FINAL REPORT: CHARACTERIZING THE SPREAD AND CONSEQUENCES OF RESPIRATORY DISEASE FOR BIGHORN SHEEP

Part I: Mojave Desert Bighorn Sheep Survival and Movement Study 2013-2018

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Part II: Evaluation of Gene Flow Among Desert Bighorn Sheep Populations in the Mojave Desert, California, c. 2000-2003 and 2014-2018

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PART I: MOJAVE DESERT BIGHORN SHEEP SURVIVAL AND MOVEMENT STUDY 2013-2018

This report summarizes research conducted in the Mojave Desert, California, following the 2013 pneumonia outbreak and subsequent die-off of bighorn sheep (*Ovis canadensis nelsoni*) at Old Dad Peak (Kelso Mountains). Since the outbreak, pneumonic bighorn have been observed throughout the Mojave Desert system in California, and *Mycoplasma ovipneumoniae* (*M. ovi*) has been documented in all infected populations. Our research evaluated adult female survival, lamb survival, and bighorn movement in connection with the epizootic. The results presented herein are derived from data collected from November 2013 to December 2018; our analyses have not been finalized for all aspects of the study, and some results are preliminary as indicated.

INTRODUCTION

In May and June of 2013, a pneumonia outbreak linked to *M. ovi* led to an all-age die-off of desert bighorn sheep (*O. c. nelsoni*) at Old Dad Peak (Kelso Mountains) in the Mojave Desert, California (Epps et al. 2016). Previously, bighorn sheep in this region of the Mojave were believed to be insulated from the threat of pneumonia because of reduced connectivity with neighboring domestic and wild sheep systems. How the pathogen entered the population is unknown, but subsequent to the outbreak, clinical signs of disease were observed in multiple bighorn sheep populations throughout the system. In November 2013, 2014, and 2015, adult bighorn from Old Dad Peak and 8 neighboring ranges (i.e., South Soda, Cady, North Bristol, South Bristol, Marble, Clipper, Hackberry, and Woods Mountains; Figure 1) were captured and tested for *M. ovi* infection via polymerase chain reaction (PCR). The same strain of *M. ovi* was detected in adult bighorn from all ranges except the South Soda Mountains, where animals were PCR-negative for the pathogen but seropositive for *M. ovi* antibodies. We attempted to screen for additional respiratory pathogens as well, including leukotoxigenic *M. haemolytica and B. trehalosi*, but storage and transport of samples was difficult and resulted in field failure. To date, the incident that occurred at Old Dad Peak is the only known pneumonia induced die-off observed in the Mojave Desert ecosystem in California, although retrospective screening of banked serum collected in 2002-2005, and tested in 2014, revealed that some bighorn in the South Bristol and Marble Mountains were in fact seropositive for *M. ovi* antibodies as early as 2002, while bighorn at Old Dad Peak were seronegative in 2005. The strain of *M. ovi* that animals were exposed to during this earlier time remains unknown.

As yet, disease dynamics of epizootic pneumonia in bighorn sheep are not completely understood, and likely vary by region, host ecology, and causal pathogens, but pneumonia outbreaks seem to have a common pattern. An all-age die-off is often the first signal of a pneumonia invasion into a naïve population, and is typically followed by high lamb mortality in subsequent years, likely due to chronically infected adult females passing pathogens to their offspring through physical contact (Cassirer et al. 2013, 2018, Manlove et al. 2014, Wood et al. 2017). Not all surviving adults become chronically infected, however, those that do can maintain infection within populations for decades (Plowright et al. 2017, Cassirer et al. 2018), while adult survival can be relatively stable in years following an initial outbreak (Cassirer et al. 2013, Manlove et al. 2016). There also appears to be a seasonal component associated with acute pneumonia-induced mortality, whereby age-specific die-offs in adults typically occur during the breeding season when contact rates increase (Cassirer et al. 2013). Aside from seasonality, how other factors contribute to disease patterns remains unclear, but population density along with stochastic variables, such as weather and range condition, may also influence expression of the disease (Dunbar 1992, Ryder et al. 1992, Monello et al. 2001, Singer et al. 2001, Wolfe et al. 2010). For example, precipitation is strongly linked to productivity in desert bighorn sheep populations through quality and quantity of forage (Wehausen et al. 1987, Wehausen 2005), but harsh winter weather has

also been noted as a possible factor influencing pneumonia in both wild and domestic sheep (McIlroy et al. 1989, Ryder et al. 1992, Wolfe et al. 2010).

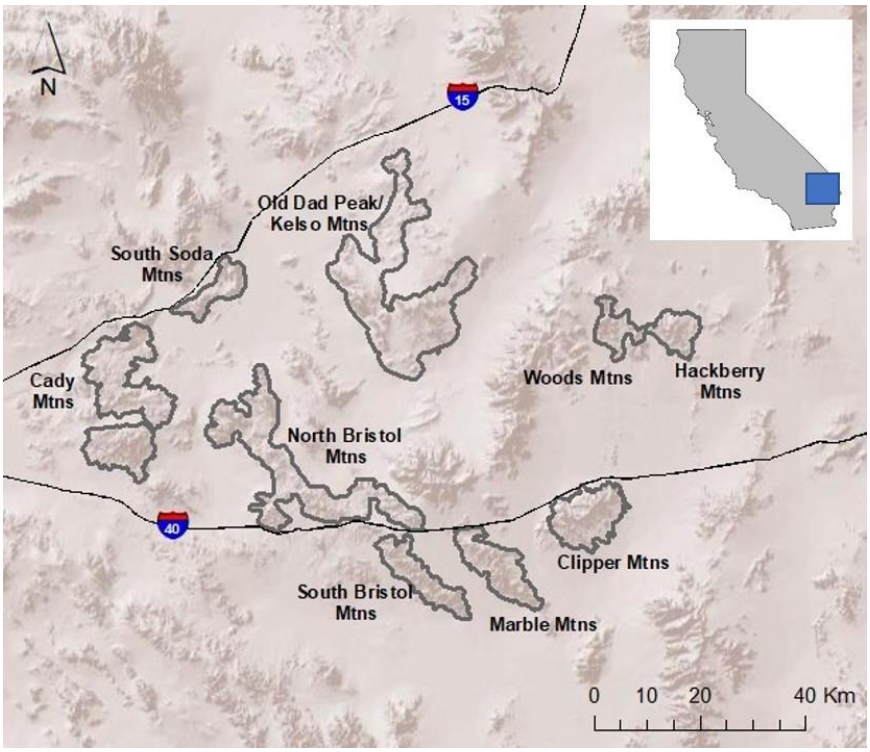

Figure 1. Map of study area.

As such, we conducted research to investigate disease dynamics in this system. Our objectives were to evaluate post-outbreak adult female survivorship (Objective 1), post-outbreak lamb survival (Objective 2), and assess individual-level potential transmission risk via potential for contact within and between populations by analyzing movement trends (Objective 3). We elaborate on these objectives below.

Objective 1: Post-Outbreak Adult Female Survivorship

We investigated post-outbreak survivorship of adult female bighorn (> 2 years old) across 9 populations for ~3.5 years (November 2013 to March 2017) in the Mojave Desert, California, and evaluated the effect of *M. ovi* exposure and infection on seasonal survival, while testing effects of range-specific factors that could potentially drive differences in adult survival and the effect of age. We had 3 hypotheses:

> *H1) M. ovi exposure and infection lowers post-outbreak survival of adult females.* We assumed that females who were carrying the *M. ovi* pathogen at capture could suffer fatality from acute infection, experience reduced health from disease post-recovery, or incur debilitating effects from prolonged infection. Mycoplasmal species are known to induce chronic and latent disease states in animals and humans (Waites and Talkington 2004), and bighorn sheep exposed to *M. ovi* can become chronic, non-clinical carriers of the pathogen (Plowright et al. 2017).

H2) Range-specific factors, specifically forage quality, population size, and inclement weather, further influence survival of adult females. We assumed that females in ranges with higher forage quality would have better nutrition and overall health (Bender and Weisenberger 2005); we predicted that higher forage quality would therefore partially offset the effect of infection (Wehausen et al. 1987, Dunbar 1992, Miller et al. 2012), but in larger populations, increased resource competition might cause a negative density-dependent effect (McCullough 1979, Clutton-Brock et al. 1997, Bowyer et al. 2014). We also considered that larger populations could have lower survival owing to higher contact rates and levels of disease prevalence if disease transmission was density-dependent (Begon et al. 2002, Lloyd-Smith et al. 2005, Cassirer et al. 2013). Finally, we predicted that inclement weather (i.e., heavy precipitation during colder months of the year) would add to the effect of infection (McIlroy et al. 1989, Ryder et al. 1992, Wolfe et al. 2010) and that higher winter precipitation would have a negative effect on winter survival.

H3) Increasing age lowers adult survival. Older age is associated with senescence and higher mortality among all species and has been linked to higher rates of *M. ovi* infection among adult bighorn (Plowright et al. 2017).

Objective 2: Post-Outbreak Lamb Survivorship

We investigated post-outbreak survivorship of bighorn neonates across 7 populations (i.e., Old Dad Peak/Kelso, Marble, South Bristol, Cady, South Soda, North Bristol and Clipper Mountains) during lambing season (spring through summer) in 2014-2016 using lamb-ewe ratios. Assuming the presence of *M. ovi* in all populations, we evaluated differences in lamb survival based on range-specific factors including seasonal precipitation, NDVI (normalized difference vegetation index), and adult female population size. We had 3 hypotheses:

H1) Higher winter precipitation increases bighorn neonate survival. Lambs born into *M. ovi*-infected populations typically contract pneumonia and die at 3-4 months of life (Plowright et al. 2017, Cassirer et al. 2018), but yearly fluctuations in lamb survival following an outbreak have also been observed. Winter precipitation contributes to diet quality during the growing season (February through June) and is positively correlated with lamb survival (Wehausen 2005). We assumed that lambs in our study populations would likely become infected with *M.* ovi within the first 3-4 months of life, but we predicted that in ranges and years with higher winter precipitation, increased diet quality might offset the impact of pneumonia and increase neonate survivorship, as many studies have shown that immune function and disease resistance are dependent on nutrition (Ullrey 1993, Lochmiller and Deerenberg 2000, Cotter et al. 2011, Brunner et al. 2014).

H2) Higher summer and autumn nutrition in the preceding year increases neonate survivorship. Maternal nutrition is a strong predictor of neonate survival (Mech et al. 1991, Bishop et al. 2009, Monteith et al. 2014), and many studies have identified summer and autumn nutrition as important correlates for overwinter survival and reproductive success in adult female ungulates (Mautz 1978, Cook et al. 2004, Monteith et al. 2014). We predicted that lambs in ranges with higher summer and autumn nutrition in the previous year (as approximated by NDVI) would be born to females better able to provision for offspring, and would therefore have higher nutritional condition and ultimately higher survival despite exposure to *M. ovi*.

H3) Smaller populations allow for higher neonate survival. Given the negative effects of density-dependence associated with higher resource competition in larger populations (McCullough

1979, Clutton-Brock et al. 1997, Bowyer et al. 2014), we predicted that neonate survival would likely be higher in smaller populations due to greater nutrient availability, and therefore neonate nutrition, that could potentially increase resistance to disease. We also considered that contact rates between infected and uninfected individuals might be lower in smaller populations, and disease transmission to neonates might be lower as well.

Objective 3: Movement and Potential Transmission Risk

We assessed individual-level potential transmission risk, or contact potential, between populations by examining bighorn movement trends and estimating the probability of intermountain movements with respect to sex, age, season, and PCR status at capture. We assumed that animals who were more likely to make intermountain movements were also more likely to spread *M. ovi* to populations in other mountain ranges, provided positive infection status did not preclude movements. We will further investigate potential transmission risk within populations by examining long-range movement patterns of individuals and seasonal population distributions, in order to assess contact potential between individuals and groups throughout the year. We have 2 hypotheses:

H1) Rams are more likely to make long-range, extra-home range or intermountain movements, which is further dictated by age and seasonality. Dispersal and long-range movements have been observed more frequently in males than females (Bleich et al. 1997, Borg 2013), and given seasonal differences in behavior and physiological requirements between the sexes and across age classes (Bleich et al. 1997, Barboza and Bowyer 2001), we predicted that age and time of year would further influence such movements and potential for disease spread.

H2) Contact potential between groups within a single population varies seasonally. Spatial structure of bighorn populations appears to have a seasonal component, whereby animals typically commingle in high concentrations during the breeding and lambing seasons (Cassirer et al. 2013), thereby increasing potential contact rates. We suppose that contact between infected and susceptible individuals therefore varies seasonally as well, and during periods when potential contact within the population is highest, transmission risk increases.

METHODS

Objective 1: Post-Outbreak Adult Female Survivorship

We used data from 115 radio-collared females to track survival across 9 populations and applied the known-fate model in Program MARK (White and Burnham 1999) to model survivorship from November 2013 to March 2017. Animals were aged based on horn growth (i.e., number of horn annuli) and tooth eruption patterns (Deming 1952, Geist 1966, Heffelfinger 1997). We collected serum and nasal swab samples which were analyzed by Washington Animal Disease Diagnostic Laboratory (WADDL; Pullman, WA) via competitive enzyme-linked immunosorbent assays (cELISA) and PCR to determine exposure and infection status at time of capture. We used seasonal female home ranges estimated with 90% kernel density contours in ArcGIS 10.5 (ESRI 2016) to extract mean seasonal NDVI from Landsat 4, 5, 7, 8, and winter precipitation data from CHIRPS (Climate Hazards Group InfraRed Precipitation with Station data; Funk et al. 2015, Climate Engine 2017); seasons were defined based on a climograph for the Mojave National Preserve (McKee et al. 2015).

Population estimates were derived from camera data collected at point source water features from June through September 2016, using Bushnell Trophy cameras (standard and hybrid 8MP) set to a 1-second delay. Photos and videos were sorted into activity periods for each day and were scored to obtain numbers of marked and unmarked individuals. We defined an activity period as beginning from the time

when the first bighorn sheep in a group appeared at water and ending at the time when the last bighorn sheep in a group left, with activity typically ceasing for $\geq$ 20 minutes between periods. We counted all marked and unmarked adult females (i.e., > 2 years old) in each activity period and calculated totals for each day, treating a single day as a sampling unit (i.e., occasion). Days were generally censored if there were any activity periods that elapsed for > 1.5 hours with > 5 adult females present, as longer time periods with more individuals could render uncertainty in count totals. We then applied the generalized form of Bowden's estimator ($\tilde{N}$; Bowden and Kufeld 1995) to the total number of marked and unmarked adult females observed in a subset of days in each mountain range to estimate population sizes of adult female bighorn.

We attempted to use at least 16 occasions for each population in order to achieve an average of marked animal sightings per marked individual > 1, with a higher proportion of the marked sample detected (> 0.7 in most cases), so as to reduce variance and increase precision (Bowden and Kufeld 1995, Diefenbach 2009), and adjusted the number of occasions depending on available data. We drew occasions consecutively beginning from July through August (when activity was highest), and subsequently from September and June to add occasions as needed. In ranges where camera data were collected from > 1 point-source water feature, occasions were distributed evenly across sites.

To ensure that unmarked individuals were not double-counted within a single occasion, we compared individuals across all activity periods throughout the day, and censored occasions if any unmarked individuals were indistinguishable (usually because of image quality). Unmarked individuals were identified by unique horn and pelage characteristics, and marked individuals were identified by ear tag combinations. We acknowledge that double-counting across activity periods may have occurred, however, despite efforts to control for replacement within occasions. We also recognize that our estimates are only representative of the number of adult females that used point-source water features and therefore serve as a relative index for the true population size, or abundance, within each mountain range over the study period.

