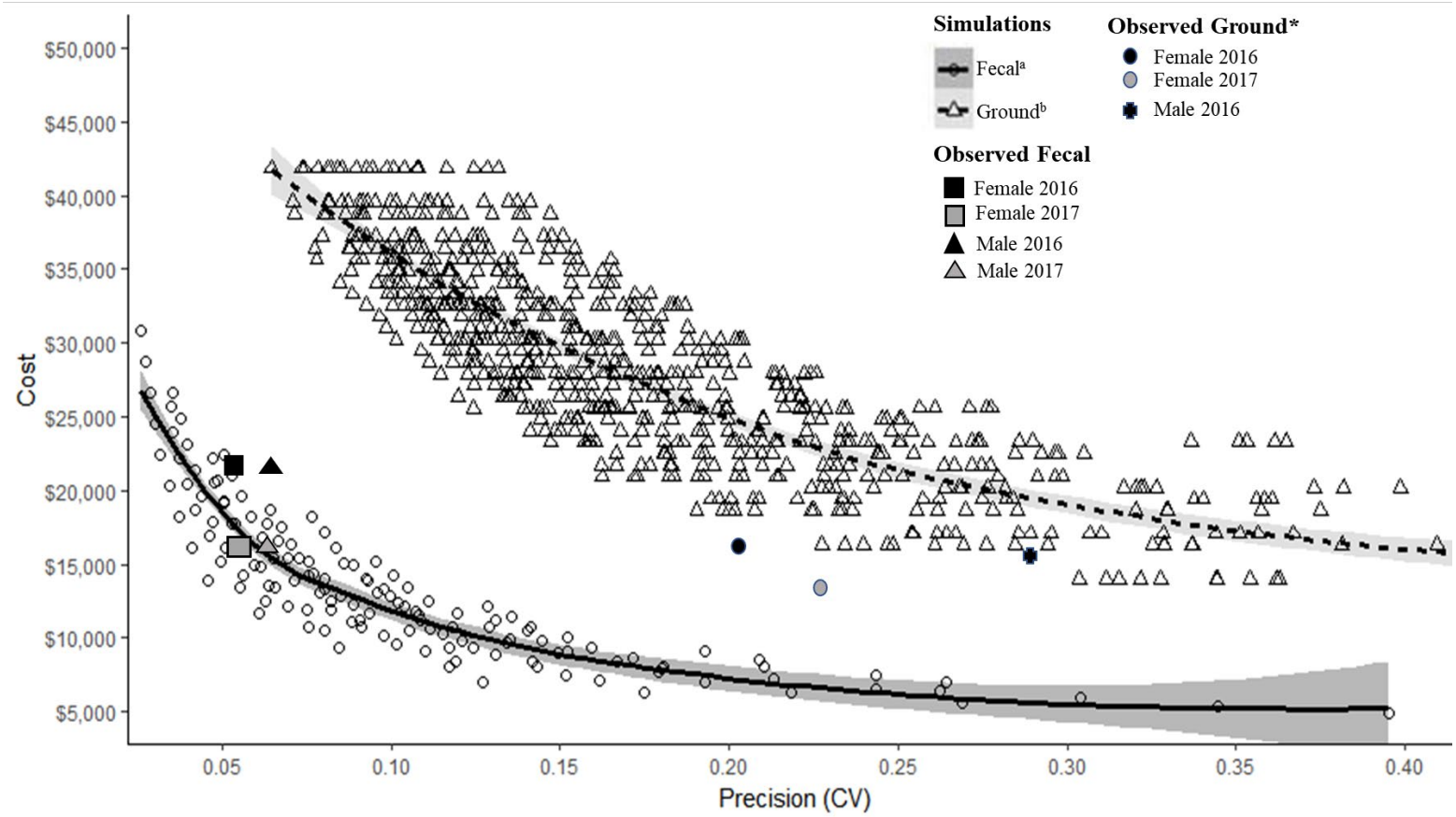

Fig. 3.5. Comparison of simulated cost and precision of ground mark-resight and fecal DNA capture-recapture population estimation methods for a range of sampling intensities and scenarios (i.e., number of marked animals, number of visits, detection probability and population size) for desert bighorn sheep in the Marble Mountains, California, USA, 2016-2017. Simulation inputs based on field data collected.

CHAPTER 4

CONCLUSIONS

For small populations of desert ungulates, fecal DNA-based capture-recapture can provide a cost-effective alternative to traditional ground mark-resight methods for estimating *N* with high precision. Previous studies have demonstrated the ability of fecal DNA-based capture-recapture to provide abundance and density estimates when applied to wild ungulate populations (Brinkman et al. 2011, Goode et al. 2014, Lounsberry et al. 2015, Woodruff et al. 2016, Furnas et al. 2017). However, few studies have evaluated the cost effectiveness (i.e. cost/level of precision) of this method when compared to traditional methods of estimating abundance (Janecka et al. 2011, Poole et al. 2011, DeMay et al. 2015). Concentrating sampling efforts at water sources during dry summer months provided an efficient way to sample nearly the entire population within our study areas . While the number of sampling occasions, sampling intervals, and the quality of collected samples all influence precision of abundance estimates, sampling design influences their accuracy. To determine the accuracy of abundance estimates, based on sampling solely around water sources, for an entire population in an area, we recommend implementing a sampling design that includes random and representative sites throughout the study area in addition to sampling around water sources.

The costs of obtaining $\hat{N}$ through fecal DNA-based capture-recapture is highly dependent on genotyping success rates (Taberlet et al. 1996), which suggests that sampling designs should aim to collect fresh samples that contain high-quality DNA (Ruibal et al. 2009). Both the age of fecal pellets and season of collection have significant

effects on amplification rates of fecal samples (Piggott 2014). Genotype success rates are typically highest for samples collected during the summer or during dry periods (Piggott 2014). Furthermore, genotyping error rates may significantly increase one week after deposition (Piggott 2014). In order to target fresher pellets and reduce the chance of collecting samples that are contaminated by multiple individuals, we recommend clearing transects after each sampling occasion. Additionally, since there is no evident way to prevent or reduce the number of duplicate samples collected, we strongly recommend that future sampling designs take this into account by assuming approximately 25% of all samples collected at water sources will be duplicates.

