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An Evaluation of the Bighorn Sheep Population in Badlands National Park

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AN EVALUATION OF THE BIGHORN SHEEP POPULATION IN BADLANDS NATIONAL PARK

BY

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THESIS ACCEPTANCE PAGE

Austin J. Wieseler

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Within the last century, bighorn sheep (*Ovis canadensis*) in the badlands ecosystem of western South Dakota have been subjected to complete extirpation, reintroduction, disease die-offs, genetic bottlenecking, and population augmentation. Subsequently, the population in Badlands National Park (BNP) appears to have recovered, but it was unknown to what degree past events had influenced the population. From 2017-2019, we conducted research on 5 subherds within 2 management units in BNP to 1) survey for the presence of respiratory pathogens and estimate the prevalence of other potentially infectious diseases; 2) assess adult and lamb survival and cause-specific mortality; 3) estimate population size and growth; 4) evaluate the risk of disease exposure from domestic livestock operations within 8 km of the North Unit of BNP; and 5) evaluate genetic variation and population structuring and differentiation. We sampled (*n* = 83) individuals for the presence of respiratory pathogens including *Mycoplasma ovipneumoniae* (*Movi*). *Movi* results were PCR negative and serology positive (18% prevalence). Bacteriology results indicated additional respiratory pathogens (e.g., *Bibersteinia trehalosi*, *Pasteurella* species, *Mannheimia* species, *Trueperella pyogenes*) were present within the population. We radio-collared 49 adults and 53 lambs to monitor survival and cause-specific mortality. Overall adult and lamb survival was 96% (95% credible interval [CI] = 89%, 99%) and 82% (CI = 65%, 92%), respectively, with

predation accounting for 56% of lamb mortalities. We documented 5 domestic sheep and goat operations within 8 km of the North Unit of BNP. Two goat operations were sampled for respiratory pathogens, one of which testing PCR positive for *Movi* (77% prevalence). We estimated population growth of $\lambda = 1.17$ in 2016-2017 and $\lambda = 1.22$ in 2017-2018 with a minimum population size count of 233 in 2018. Genetic analysis was conducted at 15 microsatellite loci from 75 individual samples. Overall genetic variation for the BNP population was consistent with other native and translocated populations of bighorn sheep across their range. We found averages of 5.80 and 0.65 for allelic diversity and heterozygosity levels, respectively. We identified three genetically distinct clusters recognized as the three source herds used to establish and supplement the BNP population between 1967 and 2014. Disease and genetic variation were not impacting the growth and survival of the BNP population. As the population continues to have high survival and growth, disease exposure from contact with domestic livestock operations appears to be the greatest risk to the population in the future.

CHAPTER 1: DISEASE, SURVIVAL, AND GROWTH: AN EVALUATION OF THE BADLANDS NATIONAL PARK BIGHORN SHEEP POPULATION

This chapter is being prepared for publication and was coauthored by Daniel P. Walsh, E. Frances Cassirer, Thomas E. Besser, Eddie L. Childers, and Jonathan A. Jenks.

ABSTRACT

Bighorn sheep (*Ovis canadensis*) populations experience irregular periods of growth and declines. The introduction of infectious agents, particularly respiratory pathogens, has been identified as a leading source of these declines, which have inhibited recovery efforts for the last century. The demographic cost and effect of disease within a population can cause severe long-term consequences on population growth. Within the last century, bighorn sheep in the badlands ecosystem of western South Dakota have been subjected to complete extirpation, reintroduction, suspected disease die-offs, genetic bottlenecking, and population augmentation. Subsequently, the population in Badlands National Park (BNP) appears to have recovered, but it was unknown to what degree past events had influenced the population. From 2017-2019, we conducted research on 5 subherds within two management units in BNP to 1) survey for the presence of respiratory pathogens and estimate the prevalence of other potentially infectious diseases; 2) assess adult and lamb survival and cause-specific mortality; 3) estimate population size and growth; and 4) evaluate the risk of disease exposure from domestic livestock operations within 8 km of the North Unit of BNP. We sampled individuals (n = 83) for the presence of respiratory pathogens including *Mycoplasma ovipneumoniae* (*Movi*). *Movi* results were PCR negative and serology positive (18% prevalence). Bacteriology results indicated additional respiratory pathogens (e.g., *Bibersteinia trehalosi*, *Pasteurella* species, *Mannheimia* species, *Trueperella pyogenes*) were present. We radio-collared 49 adults and 53 lambs to monitor survival and cause-specific mortality. Adult and lamb survival rates were 96% (95% credible interval [CI] = 0.89-0.99) and 82% (CI = 0.65-0.92), respectively, with predation accounting for 56% of lamb mortality. We estimated

population growth of λ = 1.17 in 2016-2017 and λ = 1.22 in 2017-2018 with a minimum population size count of 233 in 2018. We documented 5 domestic sheep and goat operations within 8 km of the North Unit of BNP. Two goat operations were sampled for respiratory pathogens, one of which testing PCR positive for *Movi* (77% prevalence). Our results indicate disease was not impacting the growth and survival of the BNP population, but disease exposure from contact with livestock appears to be the greatest risk to the population in the future.

Keywords: bighorn sheep, lamb survival, cause-specific mortality, wildlife disease, domestic livestock.

INTRODUCTION

Bighorn sheep (*Ovis canadensis*) were historically one of the most abundant ungulates in North America. Populations were estimated to be in the millions, and were distributed across much of the western United States, portions of Mexico, and Canada (Buechner 1960, Geist 1971). Bighorn sheep have experienced a population decline of two orders of magnitude since the settlement of western North America in the 19th century (Buechner 1960). These declines reduced the total population to <20,000 individuals across just one-third of their native range by 1960 (Buechner 1960).

A combination of environmental and demographic factors, such as unregulated hunting, domestic livestock grazing, introduced infectious agents, loss of fitness, predation, and displacement from range and migratory behavior are credited with these large-scale declines (Douglas and Leslie 1999, Miller et al. 2012). Of these, the introduction of infectious agents, particularly respiratory pathogens, has been identified as a principal cause of declines in bighorn sheep populations and has inhibited recovery

efforts during the last century (Singer et al. 2000). Although the etiology of respiratory disease in bighorn sheep is complex, pneumonia epizootics in bighorn sheep populations commonly result in significant declines and disease often persists following epizootics (Valdez and Krausman 1999, Cassirer and Sinclair 2007, Smith et al. 2015, Garwood et al. 2020).

Initial catastrophic losses in adult bighorn sheep populations are often overshadowed by long-term effects of pneumonia outbreaks on lamb survival and recruitment (Cassirer and Sinclair 2007, Wood et al. 2017). Sustained lack of recruitment is the primary impediment to bighorn sheep population recovery and demographic costs of disease persistence can be equal to or greater than the impact of the initial epizootic, potentially leading to severe long-term consequences to population growth (Manlove et al. 2016, Plowright et al. 2017, Cassirer et al. 2018). This is especially true for ungulates where juvenile survival is more variable than adult survival (Gaillard et al. 2000, Raithel et al. 2007, Smith et al. 2014a).