Age was modelled as a dummy variable (Cooch and White 2017) whereby females 2-9 years old received a covariate value of 0, and females > 9 years old received a value of 1. Females that became older than 9 years during the study were moved from the former cohort to the latter upon aging out. Infection and exposure status were also modelled as dummy variables (Cooch and White 2017) whereby individuals received covariate values of 1 if they were PCR- or cELISA-positive at time of capture, values of 0 if they were negative, and a mean value of 0.5 if a test result was indeterminate based on results from WADDL or infection/exposure status was otherwise unknown due to missing or compromised samples. All of our continuous variable inputs (i.e., NDVI, winter precipitation, and population size) were *z*-standardized; we used the Pearson correlation coefficient to assess relatedness of these variables prior to model fitting. Models were ranked using Akaike Information Criterion adjusted for small sample sizes (AICc; Akaike 1973, Hurvich and Tsai 1989). We interpreted covariate effects in our top models (ΔAICc scores < 2) based on model-averaged estimates with 90% confidence intervals (Burnham and Anderson 2010, Arnold 2010, Symonds and Moussalli 2011, Monteith et al. 2014), which we compared to results from our top 2 models. We identified predictor variables with summed AICc weights (ΣAICc *w*) closer to 1 as most important (Burnham and Anderson 2010, Symonds and Moussalli 2011).

Objective 2: Post-Outbreak Lamb Survivorship

We collected camera data as described under Objective 1 and combined camera observations with field observations to track lambing status of collared females in 3 ranges in 2014 (Old Dad Peak/Kelso, Marble, and South Bristol Mountains), and 7 ranges in 2015 and 2016 (Old Dad Peak/Kelso, Marble, South Bristol Mountains, Cady, South Soda, North Bristol and Clipper Mountains). Field

observations were gathered opportunistically (i.e., during site visits, surveys, and ground counts) and systematically (i.e., by targeting specific individuals and conducting ground searches using GPS collar location data and telemetry). Ewes were identified as having a lamb if they were seen with a lamb at heel and engaged in nurturing or otherwise associative behavior (i.e., nursing, nuzzling, traveling to and from a site together, or appearing repeatedly together), and if ewes were lactating. We aged lambs by horn length and rostrum size (Bleich 1982), and visually monitored lactation in ewes by examining udder condition and estimating size when possible (i.e., full, ½ full, ¼ full, < ¼ full). If lambs survived to 4-5 months of age, we presumed they had survived to the start of weaning and had passed the critical period of lambing when the risk of pneumonia-related mortality is highest (Cassirer et al. 2013).

We assumed that ewes had lost their lambs to mortality if they were pregnant prior to the lambing season but were never seen with lambs, or became solo over the course of the field season and their udders dried prematurely. Generally, given confirmation of positive pregnancy status (i.e., via serum testing or observation with a lamb), if 2 of the 3 following criteria were met, we assumed the lamb had died: 1) the ewe's udder went dry prematurely, 2) the lamb was never observed or stopped appearing on camera, 3) the ewe was observed alone in the field. We concluded that a ewe was without a lamb in the field if she was observed alone in areas where a lamb could be easily seen if present; these observations were typically confirmed with follow-up observations as well. If we were unable to determine pregnancy status prior to lambing, or whether a lamb had survived or died, we identified the ewe as an unknown case and removed her from the sample. The sample with unknowns removed is referred to hereafter as the adjusted sample.

For ewes that were collared in autumn (early November) preceding a field season, pregnancy status prior to lambing was determined by testing blood serum collected during capture for Pregnancy-Specific Protein B (Wood et al. 1986; lab work was performed by BioTracking, Inc., Moscow, Idaho). For ewes collared in previous years, we determined pregnancy status for that season by visually inspecting the udder for signs of swelling when ewes first appeared on camera or were observed in the field earlier in the season. Ewes identified as nonpregnant were placed in the category of unknown and removed from the sample.

Camera data were also used to estimate late season (15 August to 30 September) lamb-ewe ratios, which we compared to the proportion of surviving lambs associated with collared ewes. This step allowed us to validate lamb-ewe ratios for further analysis of survival. Lamb-ewe ratios were generated from camera count data in much the same way described under Objective 1. We treated a single day as a sampling unit (i.e., occasion) and counted adult females (i.e., > 2 years old) and lambs in each activity period per day to produce daily totals of adult females and lambs observed. We attempted to use at least 8 occasions from each camera site (4 occasions each from August and September) within a given range per year, and calculated ratios by dividing the sum of all daily lamb totals by the sum of all daily ewe totals for each range and year. We used Pearson's correlation coefficient to evaluate the relationship between proportion of surviving lambs with collared ewes and late season lamb-ewe ratios for each population and year for which we had both data types, and determined that the 2 survival metrics were strongly correlated (r = 0.92). We suspect that smaller adjusted samples sizes of marked ewes may have produced less accurate estimates of lamb survival from the proportion surviving in some cases, and lamb-ewe ratios are likely a more reliable index of lamb survival because they were generated using data from the population at large.

As such, we modelled lamb survival using standard linear regression with late season lamb-ewe ratios as a response variable, and evaluated effects of seasonal precipitation for winter, spring, and summer concurrent with pre-lambing and lambing (December through September), along with effects of

nutrition (as approximated by NDVI) from the preceding summer and autumn, and the effect of population size. We used the Pearson correlation coefficient to assess relatedness of input variables prior to model fitting. We used AICc for model ranking (Burnham and Anderson 2010) and interpreted variable effects based on our top model.

Objective 3: Movement and Potential Transmission Risk

We used data from 123 radio-collared bighorn (25 males and 98 females) from our 9 focal populations and the Newberry Mountains to analyze movement trends and estimate probability of intermountain movements with respect to sex, age, season, and PCR status at capture between November 2013 and December 2018. We used logistic regression to test effects given whether an individual made an intermountain movement over the period of the study, whereby an animal received a 1 for an intermountain movement and a 0 for no intermountain movement as the response variable. Seasons were modelled as categorical variables, and individual covariates were modelled as dummy variables (Cooch and White 2017); animals received an input value of 0 if they were < 5 years old and 1 if they were > 5 years old, 0 if they were female and 1 if they were male, and 0 if they were PCR-negative at capture and 1 if they were PCR-positive. Animals were aged as described under Objective 1. We first fit models with covariates for season and found no significant effects. We then tested candidate models with all variable combinations of age, sex, and PCR status. We used AICc for model ranking and interpreted covariate effects according to our top model (Burnham and Anderson 2010).

To assess contact potential between individuals and groups within a population throughout the year, we will examine daily step-length distributions (or daily movement rates; Morales et al. 2004, Fryxell et al. 2008) and use standard linear regression to evaluate the same individual covariates listed above, given seasonal median and upper quartile step-lengths as response variables. This approach will allow us to identify individuals that moved greater distances (based on daily displacement by season) and who were therefore potentially more likely to spread disease within the population during different seasons. We will also estimate seasonal home range size at the population-level using kernel density estimates at 50% and 90% quantiles to compare differences in spatial distribution across seasons, and thereby determine when populations were most concentrated and therefore contact potential between individuals and groups was presumably highest.

RESULTS

Objective 1: Post-Outbreak Adult Female Survivorship

We examined pairwise correlations between winter precipitation and NDVI in summer and autumn based on Pearson correlation coefficients and found no statistically significant relationships (winter precipitation/summer NDVI: $r = 0.3$; winter precipitation/autumn NDVI: $r = 0.047$). There was a small negative correlation between population size and summer NDVI ($r = -0.4$) that was significant, while the correlation between population size and autumn NDVI was also negative but lacked statistical support ($r = -0.33$). The pairwise correlation between summer and autumn NDVI was strongly supported ($r = 0.92$), and we therefore tested these variables separately in the candidate model set (Table 1).

Our top 2 models indicated that summer and autumn NDVI were associated with a positive lag effect on winter survival, winter precipitation was negatively correlated with winter survival, and population size and PCR-positive status (as determined at capture) were associated with negative effects on survival across all seasons (Table 2). Model-averaged parameter estimates substantiated these results for all but winter precipitation. Summed AICc weights indicated that the most important predictor variables were summer and autumn NDVI, which were competing although summer NDVI garnered slightly more weight (combined: ΣAICc $w = 0.98$; summer NDVI: ΣAICc $w = 0.59$; autumn NDVI: ΣAICc $w =$

0.39), PCR-status (ΣAICc *w* = 0.97), and population size (ΣAICc *w* = 0.85), with winter precipitation being comparably less important (ΣAICc *w* = 0.63). We evaluated age and *M. ovi* exposure (based on cELISA results) *a posteriori* (results not shown). The potential effects of older age (i.e., > 9 years) and *M. ovi* exposure on survival were not significant (90% CIs overlapped 0). Based on the model-averaged estimates of factor effects, survival in winter was approximately 3 times higher with every 0.03 increase in mean summer or autumn NDVI, while monthly odds of survival were approximately 30% less with every 40-animal increase in population size and approximately 70% less if an animal was PCR-positive for *M. ovi* at capture. The model-averaged estimate of winter precipitation was not significant (90% CI [-0.61, 0.16]; Table 2), indicating uncertainty in the importance of this parameter.

Seasonal NDVI averages for summer and autumn were highest in Hackberry, Woods, and Clipper Mountains, intermediate in Old Dad Peak/Kelso and Marble Mountains, and lowest in South Soda, South Bristol, Cady, and North Bristol Mountains (Table 3). Yearly winter precipitation varied widely across ranges, with totals ranging from 14mm to 118mm (Table 3). Hackberry and Woods Mountains received the highest rainfall in winter, followed by North Bristol, Old Dad Peak/Kelso, Cady, Clipper, and South Soda Mountains, which received up to 42% less (Table 3). South Bristol and Marble Mountains received the lowest levels of yearly winter precipitation, which were up to 65% less than Hackberry and Woods Mountains. Winter precipitation was highest in 2016-17 in all ranges, with totals doubling those from the previous year (Table 3).

Our adult female population estimates were lowest in Hackberry, Woods, and South Soda Mountains (< 40 adult females), intermediate in Old Dad Peak/Kelso, South Bristol, Clipper and Cady Mountains (40-80 adult females), and highest in North Bristol and Marble Mountains (> 80 adult females; Table 3). We recorded 214 marked animal sightings (detecting 78% of our total sample) and 858 unmarked animal sightings across all mountain ranges, and documented 12 occurrences of marked animals visiting the same site in 2 different activity periods during the same occasion. We tested repeatability of our method by performing 2 independent counts on 21 occasions; the 2 counts were highly correlated (*r* = 0.98), indicating that counts provided a reasonably accurate assessment of the number of animals present on each sampling occasion. The precision of our population estimates varied depending on population and sample sizes, with smaller sample sizes and larger populations having wider confidence intervals (Table 3).

Estimated annual survival was highest in the Hackberry, Woods, and Clipper Mountains, ranging from 0.958 (± 0.016 SE) to 0.983 (± 0.011 SE) for uninfected females and from 0.869 (± 0.034 SE) to 0.945 (± 0.026 SE) for infected females, intermediate in Old Dad Peak/Kelso, South Bristol, and South Soda Mountains (uninfected: 0.943 [± 0.026 SE] to 0.966 [± 0.017 SE]; infected: 0.828 [± 0.057 SE] to 0.893 [± 0.038 SE]), and lowest in the Cady, Marble, and North Bristol Mountains (uninfected: 0.896 [± 0.030 SE] to 0.953 [± 0.017 SE]; infected: 0.700 [± 0.070 SE] to 0.856 [± 0.036 SE]; Table 4). Sixty-five percent of all observed mortalities among collared females occurred during winter. In winter 2016-17, which was the most severe winter in terms of precipitation (Table 4), observed mortality among collared females was highest with a total of 10 deaths (20 mortalities occurred across all winters in the study) and estimated overwinter survival was lowest in some populations (Figure 2).

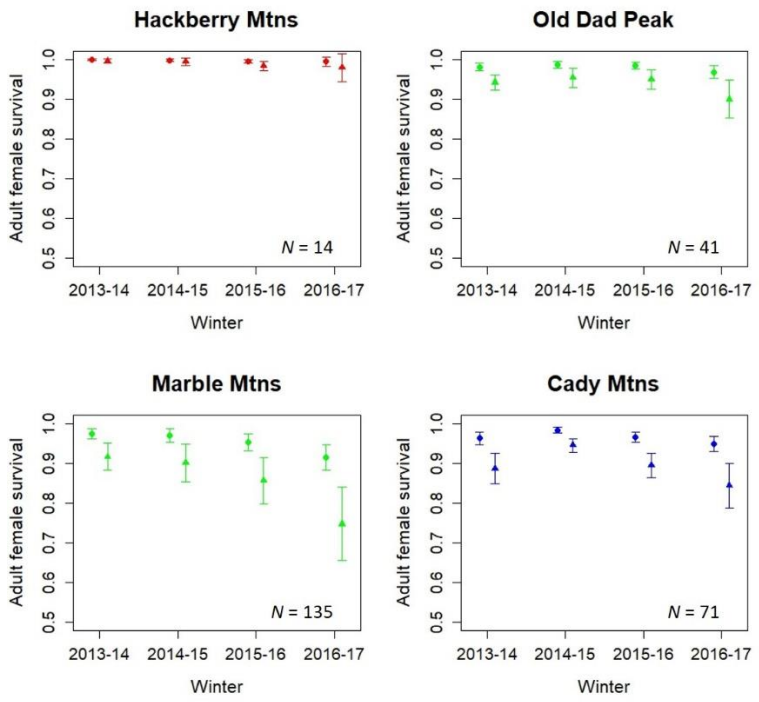

Figure 2. Estimated overwinter survival for selected populations from 2013 to 2017. Circles symbolize estimates for individuals that were PCR-negative for *Mycoplasma ovipneumoniae* infection at capture (not infected), triangles symbolize estimates for individuals that were PCR-positive at capture (infected), and error bars indicate standard errors. Colors represent levels of mean summer and autumn NDVI for a given mountain range, with red representing highest values, green representing intermediate values, and blue representing lowest values; *N* denotes population estimates of adult female bighorn.

Table 1. Top 20 models from the candidate model set for adult survival. K is the number of model parameters, w_i is the Akaike model weight.

Model No.	Model Structure	*K*	ΔAICc	w_i	Deviance
1	Summer NDVI lag + Winter precipitation + PCR status + Population size	5	0.00	0.34	322.88
2	Autumn NDVI lag + Winter precipitation + PCR status + Population size	5	1.38	0.17	324.25
3	Autumn NDVI lag + PCR status + Population size	4	1.46	0.16	326.35
4	Summer NDVI lag + PCR status + Population size	4	1.84	0.14	326.72
5	Summer NDVI lag + Winter precipitation + PCR status	4	3.38	0.06	328.27
6	Summer NDVI lag + PCR status	3	4.87	0.03	331.75
7	Autumn NDVI lag + Winter precipitation + PCR status	4	5.18	0.03	330.06
8	Autumn NDVI lag + PCR status	3	5.33	0.02	332.21
9	Winter precipitation + PCR status + Population size	4	6.07	0.02	330.95
10	Summer NDVI lag + Winter precipitation + Population size	4	7.68	0.01	332.56
11	Summer NDVI lag + Population size	3	7.77	0.01	334.66
12	Autumn NDVI lag + Population size	3	8.13	0.01	335.02
13	Autumn NDVI lag + Winter precipitation + Population size	4	9.05	0.00	333.93
14	Summer NDVI lag	2	9.76	0.00	338.65
15	Summer NDVI lag + Winter precipitation	3	9.90	0.00	336.78
16	Autumn NDVI lag	2	10.70	0.00	339.59
17	PCR status + Population size	3	11.19	0.00	338.08
18	Autumn NDVI lag + Winter precipitation	3	11.61	0.00	338.49
19	Winter precipitation + Population size	3	12.67	0.00	339.55
20	Winter precipitation + PCR status	3	12.69	0.00	339.58

Table 2. Parameter estimates from our top 2 models, and model-averaged parameter estimates, for monthly adult female bighorn survival from 2013 to 2017.