Expanding fecal DNA mark-recapture techniques to adjacent mountain ranges can potentially provide demographic estimates and movement data at the metapopulation level. It is important to note that this method may not be the best alternative for other populations in different systems where DNA genotyping does not have as high a success rate or samples are sparse or cryptic and more time consuming to collect. In such situations, traditional approaches may be the more cost-effective alternative. Further, traditional methods that involve physically capturing and collaring animals provide data on disease, spatial movements, age structure, and habitat use which is not easily-obtainable via fecal DNA-based capture-recapture. Regardless of management goals we recommend using a simulation-based approach for determining which method(s) is most cost effective (i.e., $ per/%CV) in other systems. In general, we recommend a simulation approach to provide a cost comparison of different methods, as well as for a cost comparison within methods for a wide variety of potential sampling scenarios.

The bacterial pathogen, *M. ovi,* continues to be a major threat to desert bighorn sheep populations in the Mojave Desert. The 30% drop in $\hat{N}$ in the Marble Mountains bighorn sheep population from 2016 to 2017 demonstrates the ability of fecal DNA-based capture-recapture methods to detect changes in abundance over a short period of time. Although it is possible that the lack of precipitation in 2017 could have contributed to this population decline, we speculate that this decline is disease-related; coughing lambs (presumably infected with *M. ovi*) were observed during ground mark-resight surveys in 2016 and 2017. Assuming most infected lambs die within the first year, the bighorn sheep population in the Marble Mountains has experienced low recruitment since the disease outbreak in 2013 (CDFW, unpublished data). The use of fecal DNA-based capture-recapture alone does not provide data that allows management to monitor the prevalence of disease. However, the capability of fecal DNA-based capture-recapture to produce precise estimates of abundance can facilitate monitoring of management goals by detecting population declines over a short period of time, thus making management actions timelier and more effective.

One pitfall to our study was the inability to estimate seasonal home range size, which would have enabled us to estimate density. We did not have appropriate data to use spatial capture-mark-recapture (see Brazeal et al. 2017, Furnas et al. 2018) to estimate density, but we recommend evaluating this design for future use. There was a small population of desert mule deer that was detected in 2015 and 2017, but not in 2016. This suggests that some proportion of the population may change their seasonal home ranges from year to year. The addition of GPS and/or VHF collared individuals would provide

insight to movement patterns and seasonal home range size, while also allowing density to be estimated at a larger scale (see Rautenstrauch and Krausman 1989, Marshal et al. 2006). The 5 "new" water sources that were sampled in 2016 showed that each water source sustains its own local sub-population of mule deer during the hot-dry season. When fecal DNA-based capture-recapture is used in desert ecosystems during the dry season, it is crucial that every source of free-standing water is used as a sampling site. Similar studies that fail to identify all sources of water within the study area will likely produce estimates that are biased low.

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APPENDICES

APPENDIX A

FINAL REPORT: DESERT MULE DEER (*Odocoileus hemionus eremicus*) fecal genotyping and individual identification of samples collected in 2017, with recaptures of individuals sampled in 2015 and 2016

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Summary
We received 548 desert mule deer fecal samples, all of which we attempted to genotype at 14 microsatellite loci plus three markers used for sex identification. No samples showed evidence of contamination, and 478 samples (87%) produced data at ≥10 of the 14 microsatellite loci and were used in individual identification analyses. Forty samples (7%) amplified at nine or fewer microsatellites, and 30 samples (5%) failed completely. From the 478 complete or mostly-complete samples, we identified 211 unique individuals (103F, 89M, 19 undetermined), of which 109 were recaptured 1-15 times each in 2017. Across all three sampling years (2015-2017) we identified 447 unique individuals; there were 204 females, 212 males, and 31 deer whose sex could not be determined.

Introduction
Using protocols established in the Epps Population Genetic Laboratory (Oregon State University; Appendix 1), we extracted DNA and genotyped fecal samples from desert mule deer (*Odocoileus hemionus eremicus*) in the Chuck Walla Mountain area of southern California. We used tissue samples with known genotypes to align microsatellite calls from our laboratory with those done by Erin Meredith at the Wildlife Forensics Laboratory (California Department of Fisheries and Wildlife) to ensure that allele calls remained consistent with data generated in previous years.

Methods
During the summer of 2017, deer pellets were collected into paper bags and dried; they were stored at room temperature until processed in the spring of 2018. At OSU, we scraped cells from the surface of pellets and used a modified commercially available kit (Aquagenomics and Aquaprecipi; Multitarget Pharmaceuticals, Colorado Springs, CO) to extract DNA from these scrapings (Appendix1). For ease in labeling microcentrifuge tubes, we renamed samples with consecutive numbers and the three-letter code of the drinker or survey transect at which they were collected (Table 1, provided as a separate excel spreadsheet). For drinkers that were sampled in 2015 or 2016, the new sample number assigned at OSU began where the previous dataset ended. The electronic sample

list that accompanied the samples showed 547 fecal samples. We received one additional sample that was not included on the electronic sample sheet (20170706XX135, which we renamed EEE009). Thus in total we analysed 548 samples.

We attempted to amplify seventeen markers (14 microsatellites and three markers for sex identification) for each sample using the polymerase chain reaction (PCR). Primer sequences, concentrations, and dye labels remained the same as those in Crowhurst and Epps (2016). Locus B was removed from the primer cocktail because analyses in 2015 and 2016 failed to find more than one allele in this population.