Audubon's bighorn sheep (*O.c. auduboni*; previously described as a subspecies of bighorn sheep now Rocky Mountain bighorn sheep [*O. c. canadensis*]) historically inhabited the badlands of the Yellowstone and Missouri rivers in eastern Montana, eastern Wyoming, western North Dakota and South Dakota, and northwestern Nebraska (Valdez and Krausman 1999, Wehausen and Ramey 2000). The badlands ecosystem (present day Badlands National Park [BNP], Pine Ridge Reservation, and Buffalo Gap National Grasslands) of western South Dakota sustained a bighorn sheep population until the species was extirpated in 1924 in Washabaugh (a.k.a., south Jackson) County, near the present-day location of BNP (Gross et al. 2000, Zimmerman 2008). The badlands

ecosystem held no bighorn sheep from 1924 to 1964. In 1964, the National Park Service (NPS), in collaboration with the South Dakota Department of Game, Fish and Parks (SDGFP) and Colorado Parks and Wildlife, relocated 22 Rocky Mountain bighorn sheep from the Pikes Peak, Colorado source herd, into a 150-ha enclosure in BNP. After ~50% loss of the herd due to pneumonia-caused respiratory infections, the remaining 14 individuals were released into the park during late-summer 1967 (Ramey et al. 2000).

Slow population growth was observed for the next decade and the population separated into two subherds (i.e., South Unit and North Unit) in 1981, with the majority of the population occupying the North Unit of the park (McCutchen 1980, Singer and Gudorf 1999). A second disease epizootic in 1982 was suspected to have been due to a respiratory infection and/or bluetongue virus outbreak that further inhibited population growth and reduced the North Unit to ~50 individuals (Ramey et al. 2000). Significant population growth occurred following this decline, and by 1988 the total estimated population for both subherds was ≈160 individuals. A third disease epizootic occurred in the early 1990's with an estimated >50% loss occurred; this outbreak reduced the population to ~60 individuals by 1996 (National Park Service 1998, Ramey et al. 2000). Following decades of variable growth and decline attributed to multiple suspected disease epizootics and a probable population bottleneck at founding, the population was recommended for a mixed sex augmentation ($n > 30$) from an outbred, native population of Rocky Mountain bighorn sheep (Ramey et al. 2000). In September 2004, BNP, in conjunction with SDGFP and New Mexico Department of Game and Fish, relocated 23 bighorn sheep from Wheeler Peak, New Mexico to augment genetic diversity of the remaining bighorn sheep population. The augmentation proved to be successful, resulting

in enhanced genetic diversity, recruitment, and population health, and post-augmentation estimates indicated strong population growth (Zimmerman 2008). In January 2014, SDGFP, Rocky Boy's Reservation, Montana, and the Oglala Sioux Tribe of Pine Ridge Reservation, South Dakota, captured and translocated 40 bighorn sheep from Montana to South Dakota. Twenty of these were released at Cedar Butte in the South Unit of BNP to bolster the subherd located in that unit (Parr 2015, South Dakota Game, Fish and Parks 2018).

During this study, the bighorn sheep population in BNP had formed into a metapopulation that resides in the North and South Units (Figure 1). This metapopulation structure was comprised of 5 known subherds (Pinnacles, Cedar Pass, Hay Butte, Homestead, and South Unit) (Figure 1). The North Unit (Pinnacles, Cedar Pass, Hay Butte, and Homestead subherds) is managed by the National Park Service, and the South Unit (South Unit subherd) is managed by the Oglala Sioux Tribe of the Pine Ridge Reservation. Despite declines in adult sheep, and subsequent poor lamb recruitment associated with pneumonia epizootics in several neighboring herds in western South Dakota at the time of this study, the BNP bighorn sheep population appears to have no recent or current disease as of 2016. Surveys estimated a total population ≥160 individuals in 2016, but knowledge was lacking about the current population's survival and growth following suspected disease epizootics and two augmentation efforts. Identifying current demographic and growth rates, presence and prevalence of pathogens, and risk of pathogen spillover from domestic operations was essential to providing recommendations and strategies for the short- and long-term management of the BNP bighorn sheep metapopulation. Our objectives were to 1) survey for the presence of

respiratory pathogens and estimate the prevalence of exposure to other infectious diseases within the BNP population; 2) estimate adult and lamb survival and document cause-specific mortality in the North Unit of BNP; 3) estimate population size and growth within the 4 subherds of the North Unit in BNP; and 4) evaluate the risk of disease from exposure to domestic sheep and goats within 8 km of the North Unit of BNP.

METHODS

Study Area

BNP is located in the White River badlands of southwest South Dakota in Pennington, Jackson, and Oglala Lakota counties. Our study area in BNP encompassed ~98,400 ha ranging in elevation from 700 m to 1,000 m above mean sea level (Weedon 1999). The surrounding region with suitable bighorn sheep habitat (hereafter, the greater badlands ecosystem) was comprised of USDA National Forest Service (Buffalo Gap National Grasslands), Pine Ridge Reservation, and privately owned lands (Sweanor et al. 1995, Gross et al. 2000). The topography was an ancient flood plain of highly eroded, diverse cliffs, canyons, and spires 100 m tall with steep gradients (0-71°) giving away to sod tables, crevasses, toadstools, and fragmented higher plains (Sweanor et al. 1995, Weedon 1999). Climate of the badlands was highly variable and unpredictable; total annual precipitation was 41 cm and mean annual temperature was 11°C (range: -27°C to 41°C) during 2017-2018 in Scenic, SD (National Oceanic and Atmospheric Administration 2019).

The badlands are primarily a mixed-grass prairie ecosystem dominated by buffalograss (*Buchloe dactyloides*), western wheatgrass (*Pascopyrum smithii*), and

prickly pear cactus (*Opuntia polyacantha*), with limited tree and brush species, consisting of Rocky Mountain juniper (*Juniperus scopulorum*) and eastern red cedar (*J. virginiana*) in draws and vegetated slopes (Weedon 1999). Other ungulates in the study area included bison (*Bison bison*), mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), and pronghorn (*Antilocapra americana*), along with additional herbivore competition from black-tailed prairie dogs (*Cynomys ludovicianus*). Potential predators of bighorn sheep in BNP included coyotes (*Canis latrans*), bobcats (*Lynx rufus*), golden eagles (*Aquila chrysaetus*), and mountain lions (*Puma concolor*). Mountain lion presence and impact on bighorn sheep in BNP was limited, with rare sightings and sign generally attributed to dispersing individuals from the Black Hills of South Dakota (Thompson and Jenks 2010).

Adult Capture, Data Collection, and Disease Surveillance

We captured adult ewes and rams via aerial net-gunning (Jacques et al. 2009) from a helicopter (Hells Canyon Helicopters, Clarkston, WA, USA and Quicksilver Air, Inc., Fairbanks, AK, USA) in March 2017 and February 2018. We estimated ewe age based on tooth eruption (Krausman and Bowyer 2003) and ram age based on horn annuli (Geist 1966). We fit all captured individuals with very high frequency (VHF) or global positioning system (GPS) collars with programmed release (24 months) manufactured by Advanced Telemetry Systems (ATS; Isanti, MN, USA). We fit ewes with VHF collars (M2230A; ATS) and rams with GPS collars (G2110B; ATS) to identify cause-specific mortality rates, movements, and subherd structure and interactions. Each radio-collared individual also received a unique tag installed on the radio-collar for individual identification. We evaluated pregnancy status of all ewes at time of capture using

ultrasonography (E.I. Medical Imaging, Loveland, CO, USA). Pregnant ewes were fitted with temperature-activated vaginal implant VHF transmitters (VITs; M3930; ATS) to assist with locating parturition sites and newborn lambs (Bishop et al. 2011). We collected biological samples from every captured individual for use in detecting the presence of bacterial and viral pathogens and/or the presence of antibodies. All individuals were weighed before release. The South Dakota State University Institutional Animal Care and Use Committee approved all capture and handling procedures (Approval number 17-003A).