	Summer NDVI lag			Autumn NDVI lag			PCR-positive			Population size			Winter precipitation		
	β	Lower 90% CI	Upper 90% CI	β	Lower 90% CI	Upper 90% CI	β	Lower 90% CI	Upper 90% CI	β	Lower 90% CI	Upper 90% CI	β	Lower 90% CI	Upper 90% CI
Model No. 1	0.95	0.36	1.55	-	-	-	-1.29	-1.98	-0.60	-0.40	-0.69	-0.12	-0.39	-0.69	-0.08
Model No. 2	-	-	-	1.06	0.31	1.80	-1.29	-1.98	-0.59	-0.42	-0.70	-0.13	-0.32	-0.65	0.02
Model Averages	1.03	0.42	1.64	1.24	0.48	2.01	-1.19	-1.89	-0.49	-0.34	-0.64	-0.04	-0.23	-0.61	0.16

Table 3. Estimated population sizes for adult female bighorn in 2016, mean summer and autumn NDVI for 2013-2016, and total precipitation in winter 2013-14 to winter 2016-17 by mountain range.

		2013	2013-14	2014	2014-15	2015	2015-16	2016	2016-17
Range (Population)	Adult F Population Size[a] (95% CI)	Mean NDVI Summer/ Autumn	Total Winter Precipitation (mm)	Mean NDVI Summer/ Autumn	Total Winter Precipitation (mm)	Mean NDVI Summer/ Autumn	Total Winter Precipitation (mm)	Mean NDVI Summer/ Autumn	Total Winter Precipitation (mm)
Hackberry	14 (0-28)	0.16/0.18	27.56	0.17/0.18	63.46	0.13/0.15	49.72	0.16/0.15	118.22
Woods	18 (6-29)	0.16/0.18	27.56	0.17/0.18	63.46	0.13/0.15	49.72	0.16/0.15	118.22
Clipper	65 (28-102)	0.13/0.14	20.54	0.13/0.17	38.29	0.11/0.13	34.67	0.11/0.11	82.75
Old Dad Peak	41 (18-63)	0.10/0.11	23.18	0.10/0.14	40.81	0.10/0.13	36.23	0.10/0.11	77.73
Marble	135 (67-202)	0.11/0.12	14.27	0.10/0.13	26.33	0.09/0.11	24.86	0.09/0.10	58.10
S. Soda	28 (5-52)	0.09/0.10	21.66	0.09/0.10	39.09	0.09/0.10	32.56	0.09/0.10	68.76
S. Bristol	46 (25-68)	0.10/0.12	14.05	0.10/0.12	22.22	0.09/0.10	19.43	0.09/0.09	52.15
Cady	71 (0-144)	0.09/0.10	26.35	0.11/0.12	42.27	0.10/0.10	35.64	0.10/0.10	70.32
N. Bristol	101 (26-175)	0.09/0.10	28.23	0.09/0.11	44.21	0.09/0.10	39.36	0.09/0.09	87.73

[a]Our estimates represent the population of adult females using point-source water features and serve as a relative index for the true population size; estimates were generated using Bowden's estimator and are rounded to nearest whole numbers.

Table 4. Annual survival estimates with standard errors (SE) of adult female bighorn from 2014 to 2016.

	PCR-positive						PCR-negative					
	2014		2015		2016		2014		2015		2016	
Range (Population)	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Hackberry***/+	0.945	0.026	0.941	0.028	0.932	0.033	0.983	0.011	0.981	0.012	0.978	0.013
Woods***/+	0.944	0.026	0.940	0.028	0.930	0.033	0.982	0.011	0.981	0.012	0.978	0.013
Clipper***/++	0.904	0.030	0.900	0.031	0.869	0.034	0.969	0.014	0.968	0.014	0.958	0.016
Old Dad Peak**/++	0.886	0.038	0.893	0.038	0.878	0.039	0.963	0.018	0.966	0.017	0.960	0.018
Marble**/+++	0.781	0.080	0.762	0.083	0.709	0.091	0.927	0.032	0.920	0.033	0.900	0.038
S. Soda*/+	0.869	0.051	0.863	0.051	0.854	0.052	0.957	0.023	0.955	0.023	0.952	0.024
S. Bristol*/++	0.882	0.039	0.871	0.039	0.828	0.057	0.962	0.019	0.958	0.019	0.943	0.026
Cady*/++	0.827	0.048	0.856	0.036	0.809	0.048	0.943	0.023	0.953	0.017	0.937	0.022
N. Bristol*/+++	0.754	0.070	0.762	0.060	0.700	0.070	0.917	0.031	0.920	0.026	0.896	0.030

* Lowest summer and autumn NDVI averages (0.09-0.12), **Intermediate summer and autumn NDVI averages (0.09-0.14), ***Highest summer and autumn NDVI averages (0.13-0.18).

+ Lowest population sizes (< 40 adult females), ++Intermediate population sizes (40-80 adult females), +++ Highest population sizes (> 80 adult females).

Objective 2: Post-Outbreak Lamb Survivorship[1]

Lamb survival varied across populations and years as per proportions of surviving lambs with collared ewes and late season lamb-ewe ratios (Table 5). There was a strong correlation between both metrics across populations and years (r = 0.92), with the exception of the Clipper and North Bristol Mountains. Estimated survival based on surviving lambs with collared ewes appeared spurious in the Clipper (adjusted n = 6) and North Bristol Mountains (adjusted n = 2) in 2016 and 2015 respectively, compared to late season lamb-ewe ratios, which was likely due to limited observations of some marked individuals and insufficient sampling. We therefore censored 2016 Clipper data and 2015 North Bristol data from the correlation test.

We examined pairwise correlations between seasonal precipitation, NDVI for the previous summer and autumn, and population size. Based on Pearson correlation coefficients, previous summer and autumn NDVI, winter and spring precipitation, previous summer NDVI and summer precipitation, and previous autumn NDVI and summer precipitation were the most closely correlated ($0.6 < r < 0.8$). Correlations for the remaining variables were between -0.5 and 0.1. We therefore did not test previous summer NDVI with previous autumn NDVI, previous summer and autumn NDVI with summer precipitation, or winter precipitation with spring precipitation in the same models. Initial evaluation of these covariates indicated that summer precipitation was the only statistically significant variable (90% CI did not overlap 0), although the effect was negative, contradicting our original hypothesis for why it would be biologically important. Onozuka et al. (2008) reported that *M. pneumoniae* in humans became more infectious with increasing ambient humidity and temperature as a result of prolonged airborne survival of the pathogen, leading to higher rates of aerosol transmission, which could potentially explain the negative effect of summer precipitation on lamb survival if *M. ovi* behaves the same way in bighorn. Considering that increased humidity and higher temperatures may potentially cause higher rates of infection and mortality in lambs, we tested effects of monthly precipitation from April to August on lamb survival as well (Table 6). We additionally tested effects of log-transformed precipitation totals, since increasing precipitation might have had a decreasing effect on lamb survival as more lambs became infected and mortality increased.

Our top model indicated that the total precipitation from May to August had the greatest effect on lamb survival, and the log-transformed total was the most strongly supported variable. The logarithmic effect of May to August precipitation (β = -0.33, SE = 0.095) explained 44% of the variation in the data. According to this model, estimated lamb survival dropped below 70% when total May to August precipitation exceeded 4.5 mm, dropped below 50% when total May to August precipitation exceeded 8.5 mm, and dropped below 20% when total May to August precipitation exceeded 21.5 mm based on the mean effect (Figure 3). We note that 56% of the variation was not explained by May to August precipitation, which indicates that other unknown variables were also acting on this effect and influencing lamb survival. We suspect that disease prevalence within populations was another important variable affecting disease transmission and survivorship of neonates, but we could not estimate yearly prevalence within the parameters of this study and therefore could not test the effect. We also believe that nutrition may have been an important factor in spite of our results, but given the positive correlation between previous summer and autumn NDVI with summer precipitation the following year, and the strength of the negative precipitation effect, we were unable to detect a positive signal for nutrition approximated by both NDVI and precipitation in our models.

[1] Results are preliminary.

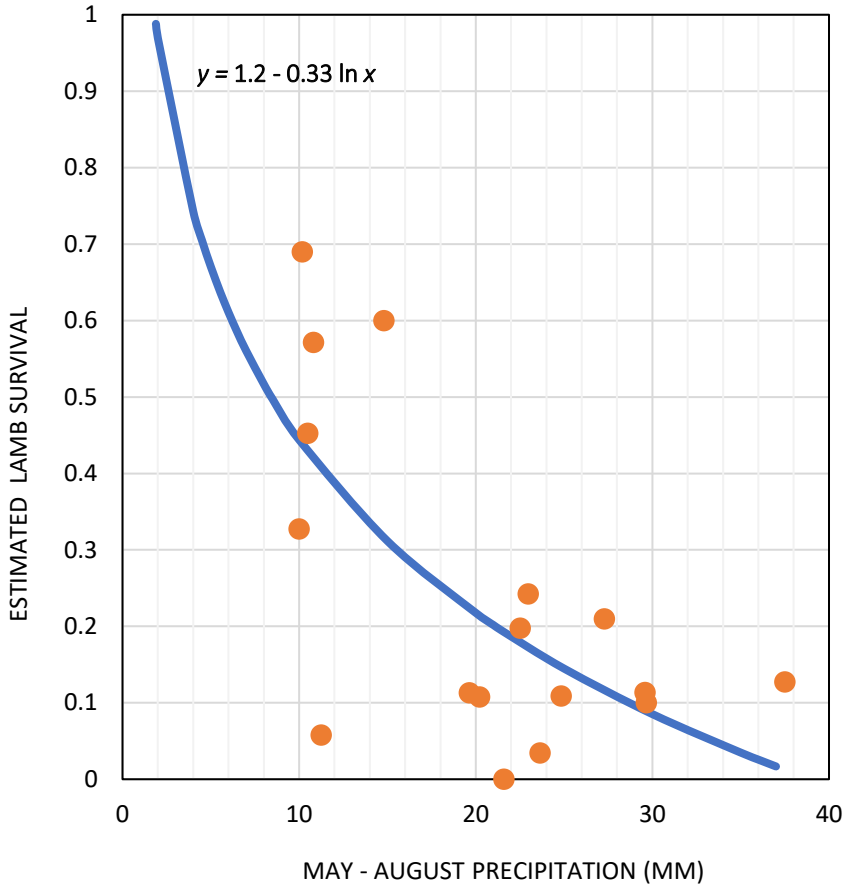

Figure 3. Plot of fitted relationship between the logarithmic effect of May through August precipitation on lamb survival (blue curve) as per our top model; orange dots represent actual data points of lamb survival derived from late season (15 August to 30 September) lamb-ewe ratios.

Table 5. Estimated bighorn lamb survival based on proportions of surviving lambs with collared ewes and late-season (15 August to 30 September) lamb-ewe ratios derived from camera data collected at point-source water features in 7 mountain ranges in the Mojave Desert, California from 2014 to 2016.

Range (Year)	Proportion Surviving	Survival Ratio
Old Dad Peak/Kelso (2014)	10%	11%
Old Dad Peak/Kelso (2015)	18%	13%
Old Dad Peak/Kelso (2016)	8%	11%
Marble (2014)	9%	3%
Marble (2015)	36%	20%
Marble (2016)	50%	45%
Cady (2015)	0%	11%
Cady (2016)	17%	33%
South Bristol (2014)	0%	0%
South Bristol (2015)	0%	11%
South Bristol (2016)	92%	57%
South Soda (2015)	33%	24%
South Soda (2016)	80%	69%
North Bristol (2015)	100%	21%
North Bristol (2016)	20%	6%
Clipper (2015)	-	10%
Clipper (2016)	17%	60%

Table 6. Top 20 models from the candidate model set for lamb survival. K is the number of model parameters, w_i is the Akaike model weight, cum. w_i is the cumulative Akaike model weight, and LL is the log-likelihood.

Model No.	Model Structure	K	ΔAICc	w_i	cum. w_i	LL
1	LN(May-Aug Precip)	3	0	0.24	0.24	7.56
2	LN(Jun-Aug Precip)	3	0.95	0.15	0.38	7.09
3	May-Aug Precip	3	1.72	0.1	0.48	6.7
4	Jun-Aug Precip	3	1.93	0.09	0.57	6.59
5	Jul-Aug Precip	3	2.07	0.08	0.66	6.52
6	LN(Apr-Aug Precip)	3	2.11	0.08	0.74	6.5
7	Summer Precip	3	2.27	0.08	0.81	6.42
8	LN(May-Aug Precip) + Winter Precip	4	3.28	0.05	0.86	7.66
9	Apr-Aug Precip	3	3.69	0.04	0.9	5.71
10	Summer Precip + Population Size	4	4.56	0.02	0.92	7.02
11	Spring Precip + Summer Precip	4	5.58	0.01	0.93	6.51
12	Winter Precip + Summer Precip	4	5.74	0.01	0.95	6.43
13	Null	2	6.85	0.01	0.96	2.64
14	Jul Precip	3	6.93	0.01	0.96	4.09
15	Previous Autumn NDVI	3	7.93	0	0.97	3.59
16	Spring Precip + Summer Precip + Population Size	5	8.12	0	0.97	7.3
17	Previous Summer NDVI	3	8.5	0	0.97	3.31
18	Winter Precip + Summer Precip + Population Size	5	8.68	0	0.98	7.02
19	Spring Precip	3	8.85	0	0.98	3.13
20	Population Size	3	9.23	0	0.98	2.94

Objective 3: Movement and Potential Transmission Risk[2]

Our top model indicated that age, sex, and PCR status at capture were all important variables influencing intermountain movements (Table 7), however, the estimated effect for PCR status at capture was not statistically significant (β = -1.05, 90% CI [-2.30, 0.20]), which suggests that the signal was very weak. Age was positively correlated with intermountain movements (β = 1.44, 90% CI [0.39, 2.49]), whereby the odds of an individual > 5 years old making an intermountain movement were 4 times higher than an individual < 5 years old. The effect of being a male was also positive (β = 1.85, 90% CI [0.92, 2.77]), whereby the odds of a male making an intermountain movement was 6 times higher than a female. Although the effect for PCR status was weak, it suggests that individuals who were PCR-positive at capture were less likely to make an intermountain movement than individuals who were PCR-negative, but age and sex were stronger predictors. Based on the mean effects estimated by our top model, the probability of a male > 5 years old and PCR-negative at capture making an intermountain movement was 55%, while the probability for a male > 5 years old and PCR-positive at capture was 30%. Alternately, for a male < 5 years old and PCR-negative at capture the probability of an intermountain movement was 22%, and the probability for a male < 5 years old and PCR-positive at capture was 9%. In contrast, the probability of an intermountain movement for a female > 5 years old and PCR-negative at capture was 16%, while the probability for a female > 5 years old and PCR-positive at capture was 6%. Alternately, for a female < 5 years old and PCR-negative at capture the probability of an intermountain movement was 4%, and the probability for a female < 5 years old and PCR-positive at capture was 2%. As such, granted the weak effect of PCR status at capture, males > 5 years old were the most likely individuals to make intermountain movements and potentially spread disease across populations, and females < 5 years old were least likely.

Across our study, 20 individuals (10 males and 10 females) made 30 intermountain movements, and 103 individuals (15 males and 88 females) did not make intermountain movements. Seasons did not appear to have an effect on movement in our models likely because of differences between movers and nonmovers, males and females, and sampling disparity. Among animals that made intermountain movements, however, there appeared to be some indication of seasonal effects as per the proportions of movements occurring by season (Figure 4). Most intermountain movements occurred during autumn and winter among females, and during summer and autumn among males, with the lowest proportion occurring in summer for females and in spring for males. We cannot offer a biological explanation for seasonal differences in observed movement trends at this stage but speculate that summer and autumn movements by males may be associated with mate-seeking during the rutting period, while autumn and winter movements by females may be spurred by physiological changes during pregnancy and pre-lambing. Regardless, among males and females collectively, intermountain movements occurred most during winter, summer, and autumn, which suggests that disease transmission potential across populations was highest during these seasons.

[2] Results are preliminary.