Samples were initially amplified in three separate PCR reactions each; those that produced data at <50% of the loci in the first three replicates were considered poor quality and were not rerun. Samples that produced partial genotypes at ≥50% of microsatellite loci were rerun 2-6 times depending on the completeness of initial replicates, while samples that produced complete genotypes in the first three replicates were considered finalized. For a genotype to be accepted for a particular locus, each allele in a heterozygous genotype had to be observed twice, while the single allele in a homozygous genotype had to be observed three times. In the event that a homozygote allele occurred only twice despite reruns, this genotype was accepted but bolded in the final data set. Sexes were assigned using the markers SRY-OSU (a 120bp fragment), SRY-WFL (a 220bp fragment) and GAPDH (a 218 bp fragment). If a particular sample amplified all three times at GAPDH but not at either SRY fragment, it was classified as a female. Samples that amplified three times at either SRY fragment (or both) were considered to be males. If a sample amplified three times at SRY-OSU but was inconsistent at SRY-WFL we considered it a male, as larger fragments have a higher rate of allelic dropout. Lastly, if a sample amplified at least once but less than three times at both SRY fragments combined, then we considered it a sample of unconfirmed sex. We used GenAlEx (Peakall and Smouse 2006, 2012) to calculate number of alleles, expected and observed heterozygosity for each locus, and to test whether loci were in Hardy-Weinberg equilibrium; we repeated these analyses using all unique deer across all three sampling years.

Using the online individual-identification program CERVUS version 3.0.3 (Kalinowski et al. 2007) and the population-specific allele frequencies tabulated for this population, we estimated the cumulative probability of identity for unrelated deer (P_{ID}) and for siblings (P_{IDsibs}) for all 14 microsatellites. We analysed all samples as one herd and searched for recaptures across all 16 drinkers and transects. When identifying recaptures we used a maximum P_{ID} of $1x10^{-4}$ and P_{IDsibs} of $1x10^{-2}$. After identifying unique individuals within the 2017 data set, we compared these genotypes with those of the 2015 and 2016 data sets to investigate cross-year recaptures.

Although not requested by the funders, we investigated the degree of genetic differentiation (population structure) among deer using different drinkers, in part to inform later analytical choices during demographic analysis, and in part to consider the

appropriateness of conducting genetic analyses (e.g., estimating P_{ID}, testing for Hardy-Weinberg Equilibrium) on the study area under the assumption that all drinkers are part of a single population. To investigate population structure among the drinkers we used STRUCTURE (Pritchard *et al.* 2000), a clustering program that assigns individuals into groups of genetically similar individuals while minimizing deviations from Hardy-Weinberg equilibrium. We included samples from 12 drinkers that were represented by ≥5 unique deer in the 2015-2017 pooled data set. We used a burnin of 500,000 with 1,000,000 replicates and ran eight iterations for each cluster value (k), where $k = 1–6$. The location (drinker) for each sample was included as a prior; individuals sampled at more than one drinker over the course of the study were assigned to the drinker at which they were first captured. We used the Evanno *et al*. (2005) Δk method to determine the number of clusters with the most support, but also examined the curve of the Δk to see whether other k values showed secondary support (i.e., if additional substructuring was present). In addition, we investigated population structure by calculating population pairwise F_{ST} in Genepop (Raymond and Rousset 1995; Rousset 2008) among the same 12 drinkers.

Results and Discussion

Of the 548 samples received and analysed, 49 (9%) amplified at fewer than half of the loci in the first three replicates; these were considered poor quality and not rerun. This value was considerably lower than seen in the 2016 data set (24%), but on par with that of the 2015 sample set (6%).

Failure rate was variable across drinker (Table 2) and sampling date (Table 3). Failure rates were highest at drinkers/survey transects CCC, DDD, DDM, EEE, GGG, and PRW, although sample sizes were considerably smaller for these areas. Amplification rates were higher for samples collected later in the sampling season, with the exception of those collected on 2017-07-06. Lower amplification success could be related to environmental conditions (e.g., rainfall or more extreme temperature fluctuations before the sampling season began) or age of samples (e.g., failure to clear older samples from transects before beginning first sampling session).

Table 2. Number of desert mule deer samples collected and genotyped at ten or more microsatellite loci (excluding monomorphic locus B) in 2015, 2016, and 2017. Not all drinkers were sampled every year.

Samples collected in 2017, genotyped in 2018				**Samples collected in 2016, genotyped in 2017**				**Samples collected in 2015, genotyped in 2016**			
Drinker	n	≥ 10 Loci	% ≥10 loci	Drinker	N	≥ 10 loci	% ≥10 loci	Drinker	n	≥10 loci	% ≥10 loci
BEN	-	-	-	BEN	6	2	33	BEN	-	-	-
BGT	51	48	94	BGT	79	13	16	BGT	82	69	84

BHW	-	-	-	BHW	35	26	74	BHW	-	-	-
BKH	25	16	64	BKH	37	15	40	BKH	68	57	84
CCC	2	0	0	CCC	-	-	-	CCC	-	-	-
CKS	94	89	95	CKS	61	36	59	CKS	94	89	95
CNH	-	-	-	CNH	4	1	25	CNH	-	-	-
CRW	6	4	67	CRW	20	15	75	CRW	22	20	91
DDD	4	2	50	DDD	-	-	-	DDD	-	-	-
DDM	5	2	40	DDM	18	14	78	DDM	36	32	89
EEE	9	0	0	EEE	-	-	-	EEE	-	-	-
GGG	2	1	50	GGG	-	-	-	GGG	-	-	-
HHH	15	11	73	HHH	-	-	-	HHH	-	-	-
JJJ	3	3	100	JJJ	-	-	-	JJJ	-	-	-
KNB	-	-	-	KNB	32	11	34	KNB	-	-	-
LBN	25	16	64	LBN	50	21	42	LBN	23	15	65
MED	-	-	-	MED	13	7	54	MED	-	-	-
MYW	97	95	98	MYW	81	58	72	MYW	100	94	94
PRW	13	7	54	PRW	14	6	43	PRW	23	19	83
RNY	50	46	92	RNY	41	20	49	RNY	63	57	90
YDR	147	138	94	YDR	59	42	71	YDR	80	64	80
Total	548	478	87	Total	550	287	52	Total	591	516	87

Table 3. Total number of desert mule deer fecal samples collected in 2017, number of samples for which amplification failed (data at <50% of loci for first 3 PCR replicates, samples not rerun), and percent failures broken down by sampling date.