To extend disease surveillance throughout the entire population of bighorn sheep in BNP, we collaborated with Oglala Sioux Park Resource Agency (OSPRA) to evaluate the presence of respiratory pathogens and/or the presence of antibodies indicative of exposure to respiratory and viral pathogens in the South Unit subherd in BNP. We assisted with capturing adult ewes and rams via aerial net-gunning (Jacques et al. 2009) from a helicopter (Helicopter Wildlife Services, Austin, TX, USA) in February 2019 and collected biological samples from captured individuals.

Respiratory Bacteria and Viral Testing

We collected nasal and oropharyngeal swabs for detecting respiratory pathogens associated with polymicrobial pneumonia in bighorn sheep (Besser et al. 2013), with particular focus on *Mycoplasma ovipneumoniae* (*Movi*) (Besser et al. 2008). Nasal swabs (n = 33) were inserted and gently rotated in both naris, with 2 swabs returned to their original swab sheath and 1 swab stored in Tryptic Soy Broth (TSB) media with 15% Glycerol (Hardy Diagnostics) (Drew et al. 2014, Butler et al. 2017). Oropharyngeal

swabs (n = 3) were rotated across the tonsillar crypts with 2 swabs returned to their original swab sheath and 1 swab stored in TSB media with 15% Glycerol. We collected whole blood for analyzing serum to detect *Movi* antibodies. We stored nasal swabs in TSB media and froze all serum upon collection and refrigerated remaining nasal and oropharyngeal swabs. We transported all samples on dry ice within 48 hours of collection to Washington Animal Disease Diagnostic Lab (WADDL) or to Dr. Thomas Besser's lab at Washington State University (Pullman, Washington, USA) for analyses. We used real-time polymerase chain reaction (PCR) to analyze nasal swabs to detect the presence and the abundance of *Movi* (McAuliffe et al. 2003, Besser et al. 2008). We evaluated oropharyngeal swabs via bacteria culture conducted by WADDL to detect additional respiratory pathogens (Besser et al. 2008). Serum was analyzed by WADDL for the presence of *Movi* antibodies using competitive enzyme-linked immunosorbent assays (ELISA) (Ziegler et al. 2014).

We collected whole blood for analyzing serum in order to detect antibodies to 5 viruses known to infect bighorn sheep populations and presumed to be found across the range of bighorn sheep (Miller et al. 2012). Specifically, we submitted sera for analysis of exposure to bluetongue (BT), epizootic hemorrhagic disease (EHD), parainfluenza 3 (PI-3), bovine viral diarrhea I/II (BVD I/II), and ovine progressive pneumonia (OPP) to the South Dakota State University Animal Disease Research and Diagnostic Laboratory (ADRDL) in Brookings, South Dakota. The ADRDL tested BT and EHD exposure via ELISA and agar gel immunodiffusion assay (AGID), PI-3 exposure via serum virus neutralization assay (SVN), and BVD I/II and OPP exposure via ELISA. Standard serum serological assay protocols were used by ADRDL for detecting presence of antibodies.

Lamb Capture and Data Collection

Beginning on 15 April 2017 and 2018, we conducted radio-telemetry ground-based monitoring of VIT implanted ewes twice daily (morning and evening) to detect VIT expulsion, indicative of parturition. Additionally, we opportunistically monitored breeding-aged ewes without VHF collars or VITs for signs of post-partum behavior (Lent 1974) or new-born lambs at heel, with the goal of capturing lambs from these ewes. In all lamb capture events, we attempted to minimize lamb abandonment by ensuring adequate post-parturition bonding period between ewe and lamb had taken place (>4 hours) (Livezey 1990). This period of adequate bonding was determined two ways: 1) parturition event calculated from time of VIT expulsion; and 2) observational and behavior signs of ewe/lamb pairs. We detected parturition event times via expelled VITs, which had built-in Precise Event Transmitters (PETTMP01/PETTMPF1; ATS) that emitted coded transmissions. We used these transmissions to calculate the amount of time (within 0.5 hours) the VIT had been expelled, which allowed us to focus our capture attempts when we knew the VIT had been expelled for >4 hours. Observational and behavioral signs also were used to help inform and estimate the age and bonding period between ewe and lamb. These signs included wet versus dry pelage, presence of afterbirth, nursing attempts, and degree of mobility (e.g., recumbent, stability while standing, coordination while moving). If observational and behavioral signs indicated sufficient age and bonding had occurred, we attempted to capture the lamb.

Once the VIT was expelled, we used radio telemetry to locate the ewe and check for presence of a lamb. Upon locating lamb and ewe pairs, we would wait for the lamb to bed down or for the lamb to be in suitable terrain for capture. Ground capture techniques

and methods were similar to those of Smith et al. (2014b). We captured lambs by hand wearing latex gloves, recorded weight, sex, and age, and minimized handling times to <5 minutes. Age was based on presence of afterbirth, wet pelage, umbilicus condition, and/or length in hours between VIT expulsion and lamb capture. We radio-collared lambs with expandable VHF collars (M4210; ATS) and released them in the direction of the ewe, if lambs were mobile, or placed lambs in vicinity of the ewe's last observation of the lamb. We recorded behavior of ewe and lamb pairs pre-, during, and post-capture. Terrain type, habitat features, and parturition and capture location were recorded when available. The South Dakota State University Institutional Animal Care and Use Committee approved all capture and handling procedures (Approval number 17-003A).

Adult and Lamb Monitoring

We collected information on sources of mortality as well as subherd interactions and dispersal events. We excluded all adult mortalities occurring ≤2 weeks of capture to avoid capture related mortalities in survival analyses. We monitored radio-collared adults ≥3 times per week. We began lamb survival monitoring at time of capture and ended in December or upon radio-collar failure. To detect cause and timing of mortality, we monitored radio-collared lambs daily for the first 3 months of life and ≥3 times per week from 3 to 6 months of age. We monitored all radio-collared individuals via handheld directional antennas with visual observations.

Trained personnel investigated all mortalities of radio-collared individuals as soon as a mortality was detected. We right-censored individuals that survived until the end of the study, experienced collar failure, or if their collar fell off due to a timed-release. We

assigned cause-of-death based on evidence observed in the field (Table 1). We then accounted for uncertainty in this assignment by incorporating a data augmentation approach outlined in Walsh et al. (2018) using prior predictive (PP) values for each cause-of-death. The use of PP values allows the incorporation of expert knowledge regarding each mortality case in the cause-of-death survival modeling framework. The specification of PP values for each mortality case involved recording the observer's belief, captured as a probability, that each cause of mortality was the true cause conditional on the observer's field-assignment of cause-of-death (Walsh et al. 2018). For each mortality, the PP's summed to 1 across mortality categories, creating a vector of probabilities or PP values. In cases where the cause-of-death was certain, a value of 1 was assigned to that mortality category while the remaining causes received values of 0. In the event that the observer could not assign a cause-of-death (i.e., observer believed all causes were equally likely), all causes were assigned the same probability (Table 2). Adult mortality cases were classified into 4 categories that represented the majority of mortality sources for adult: Predation, Accidental, Vehicle, and Other. Lamb mortality cases were designated into 3 categories that accurately represented the majority of mortality sources for lambs: Predation, Accidental, and Other.