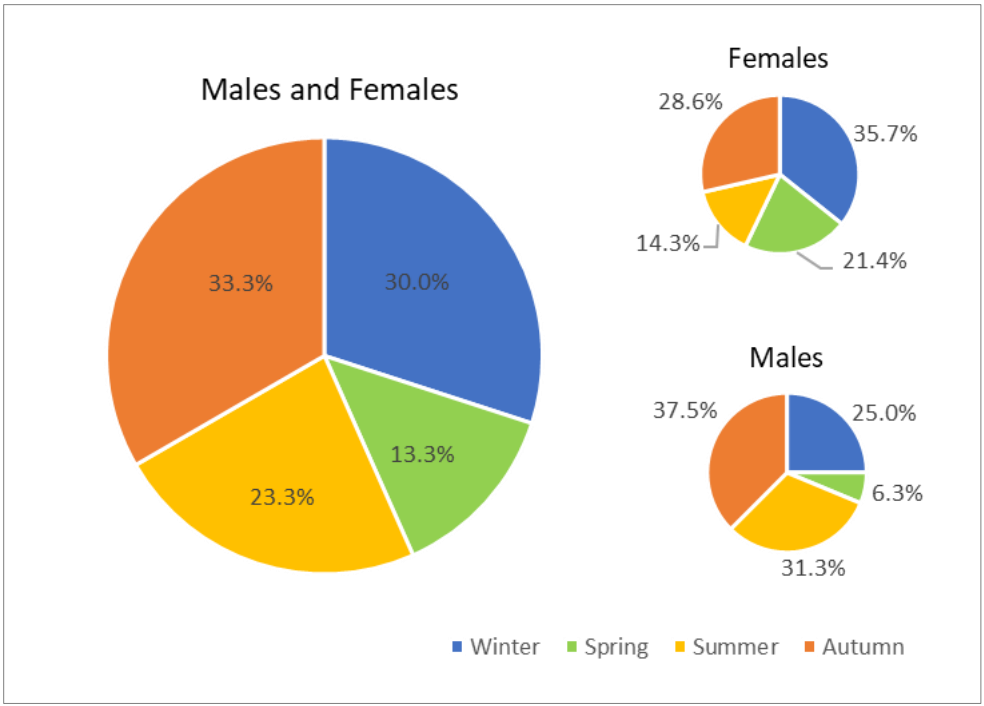

Figure 4. Proportions of intermountain movements by season.

Table 7. Ranked candidate models evaluating effects on bighorn intermountain movement based on logistic regression. K is the number of model parameters, w_i is the Akaike model weight, cum. w_i is the cumulative Akaike model weight, and LL is the log-likelihood.

Model No.	Model Structure	*K*	ΔAICc	w_i	cum. w_i	LL
1	Age + PCR + Sex	4	0.00	0.44	0.44	-47.27
2	Age + Sex	3	0.07	0.43	0.87	-48.37
3	PCR + Sex	3	4.00	0.06	0.93	-50.33
4	Sex	2	4.05	0.06	0.99	-51.40
5	Age + PCR	3	9.01	0.00	1.00	-52.83
6	Age	2	10.42	0.00	1.00	-54.59
7	PCR	2	11.52	0.00	1.00	-55.14
8	Null	1	13.40	0.00	1.00	-57.11

DISCUSSION

We found that *M. ovi* infection was associated with a substantial reduction in survival of adult female bighorn following a pneumonia outbreak in 2013 in the Mojave Desert, which is consistent with many studies that have linked *M. ovi* to pneumonia epizootics and acute mortality in bighorn sheep populations (Besser et al. 2012*a*, *b*, Cassirer et al. 2017, 2018). Environmental variables also appeared to influence survival, and in particular, forage quality in summer and autumn (as approximated by NDVI), and May to August precipitation, were strongly correlated with adult and neonate survival respectively, and likely mediated effects of *M. ovi* in infected populations. We found that winter survival was higher in mountain ranges with higher summer and autumn NDVI, for both infected and uninfected adult females, suggesting that better nutrition might partially offset negative effects associated with infection. Lamb survival appeared to decrease with higher precipitation in May to August across infected populations and years, which may have been a function of increased pathogen infectiousness associated with higher ambient humidity and temperature. We also found that intermountain movements may have been influenced by *M. ovi* infection, as the probability of an intermountain movement for a male > 5 years old was 25% less if the individual was PCR-positive at capture based on our top model, although infected males > 5 years old were still more likely to transmit disease across populations via intermountain movements compared to infected individuals in other sex and age classes.

Our study suggests that many adult females exposed to *M. ovi* may have remained chronically infected, given that previous exposure to *M. ovi* (as per cELISA testing and demonstration of *M. ovi* specific antibodies), barring infection status at capture, did not appear to affect adult female survival, while positive infection status at capture had a negative effect. Additionally, eighty-four percent of mortalities in the study occurred > 1 year after capture, and we confirmed *M. ovi* infection at time of death in 2 recovered mortalities that were *M. ovi*-positive at capture 1 and 2 years prior (we were unable to determine infection status at death in 21 other cases). Recent studies have reported that bighorn sheep can become chronically infected with *M. ovi* and infectious periods can last up to 3 years or longer (Plowright et al. 2017). As such, surviving adults that remain chronically infected can continue to spread the pathogen to other animals, promoting disease persistence in the system (Plowright et al. 2017, Cassirer et al. 2018). Prevalence may therefore be largely controlled by the number of chronically infected individuals that remain in the population year after year, which would ultimately influence exposure rates and transmission risk among adults and neonates within and between populations.

The occurrence of respiratory disease in neonates has been identified as a major threat to the persistence of bighorn populations infected with pneumonia and has been largely attributed to contact with chronically infected individuals in the years following an initial disease outbreak (Cassirer et al. 2013, 2018, Manlove et al. 2016, Plowright et al. 2017). Selective culling of chronically infected animals has therefore been identified as a way to control disease persistence and improve population performance (Cassirer et al. 2018). Given that chronically infected individuals may also be driving transmission risk multilaterally within and across populations, as a next step, we recommend programmatic re-testing of infected animals to evaluate the propensity for chronic infection among bighorn in the Mojave Desert and to assess whether selective culling could be an effective management tool for reducing prevalence and transmission of *M. ovi* within the system.

On a final note, summer and autumn nutrition (as approximated by NDVI) and *M. ovi* infection appeared to be major factors influencing adult female survival in our study, whereby female populations in ranges with lower NDVI experienced lower survival and females infected with *M. ovi* experienced lowest survival. Population distributions were also concentrated around point-source water features in summer and autumn, likely increasing pressure on local resources, potentially lowering nutrient

availability per capita. We speculate that if distributions were more diffuse during these seasons, use of forage would become more evenly distributed and nutrition less limited for adult females. A more diffuse distribution might also reduce contact rates between infected and uninfected individuals, and reduce transmission of *M. ovi* within populations. We propose that one way to achieve a broader spatial distribution of populations during summer and autumn might be to reconfigure placement of artificial water features, and perhaps add new structures, in a way that would give animals greater access to good quality forage and allow for greater use of forage throughout the range.

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PART II: EVALUATION OF GENE FLOW AMONG DESERT BIGHORN SHEEP POPULATIONS IN THE MOJAVE DESERT, CALIFORNIA, C. 2000-2003 AND 2014-2018

The following reports are provided to fulfill Objective 4 of the grant agreement: expand genetic analysis from blood and fecal samples to update estimates of genetic structure, gene flow, current connectivity, and genetic diversity. The first attachment is a report of gene flow between the Sheephole, Bullion, and Newberry populations. The second is a published manuscript (Epps et al. 2018) describing updated genetic diversity, gene flow, connectivity, and genetic structure for the central Mojave study area and detailing changes observed since genetic sampling conducted in prior research in the early 2000s (Epps et al. 2005, Epps et al. 2006).

Evaluation of gene flow among desert bighorn sheep populations in the Newberry, Bullion, and Sheephole Mountains, California, c. 2000-2003 and 2014-2018

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Introduction: This document is intended to serve as a brief report of our investigations of gene flow among desert bighorn sheep (DBS) in the Newberry/Ord/Rodman, Bullion, and Sheephole Mountains of California. The purpose of this analysis was to determine whether DBS in the Newberry Mountains/Ord/Rodman Mountains population have recently begun interacting with DBS in the Bullion Mountains and the Sheephole Mountains. Here, we use the name "Bullion Mountains" population to describe potential and occupied bighorn habitat on the 29 Palms Marine Corps Base. Recent development of artificial water catchments on the 29 Palms Marine Corps Base may have helped establish such links between the Newberry/Ord/Rodman population and bighorn sheep in the Sheephole Mountains and the eastern end of the Bullion Mountains. Bighorn sheep were reintroduced into the Sheephole and Bullion mountains decades ago from a source population at Old Dad Peak, located north of Interstate 40, and thus the bighorn sheep currently occupying the Sheephole Mountains are quite genetically distinct from other bighorn in the area. Thus, this situation created the potential for an unusually high-resolution analysis of genetic origins of individual bighorn sheep throughout this system.

Methods: For this analysis, we genotyped samples collected at two time points: 2000-2003 and 2014-2018 (Table 1). Samples collected in 2000-2003 were collected by Clint Epps and Carlos Gallinger in the Sheephole Mountains and Newberry and Ord Mountains (Figure 1). Newberry/Ord samples were genotyped in 2004 at 14 microsatellite loci (Epps et al. 2005); those samples were genotyped at 6 additional loci and aligned to more recent data during 2015-2018 to create a 16-locus dataset (4 of the original loci are no longer used) for comparison to later data. Sheephole Mountain samples collected 2000-2003 were stored and not genotyped until 2016. During 2014-2018, we collected bighorn fecal samples, blood samples from captured animals, and snips of tissue from carcasses from the Newberry Mountains and the Sheephole Mountains. Pellet samples from several locations in the Bullion Mountains were provided by 29 Palms Marine Corps Base and the Society for Conservation of Desert Bighorn Sheep (Figure 1, Table 1). These samples likewise were genotyped at the same 16 variable microsatellite loci (Epps et al. 2018).

We conducted three types of analyses on these data. First, we evaluated population-level genetic structure between the Newberry and Sheephole, using GENEPOP to estimate pairwise FST (a metric of genetic structure that varies from 0, indicating no difference between two sets of samples, and a maximum value that likely approaches 0.4 in this system for two populations with no recent history of gene flow). Second, we used STRUCTURE to evaluate genetic assignment of individuals to one population or another at both time periods (50,000 burn-in, 100,000 run, admixture and correlated allele frequencies, no prior based on sampling location). This approach

allows identification of individual migrants or offspring of migrants. Third, we evaluated genetic diversity (expected heterozygosity, H_e) in each population at each time period using FSTAT.

Results:

Gene flow and genetic structure in 2003: Estimates of genetic structure at 2003 showed very strong genetic differentiation between the Newberry and Sheephole populations (Table 2). Assignment tests supported earlier conclusions (Epps et al. 2005) that the Newberry population was completely isolated during the 2000-2003 sampling . However, we found one instance of a Newberry-type bighorn sheep sampled in the Sheephole Mountains population (Figures 1 & 2). We cannot conclude that the Newberry-lineage animal sampled in the Sheephole Mountains traveled all the way from the Newberry Mountains, as a small number of bighorn sheep of that lineage could have persisted in the western portions of the Bullion Mountains, but this analysis demonstrates that the Sheephole Mountains had received at least one migrant from that lineage at that time.

Gene flow and genetic structure in 2014-2018: Estimates of genetic structure in 2014-2018 showed the Newberry and Sheephole populations are still strongly differentiated (Table 2). However, the Bullion Mountains shows an intermediate relationship, indicating that stepwise gene flow at least is occurring (Table 2).

Individual assignment tests were much more revealing, and demonstrate that two-way gene flow between the Sheephole and Newberry Mountains has now been established (Figures 1 & 2. Multiple individuals in the Newberry Mountains population showed complete (n = 1) or partial (n = 5) genetic affiliation with the Sheephole Mountains population (Figure 1). In the Sheephole Mountains, likewise, 5 individuals showed at least partial genetic affiliation with the Newberry Mountains population.

Genetic assignment of samples collected within the Bullion Mountains population (i.e., on the Marine Corps Base) showed at least two subpopulations: a western population of Newberry lineage, and eastern population of Sheephole lineage, and then an area of contact in the middle. The contact area is the location to which two GPS-collared ewes have made repeated seasonal movements from the Newberry/Ord Mountains. Interestingly, both of those GPS-collared ewes were of Newberry Mountains ancestry.

Genetic diversity: In 2000-2003, the Newberry/Ord/Rodman population had the lowest genetic diversity (H_e = 0.50) of 27 populations sampled across the Mojave Desert (Epps et al. 2005). In fact, this population showed no genetic variation at one locus analyzed here: BL4, known to be linked to an immune system gene (Table 3). A recent study of adaptive genetic variation in the Mojave showed evidence of positive selection at that locus (Epps et al. 2018) and other recent analysis in the Mojave Desert populations suggests correlation with variation at that locus and immune phenotype (Dugovich, Epps, Crowhurst, Gonzales, Beechler, Jolles, *In prep*). In 2014-2018 sampling, average genetic diversity of the Newberry/Ord/Rodman population had increased only slightly (H_e = 0.51). However, that population has now gained two new alleles at the BL4 locus, suggesting that the gene flow link established with the Bullion and Sheephole Mountains populations has increased genetic diversity at this potentially important locus linked to immune function. The Bullion Mountains population shows relatively high genetic diversity, although that may not reflect high genetic diversity of individuals given that two lineages are present in

that “population.” The Sheephole Mountains population appears to have gained significant genetic diversity since 2000-2003, perhaps in part due to gene flow from the Newberry and Bullion populations (Table 3).

Implications for disease: Respiratory disease has been detected in locations around the Mojave Desert in California; in particular, *Mycoplasma ovipneumoniae* (*Movi*), which has been associated with fatal pneumonia in bighorn sheep around western North America (Besser et al. 2008). This pathogen can be spread by bighorn sheep to other bighorn sheep by direct or close contact, causing strains to spread widely among populations via occasional intermountain movements. One strain (hereafter, the Mojave strain) has been detected in the South Bristol, Marble, and Clipper Mountains (north of the Sheephole and Bullion populations). A separate strain has been detected in Joshua Tree National Park (hereafter, the Joshua Tree strain). Sampling of bighorn sheep in the Newberry/Ord area in 2014, 2016, and 2018 detected no evidence of *Movi* infection or exposure. Although one of the least genetically diverse populations in the Mojave (Epps et al. 2006), the Newberry population may also have been partially protected from disease spread by its isolation as a result of human-made barriers and distance to other occupied habitats. Novel exposure could result from bighorn sheep contacting domestic sheep that are occasionally grazed on irrigated lands near Newberry Spring, or bighorn sheep in the Sheephole Mountains may eventually come into contact with the Joshua Tree strain of *Movi* due to previously document gene flow between the Sheephole and Coxcomb Mountains (Epps et al. 2010), which in turn is linked by high gene flow to other Joshua Tree National Park populations now affected by respiratory disease. The current disease status of bighorn sheep in the Sheephole and Bullion mountains is unknown. But, we recommend staying alert for any sign of respiratory disease in bighorn sheep in the region and notifying California Department of Fish and Wildlife if disease is suspected so that the pathogens involved could be confirmed and characterized. Given the frequent opportunity for disease spillover from domestic sheep or goats even in arid systems such as this, the increase in genetic diversity observed in these populations may help preserve their ability to persist and recover from such an outbreak.

Conclusions: We conclude that gene flow between the Sheephole and Newberry/Ord populations has increased significantly between 2003 and 2014-2018, perhaps in part because of water developments on the 29 Palms Marine Corps Base and an expanding population of bighorn sheep in the Newberry/Ord mountains as observed by recent surveys (CDFW, unpublished). Bighorn sheep samples provided from the 29 Palms Marine Corps Base showed that multiple subpopulations exist in the “Bullion Mountains” population, and originated from Newberry-lineage bighorn sheep in the west and Sheephole-lineage in the east. However, mixing among those subpopulations is occurring (note mixed individuals in the central portion of the range, Figure 2). Genetic diversity of both the Sheephole and Newberry/Ord populations has increased, on average, during this time period, and the Newberry/Ord population now exhibits new alleles (genetic variants) at a locus potentially linked to immune function.