Sampling date	n	# failed amplifications	% failed
20170613	33	7	21
20170614	47	7	15
20170620	58	8	14
20170621	65	3	5
20170627	66	4	6
20170628	102	2	2
20170706	114	17	15
20170707	63	1	2
Total	548	49	

For this population, the cumulative P_{ID} for all 14 microsatellite loci was 2.81×10^{-8}, and P_{IDsibs} was 3.53×10^{-4}, well below the study goals (Table 4). However, many samples were not successfully genotyped at all loci. We then ranked loci from least to most variable, and calculated the P_{ID} and P_{IDsibs} again, each time removing the next most variable locus (to simulate the worst case scenario of having one less locus typed). To obtain P_{ID} and P_{IDsibs} values at study goals, we therefore used only samples that had been genotyped at ≥10 loci (Table 4).

We ran multiple iterations of the identity-matching test in CERVUS, initially identifying samples that matched at 10 or more loci with no mismatches. For each set of identical samples we assigned a unique Deer ID and removed all but one of the replicates. In subsequent runs we relaxed the match stringency, allowing for a mismatch at up to two loci, as long as ≥10 loci were identical. If a mismatch could be explained by allelic dropout we kept the heterozygote, as allelic dropout is more common than spurious amplification in these markers. If a mismatch was not easily explained by allelic dropout, or if the two samples were different sexes, we did not consider the samples to be from the same individual and retained both in the final data set. If two or more samples from the same individual amplified at different loci we built a composite genotype to have the most complete possible genotype for that individual; these composite genotypes were denoted with "comp" suffix.

After unique individuals were identified within the 2017 data set, we compared these genotypes with those of the 2015 and 2016 data sets to investigate cross-year recaptures.

In the final 2017 data set, 478 samples (87%) produced data at ≥10 loci, 40 (7%) produced genotypes at ≤9 loci (insufficient data to analyse), and 30 (5%) failed completely at all 14 loci (Table 1). Genotype success was higher than that reported for the 2016 samples (52%) but identical to that of the 2015 samples. Unlike in 2016, sample genotype success in 2017 did appear to be predicted by the condition score assigned in the field, with 96% of samples classified as "good" in the field amplifying at ≥10 loci, versus only 25% of those considered "poor" (Table 5).

Table 4. Cumulative probability of identity for unrelated individuals (P_{ID}) and siblings or parent-offspring pairs (P_{IDsibs}) for desert mule deer samples collected from 2015-2017, with absolute numbers and percentages of samples retained in the final data set, for successively reduced numbers of loci.

	2017 (n = 548)				**2016 (n = 550)**				**2015 (n = 591)**			
Number of loci typed (excluding locus B)	P_{ID}	P_{IDsibs}	Samples retained	% retained	P_{ID}	P_{IDsibs}	Samples retained	% retained	P_{ID}	P_{IDsibs}	Samples retained	% retained
14	2.81E-08	3.53E-04	276	50	3.72E-08	3.94E-04	86	16	2.79E-08	3.44E-04	378	64
13	2.65E-07	8.76E-04	353	64	3.20E-07	9.54E-04	152	28	2.73E-07	8.63E-04	468	79
12	1.95E-06	2.05E-03	408	74	2.46E-06	2.26E-03	196	36	2.01E-06	2.02E-03	494	84
11	1.33E-05	4.71E-03	438	80	1.76E-05	5.24E-03	237	43	1.45E-05	4.69E-03	506	86
10	**7.1E-05**	**9.97E-03**	**478**	**87**	**9.17E-05**	**1.10E-02**	**287**	**52**	**6.98E-05**	**9.57E-03**	**516**	**87**
9	3.35E-04	2.03E-02	480	88	4.07E-04	2.19E-02	341	62	3.19E-04	1.92E-02	527	89

Table 5. Desert mule deer sample success (≥10 loci genotyped) versus total failure (0 loci genotyped) by field condition for samples collected in 2017.

Sample Condition (Field Notes)	total collected	amplified at ≥10 loci, excluding Locus B	% ≥10 loci	amplified at 0 loci (total failure)	% total failure
Good	421	403	96	7	1.7
Fair (or Fair/Good)	112	69	62	18	16.1
Poor (or Poor/Fair)	12	3	25	4	33.3
Blank	3	3	100	0	0

Within-year (2017) recaptures

We identified 211 unique deer individuals (103F, 89M, 19 undetermined), of which 109 (52%) were recaptured 1-15x during the 2017 sampling season. The individual deer captured the most times was a buck captured 16 times at drinker BGT, although these captures occurred across only three sampling dates. One hundred and two of these deer were not previously sampled in 2015 and/or 2016 (Table 6).

Across-year (2015-2017) recaptures

In total, 447 unique deer were sampled across the three years of this study (Table 6). There were 204 females, 212 males, and 31 deer whose sex could not be determined. Forty-two deer (9%) were sampled in all three years (25 females, 17 males).

Table 6. Number and sex of unique deer sampled within and across three years of sampling efforts (2015-2017) in the Chuckwalla Mountains of southern California.

Year(s) captured	n	Female	Male	Undetermined
2015 only	113	52	56	5
2016 only	84	27	50	7
2017 only	102	38	45	19
2015 and 2016	39	22	17	0
2015 and 2017	40	28	12	0
2016 and 2017	27	12	15	0
2015, 2016, and 2017	42	25	17	0
Total	447	204	212	31

Across all 447 unique deer for the three sampling seasons, the number of alleles per locus ranged from 2 (Table 7; loci F, H, J, and L) to 7 (locus N). Marker failure was most commonly seen at microsatellite loci C and H, and the sexing markers, ZFX and SRY-WFL. Three of these four markers have fragment sizes of 300bp or larger (excluding SRY-WFL, which is 220bp but is located on the Y chromosome), thus it is not unexpected that they would not work as well on fecal DNA, which is often degraded compared to DNA derived from tissue. No loci deviated from expectations of Hardy-Weinberg equilibrium (HWE) when data from all three years were pooled.