We completed mortality investigations by locating the radio-collar of the individual and evaluating the mortality site for cause-of-death. Evidence used to evaluate adult and lamb mortality sites and inform PP values included but were not limited to the following: signs of predation (e.g., caching, bite marks, plucking, blood, predator scat), scavenging, disease (e.g., diarrhea, internal/external parasites), poor condition (e.g., bone marrow, body condition, fat reserves), accidental (e.g., broken bones consistent with

falling, entrapment), vehicle collision (e.g., abrasions, broken bones, blunt force trauma, distance to road), known life history of individual, and environment factors (e.g., precipitation and temperature). When a carcass was found and cause of death was unknown, the carcass was sent to the ADRDL at South Dakota State University for complete necropsy.

Survival Analysis

We analyzed adult and lamb survival in a Bayesian time-to-event framework (Cross et al. 2015, Walsh et al. 2018, Garwood et al. 2020) with Nimble in Program R (NIMBLE Development Team 2018). This survival analysis framework uses a two-component model. The first component estimated the overall hazard of dying irrespective of cause-of-death. The second component estimated the cause-specific probabilities of each mortality event leveraging the observer's expert knowledge via PP values (Table 2). We followed the methods of Cross et al. (2015) to calculate cause-specific mortality while simultaneously accounting for observer uncertainty in the cause-of-death assignments (Walsh et al. 2018). We treated true cause of death as a latent unknown variable that utilized PP values determined by mortality investigators, as described previously.

We used Markov Chain Monte Carlo (MCMC) numerical techniques to estimate the posterior distributions of the parameters. Specifically, for each analysis, we ran 3 MCMC chains with diffuse starting values for 100,000 iterations and removed the first 10,000 repetitions for burn-in prior to making inference. We evaluated evidence of

nonconvergence among chains through graphical checks and determined no evidence of nonconvergence for the adult and lamb analyses.

We analyzed adult survival by modeling the baseline, log unit cumulative hazard through time, and cause-specific mortality probabilities. Our baseline model calculated log unit cumulative hazard as $ln(\Lambda_{i,j}) = \gamma + \rho_j$, where γ was the baseline, log unit cumulative hazard rate and the week effect from the start to end of survival monitoring was represented by ρ_j (Table 3).

Recent investigations into bighorn sheep in western South Dakota, on the eastern fringe of their distribution, evaluated lamb survival in declining, increasing, or stable populations (Smith et al. 2014a, Parr et al. 2018, Garwood et al. 2020). We assessed covariates utilized in their models and explored covariates specific to the badlands ecosystem that we deemed biologically meaningful for survival of lambs to 6 months of age. The intrinsic covariates we investigated were subherd (i.e., 3 groups: Pinnacles, Homestead, and Hay Butte), capture weight, sex, and year. Birth weight was a continuous variable and was measured with a scale to the nearest 0.1 kg.

We incorporated these covariates into models built *a priori* to test hypotheses we deemed meaningful to bighorn sheep lamb survival in the badlands ecosystem (Table 3). Our global model calculated log unit cumulative hazard as $ln(\Lambda_{i,j}) = \gamma + \beta_{herd}[herd_i] + \beta_{capture\ weight} \times capture\ weight_i + \beta_{sex} \times sex_i + \beta_{year} \times year_i + \rho_j$, where γ was the baseline, log unit cumulative hazard rate. The effect of the herd where a lamb was born was indicated as β_{herd}, where β_{herd} [1] specified the effect of the i^{th} individual born in the Homestead herd and β_{herd} [2] specified the effect of the i^{th}

individual born in the Hay Butte herd. We indicated the effect of capture weight as $\beta_{capture\ weight}$, with $capture\ weight_i$ being the specific capture weight of the i^{th} individual. We indicated the effect of lamb sex as β_{sex}, with sex_i being the indicator for males. The effect of year was represented as β_{year}, with $year_i$ being the effect of 2018 on the i^{th} individual. The age effect was represented by ρ_j.

We used diffuse priors on the baseline log cumulative hazards for adult and lamb survival. For the adult survival analysis, we used a beta prior on the baseline log cumulative hazard (specifically, $\gamma \sim \log\left[-\log\left[1-\text{dbeta}[1,1]\right]\right]$). For the lamb survival analysis, we assumed a mean annual lamb survival of 50% with a 95% credible interval of ~10% to ~ 100% (specifically, $\gamma \sim \text{dnorm}[-6.26, \text{precision} = 3]\text{T}[-10,-1]$); all priors were specified in BUGS language and were similar to those used by Parr et al. (2018) and (Garwood et al. 2020) .

To account for variability and temporal correlation in weekly (adult) or age (lamb) hazard rates, we specified an intrinsically conditional autoregressive prior (ICAR) (Heisey et al. 2010, Cressie and Wikle 2015) for the effect of each week or day on the overall hazard (ρ_j). Thus, we specified a prior with a uniform distribution ($\rho_1 \sim dunif(-0.5, 0.5)$ for the first week or day effect, and we specified the effect for the j^{th} week and j^{th} day as ($\rho_j \sim dnorm(\text{mean} = \rho_{j-1}, \text{precision} = \tau)$. Finally, we specified the prior for the precision parameter as: $\tau \sim dgamma(1,1)$ (Heisey et al. 2010). The ICAR prior provided temporal smoothing across weekly and daily hazard estimates. All the priors on covariates were diffuse ($\beta_x \sim \text{dnorm}(0, 0.01)$).

We calculated Watanabe-Akaike Information Criteria (WAIC) from each lamb model for identifying the top supported model. The top supported model that best reflected the data was used to provide parameter estimates. In addition, we considered models that were ≤2 WAIC as alternatives to the top ranked model and evaluated competing model parameter estimates (Burnham and Anderson 2002).

Population Size and Growth Estimates

BNP conducts annual bighorn sheep ground surveys during the rut to estimate population size throughout the North and South Units of the park. Surveys are completed in 1-2 days during peak rut, while bighorn sheep are congregated on wintering areas. Known occupied ranges of bighorn sheep were divided into 6 units and surveyed by 1 or 2 observers within 12 hours in 2016, 2017, and 2018. To increase detection probability of individuals for surveys, we included all marked individuals (i.e., located via telemetry) in the population to provide a more rigorous population count. Counts from each survey unit were tabulated and used as a minimum population count. We used survey counts to estimate growth rates (r) of the population assuming geometric growth (λ); $\lambda = N_{t+1}/N_t$ and $r = \ln(\lambda)$. In addition to calculating geometric growth estimates from survey counts, and to make a comparison estimate, we conducted a population viability analysis (PVA) in Vortex PVA Software (Lacy and Pollak 2020). We informed parameters in the PVA with the demographic rates from the survival analysis of radio-collared individuals in the North unit of BNP.

Domestic Sheep and Goat Respiratory Pathogen Surveillance

We documented the presence and location of domestic small ruminant operations (goat [*Capra aegagrus hircus*] and sheep [*Ovis aries*]) within an 8 km buffer around the North Unit of BNP to assess the risk of respiratory disease due to potential contact with livestock. Domestic operations were located via personal communication with area residents, visible identification on the landscape, and/or consultations with local veterinarians. We contacted owners of domestic sheep and goat operations within the 8 km buffer and discussed the goals of the risk assessment. If we received permission to include an operation in our risk assessment, we collected specific information on size, species (i.e., sheep or goat), confinement type (i.e., lot, pasture, feed regimen), biosecurity (i.e., open versus closed operation, rent or borrowed, quarantine precautions implemented), herd health (i.e., disease history, clinical symptoms, coughing/nasal discharge, parasites), and distance to known occupied bighorn sheep range. Additionally, and if permitted, we sampled animals for respiratory pathogens associated with polymicrobial pneumonia in bighorn sheep by collecting nasal swabs from a subset of the total herd or flock to test for the presence of *Movi*. We followed the same sampling and testing protocol for the domestic animals used for *Movi*-testing of bighorn sheep.