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Table 1. Sample sizes and types of samples used for DNA analysis of desert bighorn sheep sampled in the Sheephole, Newberry/Ord, and Bullion Mountains of southeastern California at two time points.

Population or Mountain Range	**Time period of sample collection**	**Sample size**	**Sample type**
Bullion	2015-16	12	Fecal pellets
Sheephole	2003	12	Fecal pellets
Sheephole	2014-18	18	Tissue from carcasses at Suds Hole (n = 6, 2014) and fecal pellets (n=12, 2018)
Newberry/Ord	2003	15	Fecal pellets
Newberry/Ord	2014-16	27	Fecal pellets (n = 21) and blood samples from CDFW captures (n = 6)

Table 2. Estimates of genetic structure among groups of desert bighorn sheep sampled in the Sheephole, Newberry/Ord, and Bullion Mountains of southeastern California at two time points.

Population Comparison	**Genetic structure (FST) c. 2003**	**Genetic structure (FST) c. 2014-2018**
Sheephole-Newberry/Ord	0.208	0.205
Sheephole-Bullion	NA	0.064
Newberry/Ord-Bullion	NA	0.087

Table 3. Estimates of genetic diversity (expected heterozygosity, H_e) based on 16 microsatellite loci for populations of desert bighorn sheep sampled in the Sheephole, Newberry/Ord, and Bullion Mountains of southeastern California at two time points (2000-03 and 2014-18).

Locus	Newberry/Ord		Sheephole		Bullion (29 Palms Marine Corps Base)	
	2000-03	2014-18	2000-03	2014-18	2000-03	2014-18
AE129	0.67	0.71	0.74	0.84	-	0.73
AE16	0.67	0.58	0.52	0.71	-	0.73
BL4	0.00	0.21	0.69	0.69	-	0.56
FCB11	0.51	0.51	0.30	0.47	-	0.42
FCB193	0.31	0.32	0.57	0.69	-	0.66
FCB266	0.49	0.50	0.00	0.47	-	0.46
FCB304	0.48	0.59	0.36	0.60	-	0.61
HH62	0.71	0.66	0.77	0.86	-	0.89
JMP29	0.56	0.62	0.53	0.55	-	0.77
MAF209	0.68	0.59	0.66	0.80	-	0.75
MAF33	0.20	0.24	0.65	0.68	-	0.59
MAF36	0.64	0.63	0.42	0.68	-	0.64
MAF48	0.35	0.34	0.70	0.74	-	0.72
MAF65	0.68	0.65	0.71	0.62	-	0.46
TCRBV62	0.59	0.57	0.60	0.68	-	0.61
TGLA387	0.45	0.43	0.72	0.67	-	0.80
Average	0.50	0.51	0.56	0.67	-	0.65

Figure 1. Map of the study area, including established populations of bighorn sheep used in the 2014-2015 analysis of genetic structure among bighorn sheep in the Newberry/Ord Mountains (red polygon), Bullion Mountains (bracket), and Sheephole Mountains (green polygon). Red star denotes location east-most extent of movements from two ewes collared in the Newberry/Ord Mountains. Individual genetic samples are indicated by vertical bars, in some cases linked by grey lines to sampling locations when multiple samples occurred in close proximity. Proportional genetic assignment to lineage (Sheephole or Newberry) is indicated for each individual by red or green coloring; two colors in an individual indicate ancestry from both lineages.

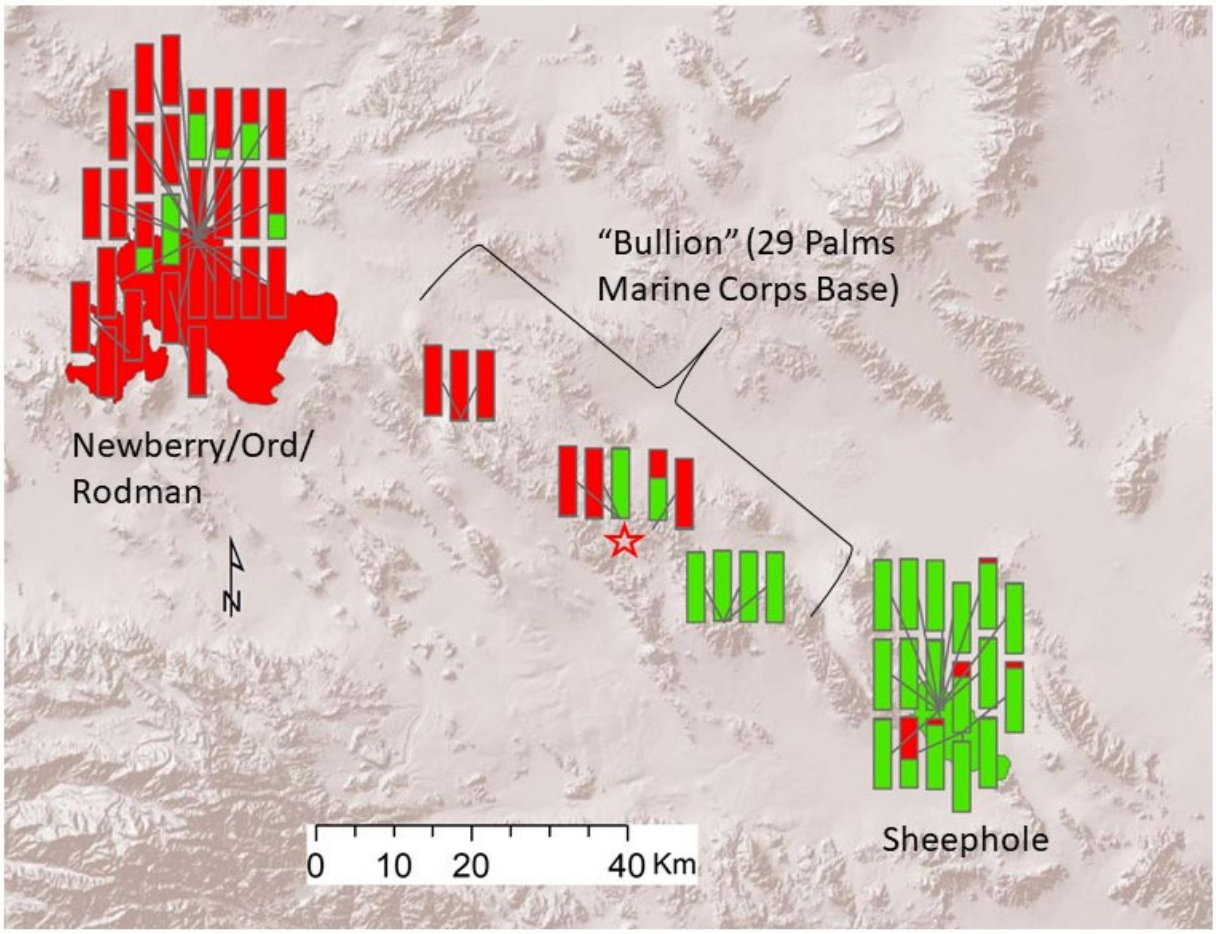
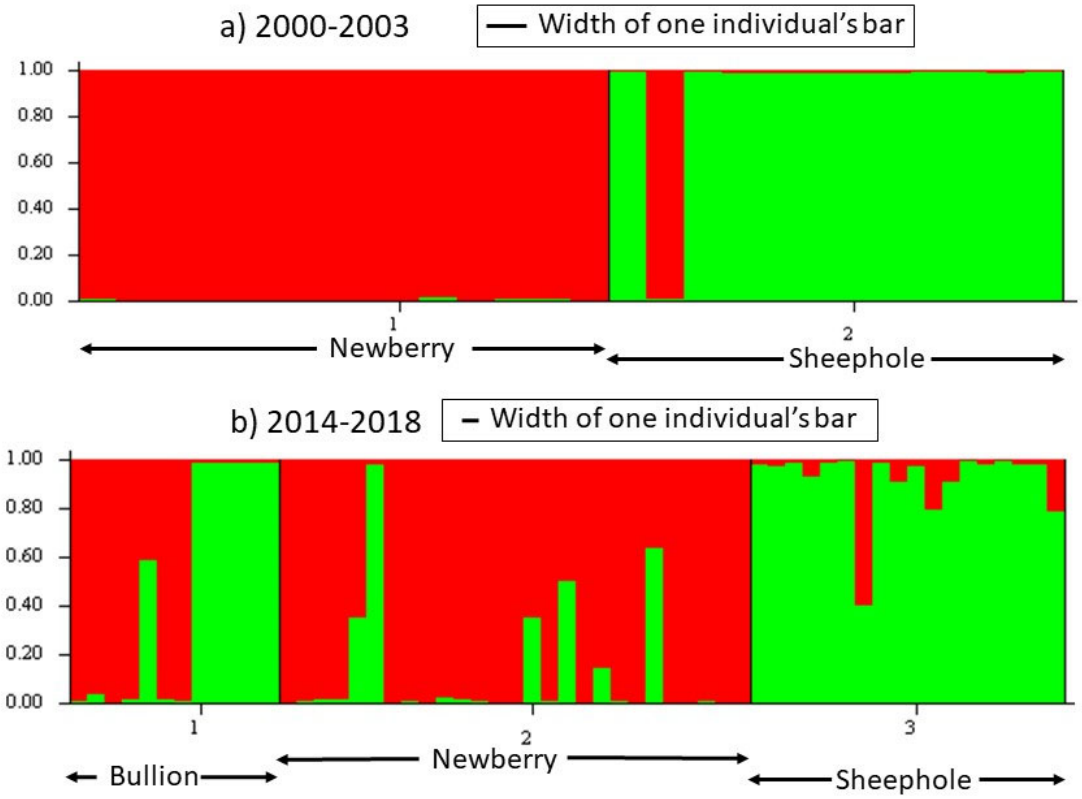


Figure 2. Individual genetic assignments of desert bighorn sheep to Newberry (red) or Sheephole (green) ancestry using Program STRUCTURE, including in 2000-2003 (a) and 2014-2018 (b). Each vertical bar represents proportional assignment of a single individual to one or both lineages; note that the width of the bar changes between the figures.



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ORIGINAL ARTICLE

WILEY MOLECULAR ECOLOGY

Assessing changes in functional connectivity in a desert bighorn sheep metapopulation after two generations

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Abstract

Determining how species move across complex and fragmented landscapes and interact with human-made barriers is a major research focus in conservation. Studies estimating functional connectivity from movement, dispersal or gene flow usually rely on a single study period and rarely consider variation over time. We contrasted genetic structure and gene flow across barriers for a metapopulation of desert bighorn sheep (*Ovis canadensis nelsoni*) using genotypes collected 2000–2003 and 2013–2015. Based on the recently observed but unexpected spread of a respiratory pathogen across an interstate highway previously identified as a barrier to gene flow, we hypothesized that bighorn sheep changed how they interacted with that barrier, and that shifts in metapopulation structure influenced gene flow, genetic diversity and connectivity. Population assignment tests, genetic structure and genetic recapture demonstrated that bighorn sheep crossed the interstate highway in at least one location in 2013–2015, sharply reducing genetic structure between two populations, but supported conclusions of an earlier study that such crossings were very infrequent or unknown in 2000–2003. A recently expanded population established new links and caused decreases in genetic structure among multiple populations. Genetic diversity showed only slight increases in populations linked by new connections. Genetic structure and assignments revealed other previously undetected changes in movements and distribution, but much was consistent. Thus, we observed changes in both structural and functional connectivity over just two generations, but only in specific locations. Movement patterns of species should be revisited periodically to enable informed management, particularly in dynamic and fragmented systems.

KEYWORDS
dispersal, genetic monitoring, habitat fragmentation, roads

1 | INTRODUCTION

Determining functional connectivity, or how species move through landscapes (Rudnick et al., 2012), has been a major focus in landscape ecology (Betts, Gutzwiller, Smith, Robinson, & Hadley, 2015) and landscape genetics (Manel & Holderegger, 2013). Empirical estimates of functional connectivity are vital for effective management of species in the face of habitat fragmentation and climate change (Creech, Epps, Monello, & Wehausen, 2014; Knowlton & Graham, 2010). In combination with assessments of structural connectivity, determining how species interact with barriers and move across fragmented landscapes has improved the ability to mitigate the impact of such landscape features on wildlife (Clevenger & Waltho, 2005). To investigate whether, and where, individuals cross barriers or human-modified habitats, researchers have employed radiotelemetry, GPS collars generating high-resolution spatial data, behavioural experiments (Moriarty et al., 2015) and remote cameras at potential crossing points (Gagnon, Dodd, Ogren, & Schweinsburg, 2011).

Landscape or population genetic approaches are also widely used for inferring functional connectivity, particularly where species are small-bodied and difficult to monitor with telemetry (Spear & Storfer, 2008), dispersal or long-distance movements are thought to be rare (Davis, Murray, Fitzpatrick, Brown, & Paxton, 2010) or studies encompass large landscapes (Cushman, McKelvey, Hayden, & Schwartz, 2006; Epps, Wehausen, Bleich, Torres, & Brashares, 2007). Both GPS collar and landscape genetic data have served as the basis for developing connectivity or movement models (Chetkiewicz & Boyce, 2009; Creech et al., 2014). Such models have proved fundamental for managing species on fragmented landscapes (Hilty, Lidicker, & Merenlender, 2006) and are preferred for predicting linkages among habitat patches (Rudnick et al., 2012).

Studies aimed at understanding interactions with barriers or animal movement in general are, however, often based on a "snapshot" of patterns on a particular landscape over a few years. Movement models based on direct observation of animal movements, as by GPS telemetry, usually reflect 2–6 years of data (Kertson, Spencer, Marzluff, Hepinstall-Cymerman, & Grue, 2011). Genetic patterns integrate movements over longer and variable timescales (Epps & Keyghobadi, 2015), but genetic investigations of the effects of barriers or fragmented landscapes are almost always based on a single estimate of genetic structure. The stability of patterns and processes inferred from any empirical movement analysis is rarely considered, yet movement or dispersal behaviours themselves may vary over time due to changes in factors such as resource availability (Bowler & Benton, 2005, 2009), parasite load (Debeffe et al., 2014) or population density (Plumb, White, Coughenour, & Wallen, 2009). Thus, models generated in a particular place and time might not capture behaviours under different conditions or newly learned behaviours. Although some studies compare models of movement and connectivity derived from different types of data, very few studies appear to have examined changes in movements or movement behaviours on decadal timescales using the same type of data.

Desert bighorn sheep (*Ovis canadensis nelsoni*) in the Mojave Desert of California are a case study of a species experiencing both natural and anthropogenic habitat fragmentation. Bighorn sheep in this region exist in metapopulations (Bleich, Wehausen, & Holl, 1990; Schwartz, Bleich, & Holl, 1986), with local populations of <25–250 individuals that experience frequent extinction and colonization events (Abella et al., 2011; Epps, McCullough, Wehausen, Bleich, & Rechel, 2004; Epps, Wehausen, Palsboll, & McCullough, 2010). Populations occur in small, sometimes isolated mountain ranges separated by desert flats and bajadas (alluvial fans), as well as fenced interstate highways and other potential anthropogenic barriers (Bleich, Wehausen, Ramey, & Rechel, 1996). Systematic investigation of population genetic structure from 2000 to 2003 and a review of known intermountain movements revealed that gene flow and thus movement of individuals between populations was strongly influenced by distance and topography, and that fenced interstate highways appeared to act as complete barriers (Epps et al., 2005, 2007). Subsequent investigations have treated such barriers as impermeable (Creech et al., 2014). Yet, in 2013, roughly two bighorn sheep generations (assuming 6 years/generation, Coltman et al., 2003) after the 2000–2003 study, an outbreak of respiratory disease associated with the respiratory pathogen *Mycoplasma ovipneumoniae* (Besser et al., 2008), hereafter *M. ovi*, was detected in the Old Dad Peak population in the central Mojave Desert. Several months later, the same strain was detected south of Interstate 40 in the Marble Mountains (T. Besser, Washington State University, and California Department of Fish and Wildlife [CDFW], unpublished data), suggesting stepwise contact by bighorn sheep had occurred across intervening regions, including across the interstate. Avenues for such crossing could include pushing through fencing and crossing at surface level, despite heavy traffic, or using washes bridged by the interstates but also fenced and typically occurring on flatter ground rarely used by bighorn sheep. While transmission of respiratory disease can occur through contact with even a single individual (Besser et al., 2014), this observation raised questions of considerable import for conservation of these metapopulations. Specifically: (i) did bighorn sheep begin crossing barriers within the last two generations, or alternately, (ii) did the spread of the disease indicate that previous genetic analyses were unable to detect ongoing but occasional movements across barriers? Additionally, how dynamic are estimates of genetic structure and genetic diversity across time points?