Table 7. Number of alleles (Na), number of samples genotyped (n), and expected (He) and observed (Ho) heterozygosity values for 14 microsatellite loci genotyped in 447 unique desert mule deer fecal samples collected from 2015-2017.

Locus	n	Na	Ho	He
C	381	3	0.54	0.61
D	446	3	0.54	0.54
F	447	2	0.08	0.08
G	421	3	0.57	0.61
H	373	2	0.43	0.47
J	427	2	0.46	0.50
K	442	4	0.67	0.70
L	418	2	0.27	0.29
M	444	5	0.34	0.34
N	428	7	0.73	0.74
P	441	6	0.72	0.72
R	408	4	0.45	0.48
S	435	6	0.63	0.64
V	445	3	0.41	0.38

Genetic structure

Although all samples were analysed as a single population every year, STRUCTURE and GENEPOP suggested that there may be some population structure (genetic differentiation) present (Fig. 1). The Evanno Δk method identified two clusters as the most likely partition (although this method often fails to identify hierarchical patterns of substructure). This suggests that two subpopulations of deer are represented in this data set; one cluster includes samples from drinkers CRW, LBN, and MYW, while the other cluster includes samples from BGT, BKH, DDM, PRW and RNY. Individuals from BHW, CKS, KNB, and YDR show higher levels of admixture between the two clusters. F_{ST} values between drinkers ranged from <0.001-0.08, with the highest values occurring for CRW versus BHW and CRW vs PRW (Appendix 2, Table S1). For reference, between small populations (25-200 individuals) of desert bighorn sheep linked by frequent inter-population movements, Epps et al. (2010) observed F_{ST} values of ≤ 0.05.

Although no deviations from Hardy-Weinberg equilibrium were seen when all data were pooled, suggesting that the degree of substructure is not severe, substructure may cause the calculated P_{ID} and P_{IDsibs} to be biased lower (i.e., suggest higher power to distinguish individuals than is actually the case) because not all alleles were seen in each subpopulation, and expected heterozygosity was lower for some loci in the CRW/LBN/MYW subpopulation relative to the whole data set (Appendix 2, Table S2). However, we recalculated the P_{ID} and P_{IDsibs} for the CRW/LBN/MYW subpopulation and found that the cutoff for minimum number of loci (≥10 genotyped) was the same.

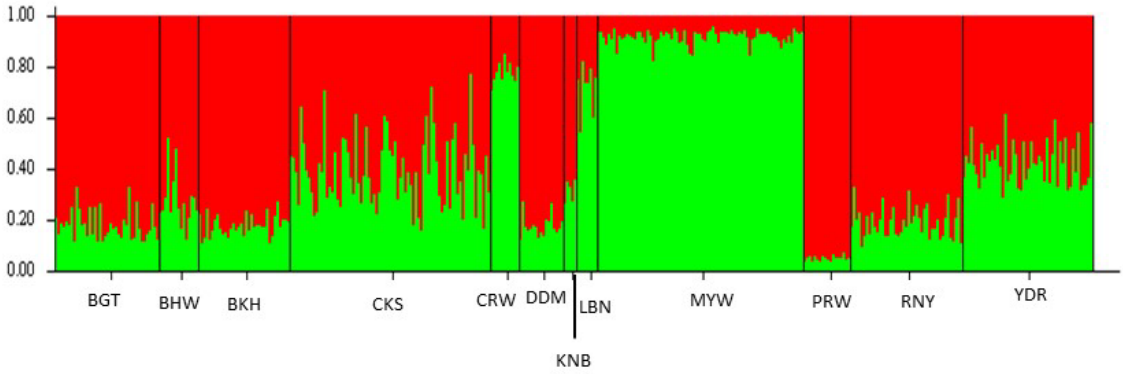

Fig. 1. STRUCTURE plots of individual assignments for deer sampled at 12 drinkers from 2015-2017, with two genetic clusters inferred. Each vertical bar represents an individual, with colors (red, green) of that bar representing proportional assignment to the two clusters.

Recommendations and future directions

The markers for this study were chosen to align with previous data sets; however, those previous studies presumably did not use fecal DNA. Because of the degraded nature of fecal DNA, some of the markers with larger product sizes suffered from allelic dropout or amplification failure, making matching analyses more difficult. Future studies that plan to use fecal DNA would be best served by using loci with amplification products <250bp.

In addition, the markers that were chosen for this study had 2-7 alleles each (mean = 3.7). Eight loci had three or fewer alleles observed across the whole data set, limiting our ability to distinguish an allelic dropout from a true homozygote in cases where two samples differed at only one locus. Future studies would benefit from choosing loci with more allelic diversity in these populations.

Sample amplification rates were high in the first and third years of the study (87% each), and much lower in the middle year (52%). It would be useful to identify factors related to sample collection/storage and environmental conditions that varied between 2015/2017 and 2016 and that might explain the differences in sample success rates.

In the 2017 data, it appears that the percent of successful samples varied by date collected, with highest failure rates on the first day of collection (20170613 and 20170614, Table 3). This could be due to differences in environmental conditions, storage of samples, or the collection of older samples. If not already incorporated, we suggest that future surveys incorporate an initial day to remove old samples from the landscape before collection begins.

Preliminary analyses using STRUCTURE and Genepop suggest that there are multiple genetically differentiated subpopulations of desert mule deer in this area, which should be taken into account when estimating demographic measures (e.g., deer density

across the landscape).