RESULTS

Respiratory Bacteria and Viral Testing

We tested 83 bighorn sheep from 5 subherds and 2 management units for *Movi* from March 2017 to February 2019 (Table 4). We did not detect *Movi* shedding from any individuals via PCR; however, 15 individuals (prevalence = 18%, mean % inhibition value = 74.01 [SE = 2.83]) had evidence of past exposure to *Movi* based on serological

testing via ELISA. We also tested 61 bighorn sheep from 4 subherds in the North Unit of BNP for other respiratory pathogens (Table 4). Via bacteria culture, we detected *Bibersteinia trehalosi* (*n* = 25; 41%), *Pasteurella* sp. (*n* = 1; 4%), *Mannheimia* sp. (*n* = 37; 61%), and *Trueperella pyogenes* (*n* = 6; 10%). A subsample of bighorn sheep (*n* = 35) tested positive via PCR for leukotoxigenic *Pasteurella* species (*n* = 23; 66%) in 2018. No samples were collected for bacterial culture from the South Unit.

We tested 54 bighorn sheep from the North Unit (*n* = 40) and South Unit (*n* = 14) of BNP for 5 viral diseases known to affect bighorn sheep populations. We detected a high prevalence of antibodies to PI-3 (93%) in both units and variable prevalence of titers to Bluetongue (North Unit 60%; South Unit 7%) (Table 5).

Capture and Data Collection Results

From March 2017 to February 2018, we captured and radio-collared 49 ewes: 5 yearlings, 10 at 2 years of age, 6 at 3 years of age, and 23 at ≥4 years of age, along with 5 mature rams (all ≥4 years of age). An additional 11 ewes were captured from BNP in 2018 for translocation to supplement the bighorn sheep population in Custer State Park, South Dakota. We recorded weights from 52 ewes and documented the overall average ewe weight=77.5 kg (SE = 1.2). We further evaluated ewe weight from each age class with average weight of yearling = 69.9 kg (SE = 3.9; *n* = 6), 2 year old=75.8 kg (SE = 2.4; *n* = 10), 3 year old=77.9 kg (SE = 2.1; *n* = 7), and ≥4 year old=77.5 kg (SE = 1.5; *n* = 29) bighorn sheep. Ewe pregnancy rates were 92% overall (2017 = 90%; 2018 = 94%). We deployed 40 VITs with a retention rate to parturition of 93% (17 retained/18 deployed in 2017; 20 retained/22 deployed in 2018). Peak parturition date was 14 May

2017 and 10 May 2018 (2017 parturition ranged 23 April–26 May; 2018 parturition ranged 19 April–31 May). We captured a total of 53 lambs (28 males; 25 females) in 2017 (*n* = 23) and 2018 (*n* = 30). Average capture weight was 4.7 kg (SE = 0.1; 2017 = 4.4 kg, SE = 0.2; 2018 = 4.9 kg, SE = 0.1) and there was no significant difference between capture weight across years (t = -1.90; p = 0.06). Males were not significantly larger than females (males = 4.7 kg, SE = 0.1; females = 4.7 kg, SE = 0.2; t = -0.21; p = 0.83). Estimated age at capture ranged from 4 hours to 3 days.

Survival Analysis

We documented 5 adult mortalities during 2017-2018 (Table 1). Based on field assigned sources, Predation accounted for 40%, Accidental (i.e., falling) for 20%, and Other for 40% of adult mortality, the latter of which included a combination of suspected mortality sources (e.g., vehicle, malnourished, unknown). We documented 18 lamb mortalities during 2017-2018 (Table 1). Using the most likely cause of death based on field and necropsy evidence for assigned sources, Predation accounted for 56% of lamb mortality with coyote (28%), bobcat (6%), and unknown predator (22%) making up the predator suite. Accidental (falling from cliff or into crevasse) accounted for 28% and Other accounted for 16% of lamb mortality due to a combination of suspected sources of mortality (i.e., environmental, non-respiratory related disease, unknown). One lamb carcass was examined at ADRDL, which determined internal bleeding/trauma, attributed to falling, as the cause of death.

Adult survival monitoring began March 2017 and ended December 2018. Adult survival analysis included 47 adults for 94 weeks of survival monitoring. Two adult ewes

were censored from analysis due to capture related deaths. We refrained from investigating covariates for our adult survival modeling due to the limited mortality cases (n = 5) and overall high survival throughout the study. We determined that covariates would have little informative power on the survival analysis and modeled baseline, log unit cumulative hazard through time, and cause-specific mortality probabilities. We evaluated our data and reported the results from the following model: $ln(\Lambda_{i,j}) = \gamma + \rho_j$. With this model, we calculated the weekly log unit cumulative hazard measurements (Figure 2) and an annual adult survival rate of 96% (95% credible interval [CI] = 89%, 99%). Given that an adult died, the probability of dying from the 4 mortality sources were as follows: Accidental = 32% (95% credible interval [CI] = 2.0%, 80%), Other = 20% (95% credible interval [CI] = 0.0%, 84%), Predation = 37% (95% credible interval [CI] = 1.0%, 85%), and Vehicle = 11% (95% credible interval [CI] = 0.0%, 55%) (Figure 3[i]).

Our lamb survival analysis included 53 lambs for ≤245 days of survival monitoring. Lamb survival analysis commenced at the estimated age upon capture and concluded on 31 December 2017 and 15 December 2018. The data best supported (w_i = 0.34) the following model: $ln(\Lambda_{i,j}) = \gamma + \beta_{sex} \times sex_i + \beta_{capture\ weight} \times capture\ weight_i + \beta_{year} \times year_i + \rho_j$ (Table 6). With this model, we calculated the daily log unit cumulative hazard measurements (Figure 4) and an annual lamb survival rate of 82% (95% credible interval [CI] = 65%, 92%). The best approximating model indicated lambs born female had a reduced daily hazard (β_{sex}= -0.01), the estimate was highly variable (95% credible interval [CI] = -0.88, 0.85). Capture weight of the lamb indicated a negative effect on daily hazard ($\beta_{capture\ weight}$= -0.04), but the credibility

interval overlapped zero (95% credible interval [CI] = -0.48, 0.41). Higher lamb daily hazard was associated with 2018 than in 2017 (β_{year}= -0.13), but the estimates was also equivocal (95% credible interval [CI] = -1.02, 0.75) (Figure 5[i]). The probability of dying from the 3 mortality sources were as follows: Accidental = 36% (95% credible interval [CI] = 14%, 61%), Other = 3.0% (95% credible interval [CI] = 0.0%, 13%), and Predation = 62% (95% credible interval [CI] = 37%, 84%) (Figure 3[ii]).