In this study, we contrast population genetic structure in a dynamic desert bighorn sheep metapopulation across two generations. By sampling the same populations ~12 years apart with the same genetic markers, we attempt to determine whether the interaction of this large mammal with anthropogenic barriers has changed, evaluate the degree of change in genetic structure and genetic diversity across populations and infer sources of recently recolonized or expanded populations. We hypothesized that changes in interpopulation movement patterns of bighorn sheep have occurred since the 2000–2003 study, including new connections formed by expanding populations and crossing of anthropogenic barriers, leading to changes in both structural and functional connectivity in localized portions of the study area. Specifically, we predicted that populations separated by Interstate 40 would show decreased genetic differentiation in 2013–2015 compared to 2000–2003. We also predicted that some individuals would be fully or partly assigned genetically to populations on the other side of the interstate barrier in 2013–2015, but not during 2000–2003, indicating that cross-interstate movements were rarer or undetected at the earlier time, and that pattern would be reflected in first-generation migrants as well. We further predicted that recently established populations in two locations would increase high gene flow linkages among populations. Finally, we consider the implications of this study for studies assessing functional connectivity at a single point in time.

2 | METHODS

2.1 | Study area

This study took place in the southern Mojave and central Mojave Desert metapopulations of desert bighorn sheep (Torres, Bleich, &

Wehausen, 1994) in southeastern California (Figure 1). Those populations were genetically sampled in 2000–2003 (hereafter, Time Point 1 or TP1) by Epps et al. (2005), Epps, Palsboll, Wehausen, Roderick, and McCullough (2006). In 2013–2015 (hereafter, Time Point 2, or TP2), we resampled 13 populations in the core of the Epps et al. (2005, 2006) study area. This sampling area was centred on the recent respiratory disease outbreak first detected at Old Dad Peak in Mojave National Preserve in 2013 (CDFW, unpublished data), as well as one apparently newly colonized population in the South Soda Mountains (Figure 1; Abella et al., 2011). Populations in the resurvey spanned a gradient of genetic diversity and isolation at TP1 (Epps et al., 2005). Interstate 40, a four-lane divided highway fenced on both sides, separated four southern populations from the remainder of the bighorn sheep populations considered in this study (Figure 1). All populations in the study area were native (i.e., never augmented by translocation), except that bighorn sheep from Old Dad Peak were translocated to the nearby North Bristol population in 1992 to mitigate an apparent population extinction in the mid-20th century (Wehausen, 1999). However, by the time of the sampling at TP1, apparently only a few transient males remained (Epps, Bleich, Wehausen, & Torres, 2003).

2.2 | Genetic sampling

We used faecal samples as a primary source of DNA in TP2, collected by visiting water sources during summer months when bighorn sheep are dependent on water and collecting opportunistically at other times of the year. We sampled at the same locations as in Epps et al. (2005) and collected faecal samples up to several weeks in age; if wet, samples were dried before storing at room temperature. We processed pellets and extracted DNA using a modified version of the AquaGenomic Stool and Soil protocol (Multitarget Pharmaceuticals

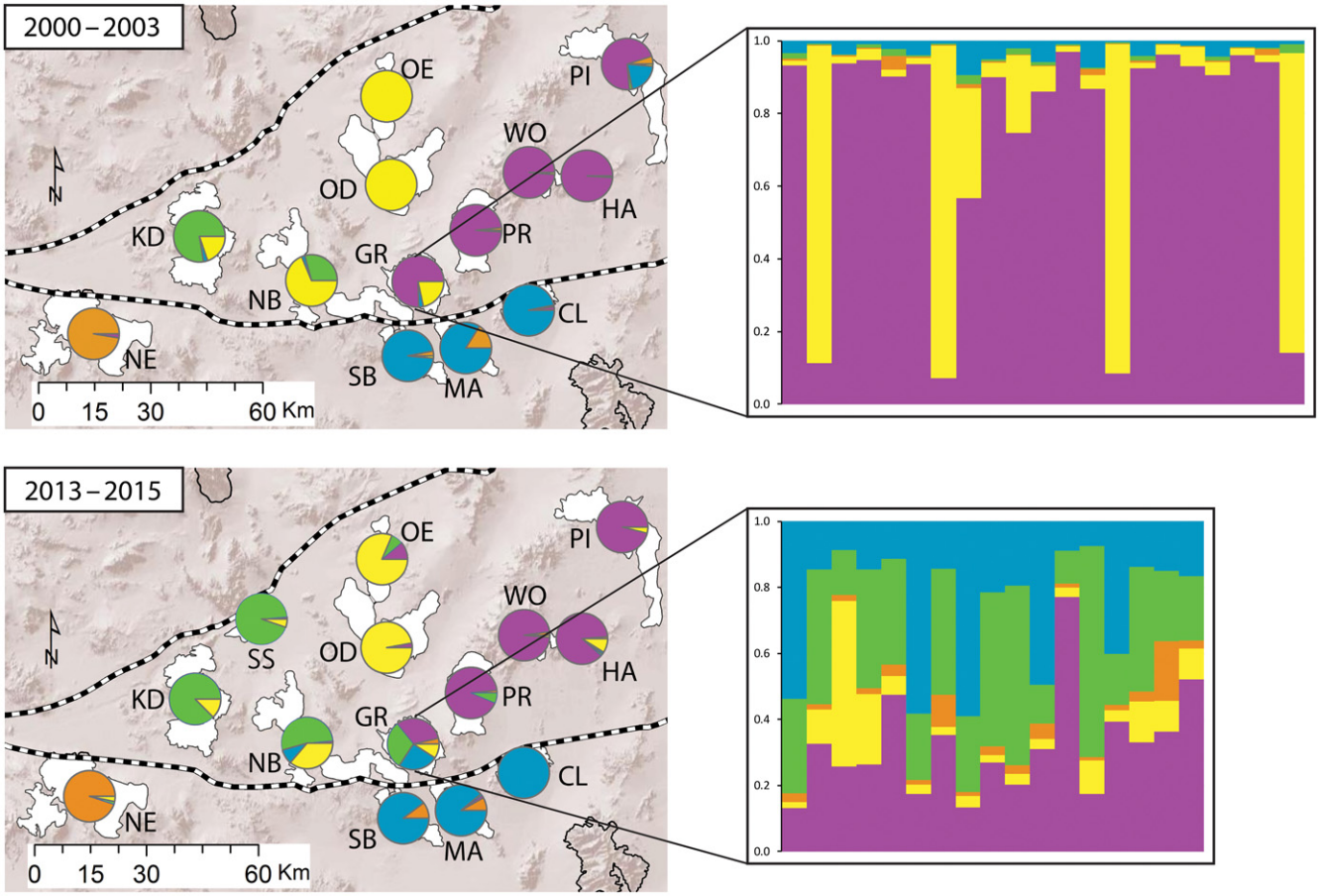

FIGURE 1 Desert bighorn sheep populations genetically sampled at two time points (2000–2003 and 2013–2015, white polygons) in the Mojave Desert of California, with other nearby populations drawn with black outlines, and shaded topographic relief. The South Soda Mountains population, an apparent recent colonization, was sampled only in 2013–2015. Interstate highways are depicted with dashed lines. Average assignments of individuals from desert bighorn sheep populations in 2000–2003 and 2013–2015 ($k = 5$) from Program STRUCTURE are shown colour-coded by proportional assignment to cluster by population (circles) and by individual (Granite Mountains [GR], where each vertical bar reflects an individual). In 2000–2003, no individuals bordering I-40 were assigned to populations on the opposite side, whereas in 2013–2015, five individuals in the Granite Mountains were at least 40% assigned to the populations south of I-40 (blue cluster). Individual assignments for all populations are presented in Figure S3. CL, Clipper Mountains; GR, Granite Mountains; HA, Hackberry Mountains; KD, Cady Mountains; MA, Marble Mountains; NB, North Bristol Mountains; NE, Newberry/Ord/Rodman Mountains; OD, Old Dad Peak/Marl/Kelso Mountains; OE, Indian Spring/Club Peak; PI, Piute Range; PR, Providence Range; SS, South Soda Mountains; WO, Wood Mountains. Polygons modified from Creech et al. (2014)

LLC, Colorado Springs, CO; see details in Appendix S1). We also used DNA extracted from blood of 159 bighorn sheep captured as part of an ongoing demographic study (2013–2015). Capture protocols were approved by the National Park Service IACUC (ACUP #PWR_MOJA_Epps.Powers DesertBighorn_2013). Whole blood was collected in EDTA tubes and spun at 4,000×*g* for 10 min to separate the buffy coat. In 16 cases, DNA was also obtained from ear tips removed from carcasses. We extracted DNA using a Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc, Valencia, CA, USA) and 30 mg of dried tissue or 200 μl of buffy coat.

2.3 | Genotyping, markers, individual identification and marker evaluation

We used 16 variable microsatellite loci to characterize genetic diversity and genetic structure at both time points (Table S1; Appendix S1). Samples at TP1 were genotyped by Epps et al. (2005, 10 loci) and Nickerson (2014, remaining 6 loci). We checked consistency of allele size identification for markers used at both time points by rerunning 16 individuals (to provide a wide diversity of allele sizes) selected across 12 populations from TP1 under laboratory conditions used in TP2 analyses, determining appropriate size corrections, and correcting allele sizes to match those in TP2. Reaction conditions and thermocycling profiles for PCR, genotyping methods, genotype matching and testing for Hardy–Weinberg equilibrium and linkage disequilibrium are described in Appendix S1.

Three of the microsatellite markers were linked to genes related to immune system function in other bovids (BL4, associated with the interferon gamma gene involved in parasite resistance; Coltman, Wilson, Pilkington, Stear, & Pemberton, 2001, TGLA387, linked to the MHC gene complex; Maddox et al., 2001, and TCRBV62, linked to genes for T-cell receptors; Buitkamp, Schwaiger, & Epplen, 1993), but have also been employed as neutral microsatellite markers in systems where they exhibited no evidence of selection (Johnson, Mills, Wehausen, Stephenson, & Luikart, 2011; Luikart et al., 2011). Therefore, we used LOSITAN (Antao, Lopes, Lopes, Beja-Pereira, & Luikart, 2008; Beaumont & Nichols, 1996) to test all microsatellites for positive and balancing selection within each time point. We conducted tests using both stepwise and infinite allele mutation models, using 1,000,000 iterations, approximated mean neutral F_{ST} by removing potential selected loci (Antao et al., 2008) and allowed LOSITAN to select the subsample size for each test. We computed 99% confidence intervals for neutral expectations; loci falling outside those intervals were considered to be potentially influenced by natural selection (Luikart et al., 2011). Because markers under selection can enhance assignment of individuals to source populations (Ogden & Linacre, 2015), all markers were retained for STRUCTURE and GENECLASS analyses. For estimates of genetic structure (F_{ST}), however, we removed markers showing evidence of positive or balancing selection across both time points (Luikart et al., 2011).

After identifying and discarding duplicate individuals and generating complete genotypes for each population (Appendix S1), we used CERVUS (Marshall, Slate, Kruuk, & Pemberton, 1998) to test for matching genotypes across populations. If a genotype was recaptured in more than one population within a time point, for subsequent analyses, we used each genotype only in the population in which it was first detected. Because desert bighorn sheep in this area can live up to ~20 years (J. Wehausen, personal communication, November 21, 2016), we also tested for matching genotypes between the data sets from the two time points. We recorded any such matches but retained matching genotypes in data sets for both time points.

2.4 | Assessing changes in genetic structure and detecting migrants

To ascertain changes in connectivity, including whether bighorn sheep moved across Interstate 40 at either time point, we used genetic recapture (above), estimates of genetic structure, assignment tests and tests for first-generation migrants (i.e., F0, Paetkau, Slade, Burden, & Estoup, 2004; hereafter referred to as migrants). For genetic structure, after removing loci with evidence of selection at both time points (Appendix S1), we used FSTAT (Goudet, 1995) to estimate pairwise F_{ST} (Weir & Cockerham, 1984) between all populations at each time point and estimated 95% confidence intervals by bootstrapping across loci for comparisons of interest. We subtracted pairwise F_{ST} values at TP1 from those at TP2 (hereafter, ΔF_{ST}) to rank changes in genetic structure among populations and compared high gene flow linkages ($F_{ST} \leq 0.05$, Epps et al., 2010) at both time points as an index of meaningful changes in patterns of connectivity. Further, we evaluated pairwise F_{ST} for each population to itself between time points to estimate within-population genetic changes, using 1,000 permutations over loci in ARLEQUIN (Schneider, Roessli, & Excoffier, 2000) to assess difference from zero. To further evaluate potential error in F_{ST} estimates resulting from variation in sample size, we selected three populations representing a gradient of low to high genetic structure and randomly subsampled individuals over a range of sample sizes, estimating pairwise F_{ST} and generating 95% quantiles from 5,000 replicates at each sample size increment (see Figure S1 for full description).

We used STRUCTURE (Pritchard, Stephens, & Donnelly, 2000) to infer individual assignments at both time points in a single analysis combining all data at both time steps, using all loci including any under selection. We used this approach to reduce impact of variation in sample sizes within populations across time steps. We examined individual assignments (q values for each individual to each cluster) within each time point to infer presence of migrants or offspring of migrants among clusters, including across Interstate 40, after estimating assignments (detailed in Appendix S1).

We used GENECLASS2 (Piry et al., 2004) to test for migrants among all populations at each time point, including those separated by Interstate 40. We used all loci including any under selection and applied the Paetkau, Calvert, Stirling, and Strobeck (1995) frequency-based criterion for likelihood computations and a default frequency for missing alleles of 0.01. To estimate the probability of each individual being a migrant, we employed the Paetkau et al. (2004) resampling algorithm, 10,000 simulated individuals, and a threshold significance of $p < .01$.

2.5 | Assessing changes in genetic diversity

We estimated genetic diversity using FSTAT (expected heterozygosity, H_e; average allelic richness, corrected for minimum sample size across loci and time points, A_r) in all populations at TP1 and TP2, using only loci showing no evidence of selection at both time points. For estimating A_r, we further excluded one locus that largely failed to amplify in one small population (see Section 3). After identifying populations of specific interest for genetic diversity comparisons (Marble Mountains and Granite Mountains, see Section 3), we re-estimated H_e and A_r for those populations alone to remove sample size constraints imposed by other populations. Finally, in each of those populations of interest, we tested whether genetic diversity was higher in TP2 than TP1 using paired one-tailed Wilcoxon ranked sum tests on corrected A_r and H_e, implemented in JMP Pro (Version 12.0.1, SAS Institute Inc., ©2015). This test allows comparison of genetic diversity within loci (Luikart et al., 2011).