DNA extraction:
We processed burro deer fecal pellets using the pellet-scraping method detailed in Wehausen et al. (2004) to collect 0.03 g of scrapings from the exterior surface of pellets. We extracted DNA from pellet scrapings using a modified AquaGenomic Stool and Soil protocol (MultiTarget Pharmaceuticals LLC, Colorado Springs, CO). Modifications included the addition of 450 µL of AquaGenomic solution to pellet scrapings, the use of 1.0 mm silica/zirconium beads (BioSpec Products Inc., Bartlesville, OK) for cell lysis, and the addition of 12 mAU proteinase K (Qiagen Inc., Valencia, CA) for recovery of mitochondrial DNA. Lastly, we added 150 µL of AquaPrecipi solution (MultiTarget Pharmaceuticals) to cell lysate to remove PCR inhibitors present in fecal samples. We did not quantify DNA concentration.

PCR recipe and cycling conditions:
Fifteen dinucleotide microsatellite markers were analyzed in a single 10 µL reaction consisting of 5x Qiagen Multiplex PCR Master Mix, 10 µg of bovine serum albumin, 100uL of a primer cocktail of 18 multiplexed loci at varying concentrations (Table 2) and 1 µL of genomic DNA. Reactions were brought to volume with nuclease-free water. Thermalcycling conditions for the multiplexed loci were as follows: initial denaturation of 15 minutes at 95 °C, followed by 35 cycles of [95 °C for 30 seconds, 60 °C for 90 seconds, 72 °C for 60 seconds], and a final elongation of 30 minutes at 60 °C. For each locus, one primer was fluorescently tagged on the 5' end with NED, PET, VIC (Applied Biosystems, Carlsbad, CA) or 6-FAM (Sigma-Aldrich, St. Louis, MO). Negative and positive controls were included on each genotyping run. PCRs were run on BioRad C1000 and MyCycler thermalcycler machines (Bio-Rad Laboratories Inc., Hercules, CA).

Genotyping:
For microsatellite genotyping, each sample was initially amplified in three replicate PCRs. We generated consensus genotypes across replicates: for a homozygous genotype to be considered verified, the allele had to be typed in three separate replicates. To confirm a heterozygous genotype, each allele had to be observed at least twice. Samples with incomplete or discrepant data were rerun in an additional 2-6 replicates.

Amplification products were visualized on a 2% agarose gel prestained with GelRed. Products were diluted accordingly, ethanol-precipitated to remove salts, and submitted for fragment size analysis on the ABI DNA 3730 DNA analyzer (Applied Biosystems) at the Oregon State University Center for Genome Research and Biocomputing (Corvallis, OR). We used GeneScan 500 LIZ dye size standard (Applied Biosystems), and called allele sizes in GeneMapper v.4.1 (Applied Biosystems).

Table S1. Population pairwise F_{ST} values for desert mule deer sampled at 12 drinkers from 2015-2017.

pop	BGT	BHW	BKH	CKS	CRW	DDM	KNB	LBN	MYW	PRW	RNY
BHW	0.0163										
BKH	0.0132	0.0082									
CKS	0.0151	0.0113	0.0003								
CRW	0.0468	0.0805	0.0467	0.0399							
DDM	0.0131	0.0055	0.0056	0.0018	0.0396						
KNB	>0.001	>0.001	>0.001	>0.001	0.0334	>0.001					
LBN	0.0194	0.0503	0.0223	0.0076	0.0294	0.0306	>0.001				
MYW	0.0249	0.0197	0.0103	0.0054	0.0305	0.0149	>0.001	0.0006			
PRW	0.0344	0.0316	0.0287	0.0268	0.0691	0.0216	0.0288	0.0429	0.0494		
RNY	0.0182	0.025	0.0059	0.0076	0.0279	0.0109	>0.001	0.0027	0.0192	0.0154	
YDR	0.0136	0.0115	0.0055	0.0039	0.058	0.0135	>0.001	0.0004	0.0144	0.0203	0.005

Table S2. Number of alleles, number of unique deer genotyped, and observed and expected heterozygosity for 14 microsatellite loci genotyped in a) all unique deer sampled across all drinkers (n=447) and b) drinkers "Crocket Walla", "Leg Bone" and "Mayer Walla" (n=98) from 2015-2017.

a) All populations

Locus	n	Na	Ho	He
C	381	3	0.54	0.61
D	446	3	0.54	0.54
F	447	2	0.08	0.08
G	421	3	0.57	0.61
H	373	2	0.43	0.47
J	427	2	0.46	0.5
K	442	4	0.67	0.7
L	418	2	0.27	0.29
M	444	5	0.34	0.34
N	428	7	0.73	0.74
P	441	6	0.72	0.72
R	408	4	0.45	0.48
S	435	6	0.63	0.64
V	445	3	0.41	0.38

b) Drinkers CRW, LBN, and MYW

Locus	n	Na	Ho	He
C	84	3	0.64	0.61
D	98	3	0.61	0.58
F	98	2	0.02	0.02
G	92	3	0.58	0.63
H	81	2	0.51	0.5
J	93	2	0.45	0.5
K	98	4	0.66	0.7
L	93	2	0.3	0.36
M	97	3	0.28	0.29
N	95	7	0.75	0.76
P	97	5	0.79	0.74
R	90	3	0.44	0.44
S	96	4	0.58	0.66
V	98	2	0.29	0.29

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APPENDIX B

FINAL REPORT: INDIVIDUAL IDENTIFICATION OF DESERT BIGHORN SHEEP (*Ovis canadensis nelsoni*) from fecal pellets collected in the Marble Mountains, Mojave in 2017, with recaptures of individuals sampled in 2016

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Summary

In total we received 277 desert bighorn sheep samples, all of which we attempted to genotype at 9 microsatellite loci plus one marker used for sex identification. Six samples showed evidence of contamination and were removed from the final data set. Of the remaining 271 samples, 269 (99%) produced data at ≥7 of the 9 microsatellite loci and were used in individual identification analyses. From the 269 complete or mostly-complete samples we identified 107 unique bighorn sheep individuals, of which 72 were recaptured in 2017 (1-9 times each) and 35 were sampled only once. Of the 107 unique individuals there were 66 females and 41 males. Seventy-seven of these bighorn were also captured in the 2016 sampling period.