Two other models were within 2 WAIC units of the top model and were considered competitive (Table 6). In comparing the effects of these models on lamb survival, the 2nd ranked model revealed no meaningful effect of herd on lamb hazard (β_{herd}[1] = 0.98, 95% credible interval [CI] = 0.00, 1.88; β_{herd}[2] = -1.04, 95% credible interval [CI] = -2.93, 0.36), and the 3rd ranked model evaluated hazard as constant and did not include covariates (Figure 5[ii]). In summary, the 95% credibility intervals of all the parameter estimates in competing models overlapped 0 and thus, the estimated effects were too variable to conclude they strongly affected lamb survival (birth to <8 months) in BNP.

Population Size and Growth Estimates

Surveys conducted in the fall of 2016, 2017, and 2018 reported minimum population counts of 163, 191, and 233, respectively, for the combined North and South Units of BNP. Using the geometric growth rate (λ) and instantaneous growth rate (r) models; $\lambda = Nt+1/Nt$ and r=ln(λ), we calculated an estimated λ = 1.17 and r = 0.15 (December 2016–November 2017) and an estimated λ = 1.22 and r = 0.20 (December 2017–November 2018). Although these growth rates were calculated using variable

minimum count population estimates from fall surveys, these growth rates were similar to growth rates and population estimates obtained in the PVA (λ = 1.21 and r = 0.19; population estimate in 2016 = 165, 2017 = 197, and 2018 = 238). The growth rate and population estimate results from both the PVA and the fall surveys from 2016-2018 were consistent with each other (A. J. Wieseler, South Dakota State University, Brookings, SD, unpublished data).

Domestic Sheep and Goat Respiratory Pathogen Surveillance

Between June 2017 and August 2018, 5 domestic sheep and goat operations were documented within 8 km of the North Unit of BNP (Figure 6). Domestic operations within the 8-km buffer ranged in size of 3-200 individuals, consisted of operation types of goats only and both sheep and goats, and were 2 km to 7 km from known bighorn sheep ranges in BNP. Two operations allowed testing for *Movi*. Operation 1 tested positive (*n*=23; 77%) and Operation 2 tested negative. Operations 3, 4, and 5 were not sampled for respiratory pathogens during this study due to lack of sampling permission from owners.

DISCUSSION

Our investigation provides a descriptive evaluation of the bighorn sheep population in BNP, given the complex history of presumed disease-induced die-offs, genetic augmentation, and variable population growth. To understand how these events may influence the current population, we assessed disease presence and prevalence, adult and lamb survival, presence of domestic operations and the disease risk they pose, and size and trajectory of the bighorn sheep population in BNP.

Infectious diseases, primarily polymicrobial pneumonia, are considered the principal cause of large-scale declines in bighorn sheep populations and continually impact recovery efforts and population growth negatively (Cassirer and Sinclair 2007, Cassirer et al. 2018). The etiology of pneumonia epizootics has been highly debated and much previous research focused on *Pasteurellacae* (*Bibersteinia trehalosi, Mannheimia haemolytica*, leukotoxigenic *Pasteurella*) as the primary causative pathogen. This paradigm has since shifted to a polymicrobial complex that is initiated by *Movi*, which predisposes affected individuals to polymicrobial pneumonia (Besser et al. 2008). Thus, we sampled for a variety of respiratory pathogens in the BNP population with particular focus on *Movi*. We did not detect *Movi* within the BNP population but found presence of or exposure to other respiratory pathogens (Table 4). However, we detected antibodies to *Movi* indicating previous exposure, although the low and declining seroprevalence along with a failure to detect *Movi* in adults or in lamb mortalities, suggests there was no current infection. We also detected antibodies indicating ongoing or previous exposure to BT and PI-3. Ramey et al. (2000) reported a disease epizootic die-off in 1967 due to a *Pasteurella* infection, a suspected second disease epizootic die-off in 1982 attributed to bluetongue and/or pneumonia, and a suspected third disease epizootic in the early 1990's; however, we can only speculate as to the cause and the roles of exposure to *Movi*, BT, and PI-3 in these epizootics. The suspected epizootics and documented die-offs in BNP were each followed by a period of growth indicating if a disease epizootic was the cause of decline, it did not have a lasting effect. This rapid recovery post die-off response is not typical of polymicrobial pneumonia epizootics. Although adult survival generally rebounds to or above previous levels in the years following all-age die-offs (Plowright et

al. 2013, Manlove et al. 2016), subsequent low recruitment due to continued high mortality outbreaks in lambs usually continues to impede population recovery (Cassirer et al. 2013). We suspect die-offs in BNP were potentially the result of some other agent or the possible result of an acute pneumonia epizootic, allowing quick recovery within the population (Coggins and Matthews 1992, Jorgenson et al. 1997).

The role of large scale die-offs associated from viral pathogens is often limited, but high seroprevalence can suggest frequent infections potentially predisposing populations to other infectious agents (Miller et al. 2012). We documented relatively high seroprevalence for PI-3 (93% prevalence) and BT (46% prevalence) overall, but higher prevalence in the North Unit than the South Unit for both PI-3 and BT (Table 5). Serologic evidence of BT exposure is common in populations of bighorn sheep and presumed to be range-wide (Miller et al. 2012). Parr et al. (2018), Noon et al. (2002), and Clark et al. (1985) investigated BT in populations of bighorn sheep and found positive seroprevalence, but limited mortality attributed to the disease. Aune et al. (1998) found high seroprevalence of PI-3 in a population of bighorn sheep in Montana prior to an epizootic occurring, but little is known of the role PI-3 played in the later epizootic. High seroprevalence to viral respiratory pathogens may suggest that infections are common and clinically mild or minor allowing population recovery (Parks and England 1974, Miller et al. 2011). The role of viral pathogens, especially respiratory viruses, in predisposing or functioning in a coinfection dynamic with other infectious pathogens is not well understood. Therefore, future disease monitoring in BNP should evaluate all pathogens and the potential roles each play in epizootics. This is especially true given the unidentified pathogen(s) that resulted in the suspected die-offs within BNP in the past.

The risk of pathogen transmission at the domestic livestock (i.e., goats, sheep) and bighorn sheep interface for the BNP population appears significant. Bighorn sheep are closely related to domestic sheep and goats, but did not evolve with them or their pathogens, making bighorn sheep highly susceptible and vulnerable to pathogens carried by domestic sheep and goats (Jessup and Boyce 1993). Contact with domestic small ruminants and bighorn sheep can be associated with respiratory disease outbreaks with high morbidity and mortality in bighorn sheep (Martin et al. 1996, Besser et al. 2008, Besser et al. 2012). Sampling efforts for this study were focused on detecting presence and prevalence of *Movi,* within the domestic operations surrounding BNP. Sampling in 2 of 5 domestic goat or mixed domestic goat and sheep operations located within 8 km of the North Unit of BNP (Figure 6) found a high prevalence of *Movi* (77%) in one domestic goat operation, the other was negative. Currently, domestic operations that are carriers of *Movi* pose a high risk to the BNP bighorn sheep population. Within the identified domestic livestock operations around BNP, we did not evaluate the presence or prevalence of bacterial (i.e., *Bibersteinia trehalosi, Mannheimia haemolytica,* leukotoxigenic *Pasteurella*) or viral (i.e., PI3, BT, EHD, OPP, and BVD I/II) pathogens that were examined in the bighorn sheep population. Future domestic livestock monitoring should explore these pathogens along with other infectious agents, assessing the risk and role in a potential pathogen spillover into the BNP population.