3 | RESULTS

3.1 | Genotyping, markers and individual identification

In TP2, samples were collected successfully at similar locations as those in TP1 except in the Piute Range, where the concentration of bighorn sheep appeared to have shifted ~30 km from the Viceroy Mine at Hart Mountain in 2003 to Piute Spring in 2015. We generated microsatellite genotypes for 206 unique individuals in 13 populations at TP1 (<4% of all allele calls missing) and 384 unique individuals in 14 populations at TP2 (Table 1; <1% allele calls missing). We detected potential positive selection (using $\alpha = 0.01$) on adaptive-linked microsatellite BL4 at TP1, and BL4 approached significant positive selection at TP2 (Table S2). At TP2, putatively neutral microsatellite OarFCB11 exhibited potential positive selection (Table S2), although this locus did not approach significance at TP1. To create data sets as parallel as possible across time points, we chose to eliminate BL4 from analyses of pairwise F_{ST} and genetic diversity given that it was adaptive-linked and was potentially under or nearly under positive selection at both time points, as well as out of HWE at TP1 (Appendix S1). We did not exclude OarFCB11 from either data set. Excepting BL4, we found no consistent evidence of any locus out of HWE or in linkage disequilibrium (Appendix S1).

Power for determining recaptures of individuals among populations and time points was high: probability of identity (P_{ID}; Waits, Luikart, & Taberlet, 2001) for the full 16-locus data set was 1.02×10^{-13}–2.00×10^{-8} (median 1.18×10^{-11}); P_{IDsibs} was 3.25×10^{-6}–2.47×10^{-4} (median: 1.64×10^{-5}). Genotype matching revealed that bighorn sheep made intermountain movements at both time points (Figure 2; Appendix S1), but crossed an interstate only at TP2: a male first detected from a faecal sample collected in the Granite Mountains in August 2014 was captured in November 2014 in the Marble Mountains on the other side of Interstate 40. Matches also occurred between time points: 5 of 384 unique genotypes sampled at TP2-matched genotypes from TP1 (Appendix S1).

TABLE 1 Population size classes and numbers of genotypes included in population genetic study of desert bighorn sheep in the Mojave Desert, California, at two time points (2000–2003 and 2013–2015)

Population	Genotyped individuals (2000–2003)	Population size-class estimate (c. 2004)	Genotyped individuals (2013–2015)	Population size-class estimate (c. 2010)
CL	16	25–50	34	No update
GR	21	25–50	17	No update
HA	13	25–50 (including WO)	11	No update
KD	12	25–50	20	201–300
MA[a]	29	101–150	47 (46)	151–200
NB	6	0 (transient males only)[b]	50	51–100
NE	14	51–100	25	151–200[c]
OD	25	201–300	48	No update[d]
OE	12	25–50	14	No update
PI	13	51–100	12	No update
PR	20	51–100	26	No update
SB	14	101–150	45	No update
SS	NA	Not known to exist	26	25–50
[a]WO	10	25–50 (including HA)	11 (10)	No update
[a]Total	206	–	386 (384)	–

[a]Sample size in 2013–2015 analyses was subsequently reduced by 1 for MA and WO because one individual in each case was first detected in a different population (GR and HA, respectively).
[b]Bighorn sheep were translocated from Old Dad Peak to North Bristol Mountains in 1992, but the translocation is thought to have failed.
[c]2016 aerial survey by CDFW, unpublished data, based on minimum count.
[d]Thought to have declined sharply in 2013 due to an all-ages die-off from respiratory disease.

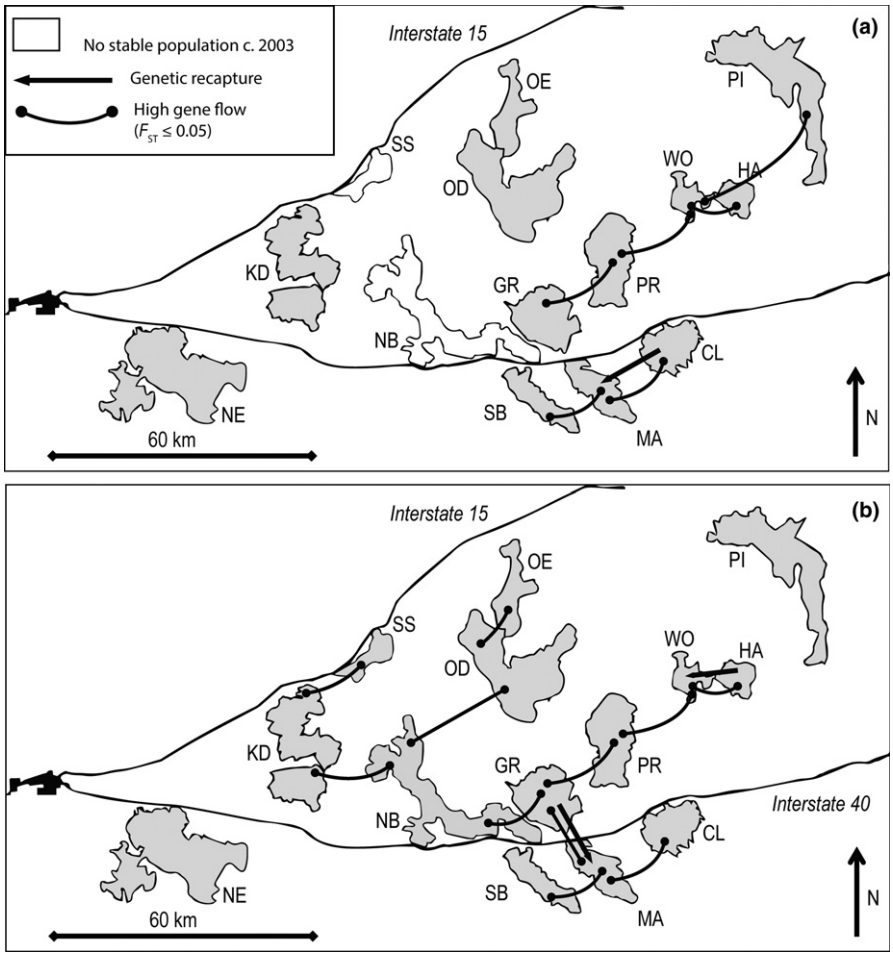

FIGURE 2 Changes in high gene flow linkages (F_{ST} < 0.05, determined by Epps et al., (2010) to be correlated with frequent movements among populations) and genetic recaptures in 2000–2003 (a) and 2013–2015 (b) for desert bighorn sheep populations in the Mojave Desert of California. Arrows represent genetic recaptures between populations within each time point, where the head of the arrow indicates the second observation of that individual. CL, Clipper Mountains; GR, Granite Mountains; HA, Hackberry Mountains; KD, Cady Mountains; MA, Marble Mountains; NB, North Bristol Mountains; NE, Newberry/Ord/Rodman Mountains; OD, Old Dad Peak/Marl/Kelso Mountains; OE, Indian Spring/Club Peak; PI, Piute Range; PR, Providence Range; SS, South Soda Mountains; WO, Wood Mountains

3.2 | Assessing changes in genetic structure and detecting migrants

Population pairwise F_{ST} estimates within the same population between time periods (hereafter, $F_{ST\_TP1:TP2}$) varied ($F_{ST\_TP1:TP2}$ = 0–0.096, Figure 3). Thus, genetic make-up of some populations changed markedly between the two sampling points, as did genetic distances between some pairs of population (Figure 4; Table S3, S4), although many comparisons showed little evidence of change across time points. Median and average genetic distances between the North Bristol Mountains and all other populations decreased the most (median: ΔF_{ST} of −0.041; Figure 4a; Table S4); genetic distances increased the most for comparisons including the Piute Range (median ΔF_{ST} of 0.053; Figure 4b; Table S4). Bootstrapping across loci suggested our power to detect differences in F_{ST} was somewhat compromised by small sample sizes (Figure 4), but experimental variation in sample size while holding loci the same resulted in much less variation in F_{ST} estimates (Figure S1). Genetic distance between populations separated by Interstate 40 declined sharply in at least one case: point estimates of F_{ST} declined from 0.11 to 0.04 (ΔF_{ST} = −0.067) between the Marble and Granite Mountains populations, and neither point estimate intersected the 95% confidence intervals of F_{ST} for the other time period (Table 2; Figure 4c). Other cross-interstate comparisons changed little (ΔF_{ST} = −0.027 to 0.034, Table 2).

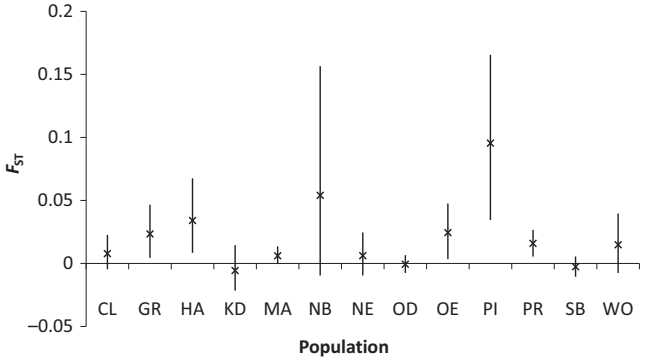

FIGURE 3 Within-population pairwise F_{ST} estimates (crosses) between sampling periods (2000–2003 and 2013–2015) in 13 populations of desert bighorn sheep in the Mojave Desert of California, from 15 microsatellite loci, with 95% confidence intervals. CL, Clipper Mountains; GR, Granite Mountains; HA, Hackberry Mountains; KD, Cady Mountains; MA, Marble Mountains; NB, North Bristol Mountains; NE, Newberry/Ord/Rodman Mountains; OD, Old Dad Peak/Marl/Kelso Mountains; OE, Indian Spring/Club Peak; PI, Piute Range; PR, Providence Range; WO, Wood Mountains

Assignment tests (STRUCTURE) and tests for migrants showed cross-interstate movements in TP2 but not TP1. For STRUCTURE analyses, we selected k = 5 (Figure S2). Results for each time point analysed separately were concordant with the combined analyses (not

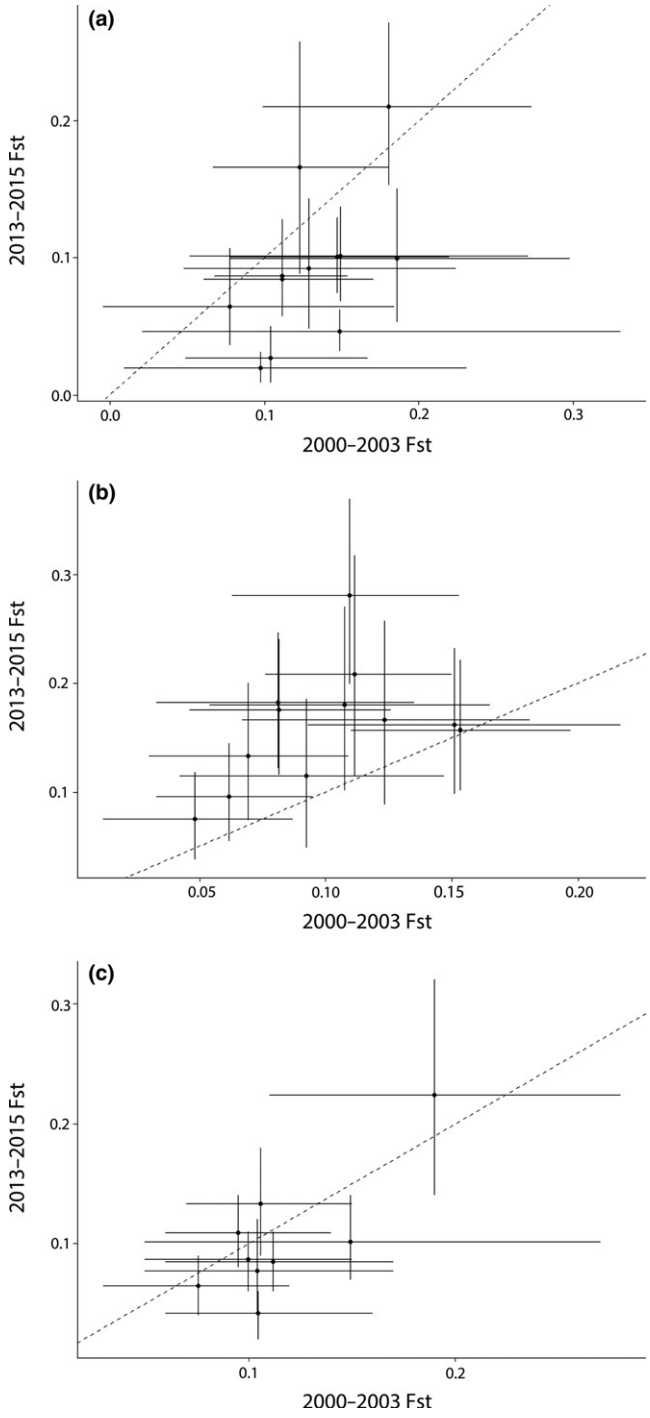

FIGURE 4 Population pairwise F_{ST} estimates among 13 populations of desert bighorn sheep in the Mojave Desert of California, based on 15 microsatellite markers, contrasted across two sampling periods (2000–2003 and 2013–2015), with 95% confidence intervals estimated by bootstrapping across loci. The dashed line marks identity between the time points, thus separating population comparisons for which genetic structure has decreased (below) and those that have increased (above). Values are shown for population pairs including the North Bristol Mountains (a), Piute Range (b) and populations near to but separated by Interstate 40 (c), including the Marble-Granite comparison (starred) where gene flow across the interstate was detected in Time Point (TP) 2 but not TP1. Although confidence intervals are large due to small sample size at TP1, most point estimates for the North Bristol Mountains (a) fall below the line, suggesting a general increase in genetic similarity with other populations in the study area, whereas all those for the Piute Range (b) all fall above the line, suggesting a general decrease in genetic similarity. Cross-interstate comparisons (c) showed little change except the Marble-Granite comparison

shown). At $k = 5$, all clusters had multiple individuals assigned at high confidence (q_{max} = 0.969–0.996). Both population average (Figure 1) and individual assignments (Figure S3) showed no evidence of cross-interstate movements among any population pairs at TP1 (e.g., North-South Bristol, Marble-Granite, Marble-North Bristol, Clipper-Providence). At TP2, however, the Granite Mountain populations showed clear contribution of individuals from populations south of Interstate 40 (Marble, Clipper or South Bristol Mountains, Figure 1), with five individuals assigned across the interstate at $q > 0.4$ (Figure 1). GENECLASS2 analyses identified one of those individuals as a migrant from the Marble Mountains (Table 3). One animal in the North Bristol Mountains showed ~40% assignment to the cluster south of the Interstate (Figure S3) and was identified as a migrant from the Marble Mountains (Table 3), but no evidence of potential direct movements between North and South Bristol Mountains was seen. Finally, in both analyses, no animals sampled south of the Interstate 40 appeared to be of northern origin (Figure 1, Table 3; Figure S3).

Other changes in connectivity were detected by assignment tests, tests for migrants and F_{ST} estimates. The North Bristol population was much more connected at TP2 (Figures 2 and 4). At TP1, this population was linked most closely to Old Dad Peak, likely due to remnant males from a prior translocation attempt (see Section 2.1). Since 2004, however, additional artificial water sources were developed, and a reproducing population was observed by 2009 (Abella et al., 2011). By TP2, genetic structure between North Bristol and nearby populations declined sharply (Figures 2 and 4), suggesting very frequent interpopulation movements were then occurring with the Cady Mountains and Granite Mountains, and to a lesser degree with Old Dad Peak (Figures 2 and 4; Figure S3, Tables S3, S4). This pattern was further supported by detection of migrants among those populations in TP2 (Table 3) and by individual assignments (Figure 1; Figure S3). The South Soda Mountains population, suspected to have been colonized from the Cady Mountains (J. Wehausen, personal communication, July 3, 2012), showed that link very clearly (Figure 1; Figure S3, Table S3, F_{ST} = 0.028).