Introduction

This study is the second consecutive yearly sampling of bighorn sheep in the Marble Mountains of the Mojave National Preserve. The study was initiated to compare the accuracy and efficacy of fecal-based abundance estimates of desert bighorn sheep with other methods, including helicopter surveys. We extracted and genotyped samples as per the protocols used in 2016.

Methods

During the summer of 2017, bighorn pellets were collected at three artificial water sources ("drinkers") in the Marble Mountains, in addition to one survey transect. Pellets were placed in paper bags and stored at room temperature until they were processed in early 2018. At OSU, we scraped cells from the surface of pellets and used a modified commercially available kit (AquaGenomics and AquaPrecipi; Multitarget Pharmaceuticals, Colorado Springs, CO; Appendix 1) to extract DNA from these scrapings. For ease in labelling microcentrifuge tubes, we renamed samples with consecutive numbers and the three-letter code of the drinker at which they were collected, or the word "survey" for the transect (Table 1, provided as a separate excel spreadsheet). Since all drinkers were sampled in 2016, the new sample number assigned at OSU began where that previous dataset ended to prevent duplicates. We received 278 samples (two envelopes had the same name, 20170708JM084, but we only analysed the first one,

which we renamed VAL 192). In total, we analysed 277 samples.

We attempted to amplify ten markers (9 microsatellites plus one marker for sex identification) for each sample using the polymerase chain reaction (PCR). Primer sequences, concentrations, and dye labels remained the same as in Crowhurst and Epps (2017), but are provided in Table 2 for convenience.

Table 2. Microsatellite loci used for individual analysis of desert bighorn sheep (*Ovis canadensis nelsoni*) fecal samples from the Marble Mountains, Mojave National Preserve, California, with fluorescent dye labels, primer concentrations and references for the original primer publication.

Locus	Reference	Dye Label	Primer Concentration (uM)
AE129	Penty *et al.* 1993	Vic	0.25
AE16	Penty *et al.* 1993	Ned	0.2
OarFCB193	Buchanan & Crawford 1993	Pet	0.25
OarFCB304	Buchanan & Crawford 1993	Pet	0.2
OarHH62	Ede *et al.* 1994	6-Fam	0.15
MAF33	Buchanan & Crawford 1992	Vic	0.175
MAF36	Swarbrick *et al.* 1991	Vic	0.1
MAF48	Buchanan *et al.* 1991	Ned	0.2
TCRBV62	Crawford *et al.* 1995	6-Fam	0.25
SE47/48*	Yamamoto *et al*. 2002	Ned	0.2

*Sex identification marker

Samples were initially amplified in three separate PCR reactions each; those that produced ≥3 alleles at any locus were considered contaminated and not rerun (n=6). Samples that produced partial genotypes at ≥50% of the microsatellite loci were rerun three more times. For a genotype to be accepted for a particular locus, each allele in a heterozygous genotype had to be observed twice, while the single allele in a homozygous genotype had to be observed three times.

Using the online individual-identification program CERVUS version 3.0.3. (Kalinowski et al. 2007) and the population-specific allele frequencies tabulated for this population, we estimated the cumulative probability of identity for unrelated bighorn sheep (P_{ID}) and for siblings or parent-offspring pairs (P_{IDsibs}) for all 9 microsatellites. We then recalculated P_{ID} and P_{IDsibs} with successively reduced numbers of loci, to determine the minimum number of loci required to identify individuals. When identifying recaptures we used a maximum P_{ID} of $1x10^{-4}$ and P_{IDsibs} of $1x10^{-2}$. We analysed all samples as one population and searched for recaptures across all three drinkers and the survey transect. After identifying recaptures within the 2017 samples, we compared those unique individuals against genotypes from bighorn sampled in 2016.
We used GenAlEx (Peakall and Smouse 2006, 2012) to calculate number of alleles, expected and observed heterozygosities for each locus, and to test whether loci were in Hardy-Weinberg equilibrium.

Results and Discussion

Of the 277 samples received and analysed, six samples exhibited three or more alleles for at least one locus, suggesting contamination or the accidental collection of pellets from multiple individuals into one envelope. Contaminated samples were collected at the Vernandyles drinker (n=4) and on the survey transect (n=2). These samples were excluded from final analyses of recaptures, resulting in a data set of 271 genotypes.

For this population, the cumulative P_{ID} for all nine microsatellite loci was 3.78×10^{-8}, while the cumulative P_{IDsibs} was 7.88×10^{-4} (Table 3). We ranked the loci from most to least-variable, and recalculated the PID metrics, each time removing the next most variable locus (to simulate the worst case scenario of having one less locus typed). To obtain P_{ID} and P_{IDsibs} values at or below the thresholds established for this study (see above), we only included in our individual-matching analyses those samples that were genotyped at ≥7 loci (Table 3). After reruns were completed, two samples were genotyped at too few loci to be retained in the individual-matching analyses, leaving a final data set of 269 samples with sufficient genetic information (Table 1). Genotype success (269 of 271 samples; 99%) was similar to that observed with the 2016 samples (97%).

Once perfect matches had been identified and removed, we reran the individual-matching software allowing one "fuzzy match" (i.e., mismatching locus). We then screened these matches by eye to determine whether the mismatch could be explained by allelic dropout in one of the samples. If so, then we considered the samples to be from the same individual, otherwise we retained both samples as unique individuals. The quality of samples was so high that very few of the mismatches could be attributed to allelic dropout, thus we retained the majority of the samples implicated in a mismatched pair, and did not relax the matching stringency any farther.

Table 3. Cumulative probability of identity for unrelated individuals (P_{ID}) and sibling or parent-offspring pairs (P_{IDsibs}) for desert bighorn sheep (*Ovis canadensis nelsoni*) samples collected in the Marble Mountains, Mojave National Preserve, in 2017.