Annual adult and lamb survival estimates for the BNP bighorn sheep population were 96% and 82%, respectively. Survival estimates for bighorn sheep in diseased and healthy populations are well documented throughout their range (Jorgenson et al. 1997, Portier et al. 1998, Cassirer and Sinclair 2007, Smith et al. 2014a, Smith et al. 2015, Parr

et al. 2018, Garwood et al. 2020, Spaan et al. 2021). Lamb survival estimates through weaning and annual adult survival estimates from multiple populations ranged 40-90% in lambs and 88-93% in adults when healthy, whereas diseased populations ranged from 0-50% in lambs and 50-93% in adults (Cassirer and Sinclair 2007, Cassirer et al. 2013, Smith et al. 2014a, Parr et al. 2018, Garwood et al. 2020, Spaan et al. 2021).

Few studies have captured true neonate survival estimates in bighorn sheep from birth through weaning due to their inaccessibility in steep and rugged terrain (Gaillard et al. 2000, Smith et al. 2014b); therefore, documentation of factors influencing survival have been limited. Garwood et al. (2020), Smith et al. (2014a), and Parr et al. (2018) evaluated capture weight, sex, and/or year from birth through weaning in their studies and found no significant relationship between these factors and survival; although by ≥ 3 months age, Festa-Bianchet et al. (1997) found a significant positive relationship between body mass and survival and no differences between sexes on size and survival of lambs. None of the factors (i.e., sex, capture weight, year, herd) included in our best approximating models were meaningful predictors of lamb survival. Our results were similar to other studies that evaluated factors influencing survival from birth through weaning of bighorn sheep.

Parr et al. (2018) and Garwood et al. (2020) reported predation as the primary source of mortality in two disease-free populations in western South Dakota. We found similar results in BNP with predation being the primary source of mortality in lambs, and the probability of mortality from predation being 62% ([95% credible interval [CI] = 37%, 84%]) (Figure 3[ii]). We found Accidental as an unexpected, but important second source of mortality for lambs. Mortality by accident was primarily attributed to lambs <4

days of age falling to their death or into inescapable crevasses resulting in starvation/abandonment (5 documented cases). All documented lamb mortalities (n = 18) occurred at <3 months of age, therefore, represent the highest hazard period to lamb survival in BNP (Figure 4).

Our annual adult survival estimates (overall = 96% [95% credible interval [CI] = 89%, 99%]) are comparable to healthy, growing populations of bighorn sheep in South Dakota and Hells Canyon, although those studies estimated survival of males and females separately (Cassirer and Sinclair 2007, Parr et al. 2018). Parr et al. (2018) evaluated survival separately between sexes but was unable to identify variables influencing male survival due to a small sample size. We speculate our survival estimation of males and females together had limited effects due the small sample size of males (n = 5) and the limited mortalities (n = 5) in adults. Despite overall cumulative hazard being low due to limited adult mortality throughout the study, adults were more likely to die during the winter months (Figure 2).

The high adult and lamb survival, in the absence of *Movi* despite the presence of other bacterial pathogens associated with pneumonia, were similar to other studies in western South Dakota. Parr et al. (2018), Werdel et al. (2019), and Garwood et al. (2020) found the absence of *Movi* resulted in healthy, disease-free populations of bighorn sheep. Garwood et al. (2020), Werdel et al. (2019), and Smith et al. (2014a) found the presence of *Movi* within populations of bighorn sheep resulted in epizootic die-offs followed by enzootic pneumonia, significantly impacting yearly recruitment. Our results further support the role of *Movi* in the polymicrobial complex of pneumonia in bighorn sheep populations.

Our results provide a baseline evaluation of the demographic and growth rates, presence and prevalence of infectious agents, and the risk of pathogen spillover from domestic operations to a metapopulation of bighorn sheep on the eastern fringe of their range. We documented high adult and lamb survival rates along with significant population growth over the course of this study. Adult mortality cases were minimal, and lamb predation was the leading source of mortality, but did not have population level effects. Disease is not currently limiting the growth and survival of the BNP population, but as the population continues to experience high survival across all age classes and expand within the greater badlands ecosystem, the risk of contracting a pathogen from the neighboring domestic operations is highly probable. Given the pathogen spillover risk at the wildlife/livestock interface along with the lack of detecting sources of previous die-offs, we recommend periodic disease monitoring within the bighorn sheep population. Additional disease surveillance should be conducted within domestic operations (i.e., sheep, goat, cattle) near BNP that include identifying both bacterial and viral pathogens detrimental to bighorn sheep. We located domestic operations within an 8 km buffer of BNP, but further research should evaluate the size of the buffer to fully determine risk of contact for the BNP bighorn sheep population. Ensuring the separation of bighorn sheep and domestic operations while educating and developing working relationships with their owners will be crucial to the future of the BNP population. Identifying additional unoccupied suitable bighorn sheep habitat within the greater badlands ecosystem and exploring the use of the BNP population as a source herd for translocations is recommended. Finally, we recommend determining a disease risk carrying capacity of

subherds in close proximity to domestic operations and the efficacy of translocations between high risk and low risk subherds within BNP.

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Table 1. Field assigned cause-specific mortality sources for adult (5/47) and lamb (18/53) bighorn sheep monitored in Badlands National Park, South Dakota, 2017-2018.

Adult		
Cause-specific mortality	*n*	%
Predation		
Coyote	2	40%
Bobcat		
Unknown Predator		
Accidental (Fall)	1	20%
Other	2	40%
Total	**5**	**100%**

Lamb		
Cause-specific mortality	*n*	%
Predation		
Coyote	5	28%
Bobcat	1	6%
Unknown Predator	4	22%
Accidental (Fall)	5	28%
Other	3	16%
Total	**18**	**100%**

Table 2. Prior predictive (PP) values assigned to all individuals included in the survival analyses. Vectors summed to one across cause-specific mortality categories for each individual that died.

Adult

Individual Identifier	Other	Vehicle	Accidental (Fall)	Predation
1	0.3			0.7
2	0.3			0.7
3	0.2	0.4	0.2	0.2
4	0.2	0.4	0.2	0.2
5			1.0	

Lamb

Individual Identifier	Other	Accidental (Fall)	Predation
8		1.0	
9		0.4	0.6
11			1.0
13			1.0
16	0.3	0.3	0.3
21		1.0	
22			1.0
24		1.0	
28	0.3	0.3	0.3
29			1.0
32			1.0
36	0.3	0.3	0.3
37			1.0
42			1.0
44			1.0
47		1.0	
48		1.0	
53			1.0

Table 3. *A priori* models constructed with intrinsic variables deemed informative to bighorn sheep adult and lamb survival in Badlands National Park, South Dakota, USA, 2017-2018. γ is baseline log unit cumulative hazard rate, β_{sex} is the effect of sex, $\beta_{capture\ weight}$ is the effect of capture weight, β_{year} is the effect of year, β_{herd} is the effect of herd, and ρ_j is the effect of a given week or age (j) with a random walk prior for temporal smoothing across estimates.