Some changes were unsuspected prior to this analysis. At TP1, both Old Dad Peak and Indian Spring populations appeared completely isolated except from each other, although North Bristol animals showed significant Old Dad Peak heritage presumably due to the reintroduction attempt. Individual assignments (Figure 1; Figure S3) and detection of migrants (Table 3) showed clear evidence of new gene flow at TP2 to Old Dad Peak and Indian Spring from populations to the east (Providence, Wood, Hackberry and Piute cluster), and at least one individual at Indian Spring with Cady or

TABLE 2 Genetic distance (population pairwise F_{ST}) between desert bighorn sheep populations along Interstate 40, based on 15 microsatellite loci, from 2000–2003 and 2013–2015, with change in mean genetic distance (ΔF_{ST}) between time periods

Population pair	F_{ST} 2000–2003 (95% CI)	F_{ST} 2013–2015 (95% CI)	ΔF_{ST}	% change in 2000–2003 F_{ST}
CL-GR	0.08 (0.03–0.12)	0.07 (0.04–0.09)	−0.01	−14
CL-PR	0.10 (0.06–0.14)	0.11 (0.08–0.14)	0.01	15
KD-NE	0.19 (0.11–0.28)	0.22 (0.14–0.32)	0.03	18
KD-SB	0.11 (0.07–0.15)	0.13 (0.09–0.18)	0.03	26
MA-GR	**0.105 (0.062–0.156)**	**0.042 (0.020–0.064)**	**−0.063**	**−60**
MA-NB	0.11 (0.06–0.17)	0.09 (0.06–0.11)	−0.03	−24
MA-PR	0.10 (0.05–0.15)	0.09 (0.06–0.11)	−0.01	−13
SB-GR	0.10 (0.05–0.17)	0.08 (0.04–0.12)	−0.03	−26
SB-NB	0.15 (0.05–0.27)	0.10 (0.07–0.14)	−0.05	−32

Only comparisons across the highway are shown. The Marble (MA) and Granite (GR) Mountains pair (bolded) showed the most direct evidence for cross-interstate movements during 2013–2015.

South Soda Mountains heritage (Table 3; Figure S3). Beyond the changes detailed above, however, genetic distances and patterns of assignment to STRUCTURE clusters showed little change among many populations (Table S4; Figure S3).

3.3 | Genetic diversity

Before estimating genetic diversity, we removed locus BL4 because of evidence of positive selection at that locus (Appendix S1). For estimating allelic richness (A_r), we also removed locus OarFCB266 because it mostly failed in the small North Bristol sample at TP1. Genetic diversity changed little in most populations (Table S5). Genetic diversity in two populations apparently linked by new movements across Interstate 40 (Granite and Marble Mountains) did not change significantly: average expected heterozygosity (H_e) across 15 loci did not increase significantly in either population (pairwise Wilcoxon rank-sum tests; Granite Mountains, mean $H_{e\_TP1} = 0.66$, mean $H_{e\_TP2} = 0.69$, $S = -22.0$, $p = .11$; Marble Mountains, mean $H_{e\_TP1} = 0.66$, mean $H_{e\_TP2} = 0.67$, $S = -19.5$, $p = .14$). Using a minimum per-locus sample size of 15 in those two populations across time points, average A_r across 15 loci also did not change significantly between time points in the Granite Mountains (pairwise Wilcoxon rank-sum tests, $A_{r\_TP1} = 4.62$, $A_{r\_TP2} = 4.83$, $S = -20.5$, $p = .12$) or in the Marble Mountains ($A_{r\_TP1} = 4.20$, $A_{r\_TP2} = 4.26$, $S = -14.0$, $p = .24$). Considering only those two populations, 17 alleles in each time step were private to one or the other population (TP1: MA, $n = 4$ alleles, GR, $n = 13$; TP2: MA, $n = 5$, GR, $n = 12$), although the identity of the private alleles varied across time points in some cases.

4 | DISCUSSION

We observed significant localized changes in genetic structure, supporting our hypothesis that both structural and functional connectivity changed among populations of desert bighorn sheep in the central Mojave Desert of California after only two generations (~12 years). These changes appeared to be driven in part by colonization and population expansion into habitats apparently unoccupied or transiently occupied c. 2000–2003 (TP1), but also by apparent changes in willingness or ability of bighorn sheep to move across or under a fenced four-lane highway (Interstate 40) in at least one location (north end of Marble Mountains). In particular, between time points, we observed a twofold decrease in genetic distance between two populations in mountain ranges separated by that highway, detected via genotype recapture one bighorn sheep using both ranges and detected two individuals assigned as migrants across the highway. In contrast, in TP1, we saw no cross-interstate assignment of individuals or migrants. Thus, we conclude that between TP1 and TP2, bighorn sheep began crossing Interstate 40 in at least one location. We know of no change in structural barriers or decrease in traffic over this time. Other populations separated by that highway still showed no clear evidence of increased gene flow or cross-assignment of individuals since 2000–2003 (Tables 2 and 3; Figure S3), suggesting that the fenced highway typically still acts as a barrier.

Although population genetic approaches often are not precise at detecting occasional or short-term interpopulation movements (Lowe & Allendorf, 2010), we suggest that the gene flow across the interstate highway between the Marble and Granite and possibly the Marble and North Bristol Mountains detected at TP2 is a new pattern of movement, and was not simply "missed" at TP1. In addition to our analyses, a summary of radiotelemetry data collected in the region over more than a decade prior to TP1 likewise showed no confirmed crossings (Epps et al., 2007). Nor do we ascribe the change in genetic structure to a time-lagged response to movements before TP1 (Epps & Keyghobadi, 2015), because individual assignments and migrant tests among these genetically distinct populations would offer immediate detection of new connections. Sample sizes were larger in some populations in TP2 (Table 1), likely increasing chances of detecting migrants by assignment tests or genetic recapture. However, the analysis of all samples from both time points in STRUCTURE would be less influenced by sample size differences. Our simulations of power to detect change in F_{ST} over different sample sizes also suggest reasonable power to resolve differences among most populations (Figure S1), particularly in the Marble-Granite

TABLE 3 First-generation (i.e., F0, Paetkau et al., 2004) migrants detected among desert bighorn sheep during 2000–2003 and 2013–2015, using GENECLASS2 and 15 microsatellite loci

Time period	Population where migrant detected	Inferred source population	Instances	*p*
2000–2003	Clipper Mtns (CL)	Marble Mtns (MA)	2	.0081, .0031
	Granite Mtns (GR)	Old Dad Peak (OD)	1	.0025
	Granite Mtns (GR)	Providence Mtns (PR)	1	.0095
	Granite Mtns (GR)[a]	Old Dad Peak (OD)	1	.0023
	Cady Mtns (KD)[a]	Old Dad Peak (OD)	1	.0001
	Marble Mtns (MA)	South Bristol Mtns (SB)	1	.0099
	Old Dad Peak (OD)	Indian Spring (OE)	2	.0041, .0025
	Indian Spring (OE)	Old Dad Peak (OD)	1	.0077
	Piute Range (PI)	Wood Mtns (WO)	1	.0019
	Providence Mtns (PR)	Piute Range (PI)	1	.0001
2013–2015	Clipper Mtns (CL)	Marble Mtns (MA)	1	.0038
	Granite Mtns (GR)	**Marble Mtns (MA)**	**1**	**.0098**
	Cady Mtns (KD)	Old Dad Peak (OD)	1	.0012
	North Bristol Mtns (NB)	Granite Mtns (GR)	2	.0010, .0038
	North Bristol Mtns (NB)	**Marble Mtns (MA)**	**1**	**.0083**
	North Bristol Mtns (NB)	Old Dad Peak (OD)	1	.0049
	Newberry Mtns (NE)[b]	Indian Spring (OE)	1	.0002
	Old Dad Peak (OD)	Indian Spring (OE)	1	.0094
	Old Dad Peak (OD)	Wood Mtns (WO)/Piute Range (PI)	1	<.0001
	Indian Spring (OE)	Old Dad Peak (OD)	1	.0087
	Indian Spring (OE)	Piute Range (PI)	1	.0099
	Indian Spring (OE)	South Soda Mtns (SS)	1	.0001
	Piute Range (PI)	Wood Mtns (WO)	1	.0008
	Providence Mtns (PR)	Hackberry Mtns (HA)	1	.0283
	Providence Mtns (PR)	South Soda Mtns (SS)/Cady Mtns (KD)	1	<.0001
	South Bristol Mtns (SB)	Marble Mtns (MA)	1	.0007
	South Soda Mtns (SS)	Granite Mtns (GR)	1	.003

We used a significance threshold of $p < .01$ to identify potential migrants. Near-ties in inferred source population (i.e., likelihood estimates differing by <1) are noted by listing >1 population. Migrants from populations across Interstate 40 are noted in bold.

[a]These assignments may be most parsimoniously explained as resulting from Old Dad Peak individuals that were translocated to the North Bristol Range in 1992 (Wild Sheep Working Group 2015) and subsequently migrated, as is common after a translocation, rather than natural movements from Old Dad Peak.

[b]This assignment results from recent gene flow between Newberry Mountains and the Sheephole Mountains (C. Epps, unpublished data), also south of Interstate 40, which received a transplant of Old Dad Peak individuals in 1984 (Wild Sheep Working Group 2015). Indian Spring is sometimes considered a subpopulation of Old Dad Peak due to movement by collared animals among those areas (Bleich, Whiting, Kie, & Bowyer, 2016).

comparison given robust sample sizes there. Thus, all lines of evidence consistently pointed to a change in movement patterns in this location. Fragmentation by roads and other linear anthropogenic features has been posited as a leading cause of habitat fragmentation and direct mortality for wildlife worldwide (Trombulak & Frissell, 2000). Our study in no way contradicts that conclusion, but does offer evidence that species may interact with such barriers in different ways over time.

This apparent change in willingness for crossing an interstate highway—albeit apparently only in one location—highlights the need for caution in extending inferences generated from a single estimate of functional connectivity forward in time (Farrington & Petren, 2011). Animal movement behaviours may be more plastic than we often recognize, particularly when such behaviours may be learned. Much attention has been given to the need to consider and estimate functional connectivity (Milanesi, Holderegger, Bollmann, Gugerli, &

Zellweger, 2017; Turgeon, Robillard, Gregoire, Duclos, & Kramer, 2010), particularly from empirical data, and individual variation in functional connectivity has likewise been recognized (Belisle, 2005). Even a single empirical estimate of functional connectivity can pose a significant challenge. Yet, the proliferation of studies of landscape ecology (Urban, Oneill, & Shugart, 1987) or landscape genetics (Manel, Schwartz, Luikart, & Taberlet, 2003) offers opportunities to revisit such estimates across a variety of systems, potentially shedding light on when repeated studies may be most warranted.

The largest shifts in genetic structure within the same populations (Figure 3) as well as between populations (Figures 2 and 4) appear to have been caused by establishment and subsequent expansion of new populations, which may be considered changes in structural connectivity. The small sizes of these populations (Table 1) make them particularly subject to rapid changes in genetic structure, as demonstrated by strong genetic structure at TP1 that apparently resulted from construction of barriers only ~7 generations before (Epps et al., 2005). An apparent recolonization of the North Bristol Mountains demonstrated how population restoration in a central location in a network can sharply increase gene flow and forge new links among populations over even a short period of time (Figure 2). Based on the genetic characteristics of this metapopulation at TP2 (Figure 1; Figure S3), we conclude the reestablishment of the North Bristol population was likely driven by expansion of the bighorn population in the Cady Mountains (Abella et al., 2011), and perhaps influenced by the installation of artificial water sources installed in the North Bristol Mountains around the time of the TP1 study. Other changes imply hitherto-unsuspected shifts in distribution and movements of bighorn sheep among mountain ranges, in some cases likely driven by dynamics outside of the study area (e.g., Piute Range, Figures 1, 3, 4).

Because no animal sampled south of Interstate 40 during 2013–2015 was assigned to populations north of the highway (Table 3; Figure S3), cross-highway gene flow seems largely driven by bighorn sheep originating south of Interstate 40 (likely, the Marble Mountains). Dispersal in bighorn sheep may best be described as facultative adult dispersal, as adults of both sexes and a variety of ages occasionally make long-distance or exploratory movements, but the behaviour is highly variable among individuals (O'Brien, O'Brien, McCarthy, & Carpenter, 2014). The gregarious nature of this species may mean that once a single individual has determined a new movement route, others will follow. Population expansion may also influence willingness of individuals to undertake potentially risky movements, as observed in other large herbivores with density-dependent dispersal (Labonte, Ouellet, Courtois, & Belisle, 1998), although larger populations may just produce increased numbers of dispersers. Populations have increased in the Marble Mountains and particularly the Cady Mountains in recent decades (Abella et al., 2011; Torres et al., 1994). Both populations served as source populations for natural recolonizations (this study, see also Epps et al., 2010). Thus, for this and other species exhibiting facultative adult dispersal, clarifying which individual- and population-level characteristics are associated with increased dispersal rates or numbers could improve our understanding of interpopulation movement or the potential for it to occur and thereby facilitate management of spatially complex systems.

Despite the dramatic increases in gene flow among several pairs of populations, only relatively small changes in genetic diversity have occurred in the affected populations over the 12-year interim between sampling, even where movement has now been established across man-made barriers present for >50 years. Genetic diversity is predicted to attain equilibrium more slowly than genetic structure after a change in migration rates (Epps & Keyghobadi, 2015); thus, we expect that genetic diversity will increase in future generations among those populations linked by new connections (e.g., Marble and Granite Mountains, Figure 1), unless influenced by other events such as population bottlenecks. The sharpest increase in genetic diversity occurred in the North Bristol Mountains, where a newly established or expanded population created a central connection among three or four populations (Figures 1 and 4), again pointing to the importance of connectivity and immigration in maintaining genetic diversity of metapopulations (Farrington & Petren, 2011). Many connections throughout the study area showed little evidence of change, however, so we do not expect a general trend of increased or decreased diversity across the study area (Table S5, Figure S3).

Our findings also shed light on the recent discovery of respiratory disease throughout much of the study system. The pattern of high gene flow links (i.e., $F_{ST} \leq 0.05$, Epps et al., 2010) observed among populations in 2013–2015 (Figure 2) corresponds exactly with the distribution of the single strain of *M. ovi* detected in the study area in 2013–2015 (CDFW, unpublished data). We documented a substantial decrease in the isolation of the Old Dad Peak/Kelso/Marl Mountains and Indian Spring populations, which we propose could in part explain the different response to respiratory disease observed there in 2013. In 2000–2003, these populations were strongly genetically distinct from other nearby populations (Figure 1; Table 3). By 2013–2015, interbreeding had occurred with the Providence-Wood-Hackberry-Piute chain of populations to the east and the North Bristol and connected ranges to the south (Figures 1 and 2, Tables 3; Figure S3, Table S4). During the recent outbreak of respiratory disease (2013-present), Old Dad Peak was the only population known to have experienced an all-ages die-off (CDFW, unpublished data). One hypothesis for this variable pattern of mortality, supported by serology tests (CDFW, unpublished data), is that other populations in the area had previously experienced *M. ovi* outbreaks, but Old Dad Peak had not because of its isolation.

Systematic genetic sampling in this metapopulation of large, long-lived mammals at time points separated by only two generations (12 years) revealed a hitherto-unsuspected degree of dynamism in genetic structure and, apparently, movement behaviour. We interpret these changes as resulting from population expansions, recolonizations and a change in functional connectivity, that is, willingness to cross an anthropogenic barrier. Our findings further support the use of population genetics as a way to obtain a high-resolution, systematic picture of metapopulation structure (Lamy, Pointier, Jarne, &

David, 2012), particularly when populations are small, but also make it clear that such characterizations may need revisiting. Moreover, we conclude that movement models based on any single estimate of movement patterns, whether genetic-based (Cushman et al., 2006; Epps et al., 2007) or telemetry-based (Chetkiewicz & Boyce, 2009), should be reviewed periodically. As future opportunities occur for recharacterizing animal movements in well-studied systems, we predict that systems with frequent population turnover, strong shifts in population density, or with long-lived species capable of learning behaviours from other individuals would be most likely to experience strong shifts in movement patterns.

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DATA ACCESSIBILITY

Sampling locations and 16-locus microsatellite genotypes of all individuals for both time periods are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.mp71t50.

AUTHOR CONTRIBUTIONS

C.W.E. designed the research; C.W.E. and R.S.C. conducted the field work; R.S.C., B.N. and C.W.E. conducted the laboratory work; C.W.E. analysed the data; C.W.E., R.S.C., B.N. wrote the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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