Number of loci typed	P_{ID}	P_{IDsibs}
9	3.78 x 10-8	7.88 x 10-4
8	6.00 x 10-7	2.19 x 10-3
7	**8.45 x 10-6**	**5.93 x 10-3**
6	6.55 x 10-5	1.41 x 10-2
5	4.34 x 10-4	3.16 x 10-2

Table 4. Number of unique bighorn sheep genotyped (n), allelic richness (Na), allele size range, and observed (H_O) and expected (H_E) heterozygosity values for nine microsatellite loci and one sexing marker analysed in desert bighorn sheep (*Ovis canadensis nelsoni*) fecal samples from the Marble Mountains, Mojave National Preserve.

Locus	N	Na	H_O	H_E	Range (bp)
AE129	107	6	0.85	0.801	167-187
AE16	107	5	0.72	0.724	84-94
OarFCB304	107	3	0.673	0.642	144-150
OarHH62	106	7	0.783	0.815	104-130
MAF33	107	3	0.533	0.613	122-126
MAF36	85	4	0.506	0.439	87-99
MAF48	107	5	0.701	0.666	120-128
OarFCB193	107	5	0.654	0.666	105-117
TCRBV62	107	5	0.738	0.692	169-179
SE47/48*	107	n/a			214 (Y chrom) 258 (X chrom)

*Sex identification marker

The number of alleles per locus ranged from 3 (loci OarFCB304 and MAF33; Table 4) to 7 (locus OarHH62). After removing all recaptures from the data set, no locus showed significant departure from Hardy-Weinberg equilibrium.

Within-year recaptures (2017)

We identified 107 unique bighorn sheep individuals, of which 72 (67%) were recaptured in 2017 (1-9 times each) and 35 (33%) were sampled only once. Of the 72 bighorn recaptured within the 2017 sampling period, 48 (66%) were females and 24 (33%) were males. Of the 35 bighorn detected only once in the sampling period, 18 (51%) were females and 17 (49%) were males. Thus, in the 107 unique individuals there were 66 females and 41 males.

The greatest number of recaptures in 2017 was for a ram sampled 10 times between the I40 and Vernandyles drinkers and the survey transect ("2016Sheep129", Table 1). This individual was captured only once during the 2016 sampling period, but was captured during seven different sampling events in 2017.

Across-year recaptures (2016-2017)

We identified 141 unique bighorn in the samples collected during the 2016 sampling season, and 107 unique bighorn sampled in 2017. There were 77 individuals sampled in both years (50F, 27M), and 30 new bighorn sampled in 2017 (16F, 14M). For individuals recaptured in 2017 we assigned the SheepID number first given to that animal in 2016 (Crowhurst and Epps 2017). Individuals sampled for the first time in 2017 were assigned a SheepID number starting at Sheep142, to continue where the 2016 data set ended. In total, 171 unique sheep were sampled across the two periods (104F, 67M).

DNA extraction:

We processed desert bighorn fecal pellets using the pellet-scraping method detailed in Wehausen et al. (2004) to collect 0.03g of scrapings from the exterior surface of pellets. We extracted DNA from pellet scrapings using a modified AquaGenomic Stool and Soil protocol (MultiTarget Pharmaceuticals LLC, Colorado Springs, CO). Modifications included the addition of 450 µL of AquaGenomic solution to pellet scrapings, the use of 1.0 mm silica/zirconium beads (BioSpec Products Inc., Bartlesville, OK) for cell lysis, and the addition of 12 mAU proteinase K (Qiagen Inc., Valencia, CA) for recovery of mitochondrial DNA. Lastly, we added 150 µL of AquaPrecipi solution (MultiTarget Pharmaceuticals) to cell lysate to remove PCR inhibitors present in fecal samples. We did not quantify DNA concentration.

A subset of samples produced very dark brown DNA that was comparable to a strongly brewed coffee (DNA samples extracted using the AquaGenomics/AquaPrecipi system are typically clear-colourless or slightly yellow tinged). Dark DNA was diluted from 1:4 - 1:10 with water (depending on colour) to dilute inhibitory agents before use in PCR.

PCR recipe and cycling conditions:

Nine microsatellite markers plus one marker for sexing were analyzed in a single 10 µL reaction consisting of 5x Qiagen Multiplex PCR Master Mix, 10 µg of bovine serum albumin, 0.1-0.25uM of each primer (Table 2) and 1 µL of genomic DNA. Reactions were brought to volume with nuclease-free water.

Thermalcycling conditions for the multiplexed loci were as follows: initial denaturation of 15 minutes at 95 °C, followed by 35 cycles of [95 °C for 30 seconds, 60 °C for 90 seconds, 72 °C for 60 seconds], and a final elongation of 30 minutes at 60 °C. For each locus, one primer was fluorescently tagged on the 5' end with NED, PET, VIC (Applied Biosystems, Carlsbad, CA) or 6-FAM (Sigma-Aldrich, St. Louis, MO). Negative and positive controls were included on each genotyping run. PCRs were run on BioRad C1000 and MyCycler thermalcycler machines (Bio-Rad Laboratories Inc., Hercules, CA).

Genotyping:

For microsatellite genotyping, each sample was initially amplified in three replicate PCRs. We generated consensus genotypes across replicates: for a homozygous genotype to be considered verified, the allele had to be typed in three separate replicates. To confirm a heterozygous genotype, each allele had to be observed at least twice. Samples with incomplete or discrepant data were rerun in an additional 2-6 replicates. Any sample that consistently showed more than two alleles at a single locus was considered contaminated and removed.

Amplification products were visualized on a 2% agarose gel prestained with GelRed. Products were diluted accordingly, ethanol-precipitated to remove salts, and submitted for fragment size analysis on the ABI DNA 3730 DNA analyzer (Applied Biosystems) at the Oregon State University Center for Genome Research and

Biocomputing (Corvallis, OR). We used GeneScan 500 LIZ dye size standard (Applied Biosystems), and called allele sizes in GeneMapper v.4.1 (Applied Biosystems).

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