Adult

Model	Description
$\gamma + \rho_j$	Hazard

Lamb

Model	Description
$\gamma + \rho_j$	Hazard
$\gamma + \beta sex + \rho_j$	Hazard varied by sex of lamb
$\gamma + \beta$capture weight$+ \rho_j$	Hazard varied by the capture weight of lamb
$\gamma + \beta year + \rho_j$	Hazard varied by year
$\gamma + \beta herd + \rho_j$	Hazard varied by subherd
$\gamma + \beta sex + \beta$capture weight$+ \rho_j$	Hazard varied by sex and weight of lamb
$\gamma + \beta$capture weight$+ \beta year + \rho_j$	Hazard varied by capture weight and year
$\gamma + \beta herd + \beta year + \rho_j$	Hazard varied by herd and year
$\gamma + \beta sex + \beta$capture weight$+ \beta year + \rho_j$	Hazard varied by sex, capture weight, and year
$\gamma + \beta sex + \beta$capture weight$+ \beta year + \beta herd + \rho_j$	Hazard varied by sex, capture weight, year, and herd (Global Model)

Table 4. Respiratory pathogen frequency and prevalence (%) in bighorn sheep in the North and South Units of Badlands National Park, South Dakota, 2017-2019.

		PCR	**ELISA**	**PCR**	**Bacteria Culture**			
	Sample Size	*Mycoplasma ovipneumoniae*	*Mycoplasma ovipneumoniae*	Leukotoxigenic *Pasteurella* (LktA)	*Bibersteinia trehalosi*	*Pasteurella* sp.	*Mannheimia* sp.	*Trueperella pyogenes*
North Unit								
2017[a]	26	0	13 (50%)	N/A	3 (12%)	1 (4%)	19 (73%)	2 (8%)
2018	35	0	2 (6%)	23 (66%)	22 (63%)	0	18 (51%)	4 (11%)
South Unit								
2019[ab]	22	0	0	N/A	N/A	N/A	N/A	N/A

[a]No tests for LktA performed.

[b]No bacteria cultures performed.

Table 5. Frequency and prevalence (%) of exposure to viruses detected in bighorn sheep in the North and South Units of Badlands National Park, South Dakota, 2017-2019.

	Sample Size	Parainfluenza 3	Epizootic Hemorrhagic Disease	Bluetongue	Ovine Progressive Pneumonia	Bovine Viral Diarrhea I	Bovine Viral Diarrhea II
North Unit	40	37 (93%)	0 (0%)	24 (60%)	0 (0%)	0 (0%)	0 (0%)
South Unit	14	13 (93%)	0 (0%)	1 (7%)	0 (0%)	0 (0%)	0 (0%)

Table 6. Top-ranked lamb models for log unit cumulative hazard $\ln(\Lambda_{i,j})$ for each individual, 15 April 2017 – 31 December 2017 and 15 April 2018 – 15 December 2018. Ranking is based upon Watanabe-Akaike Information Criteria (WAIC) and is reported with ΔWAIC (difference in WAIC between top model and model being compared) and w_i (WAIC weight).

Top Ranked Models	WAIC	ΔWAIC	w_i
$\gamma+\beta$sex$+\beta$capture weight$+\beta$year$+\rho_j$	206.0659	0.0000	0.3360
$\gamma+\beta$herd$+\rho_j$	206.7188	0.6529	0.2424
$\gamma+\rho_j$	206.9341	0.8682	0.2177
$\gamma+\beta$capture weight$+\rho_j$	208.1219	2.0560	0.1202
$\gamma+\beta$herd$+\beta$year$+\rho_j$	208.8441	2.7782	0.0838

Figure 1. Badlands National Park bighorn sheep study area with delineated North and South Units and subherd ranges in western South Dakota, USA, 2017-2019.

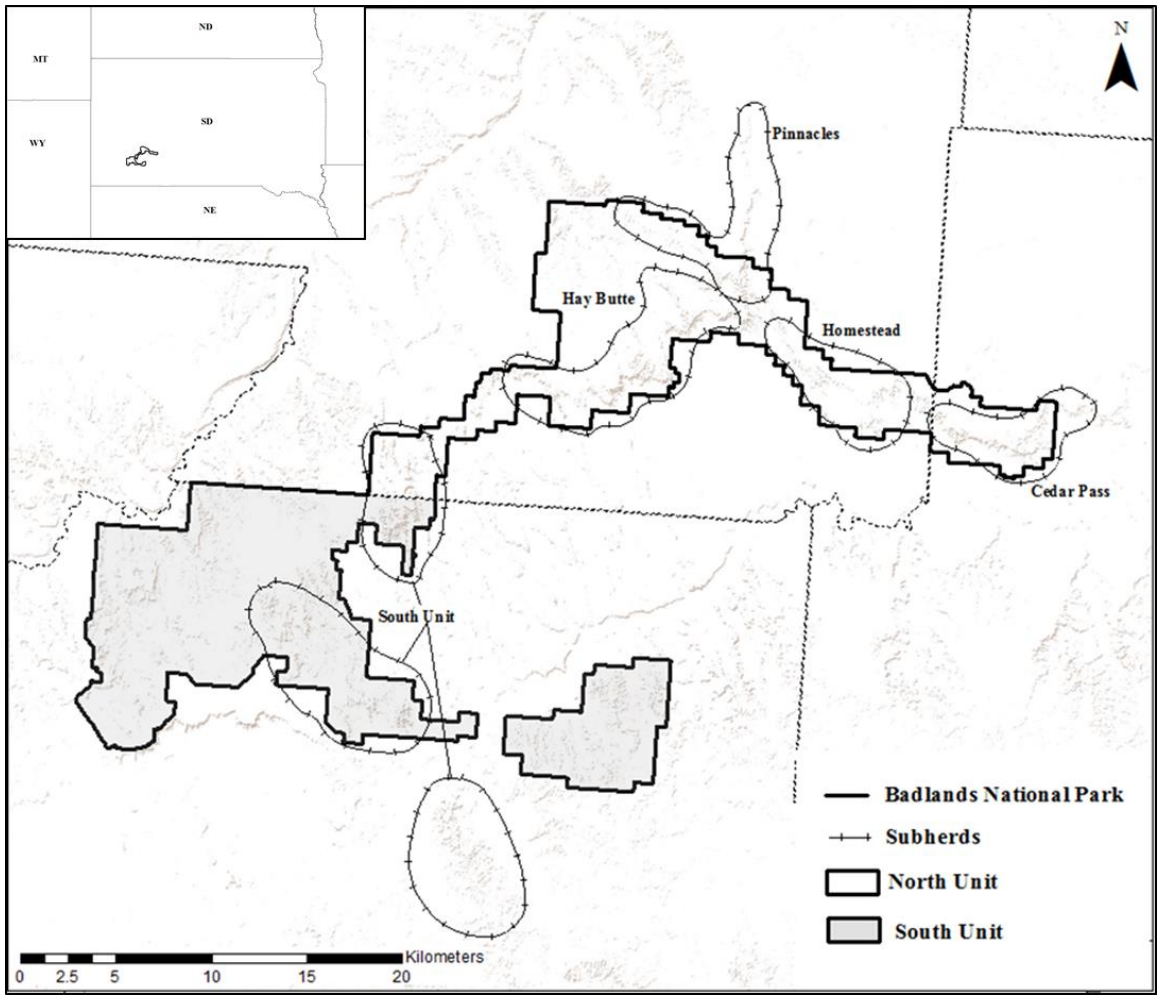


Figure 2. Overall log hazard for adult bighorn sheep in Badlands National Park (γ). Plot is based on the model, $\ln(\Lambda_{i,j}) = \gamma + \rho_j$, where $\ln(\Lambda_{i,j})$ is the unit log cumulative hazard for the i^{th} individual in the j^{th} week and ρ_j is the effect of a given week ($_j$) which is temporally smoothed via intrinsic conditional autoregressive (ICAR) random walk prior. Ninety-five percent credible intervals are shown in gray.

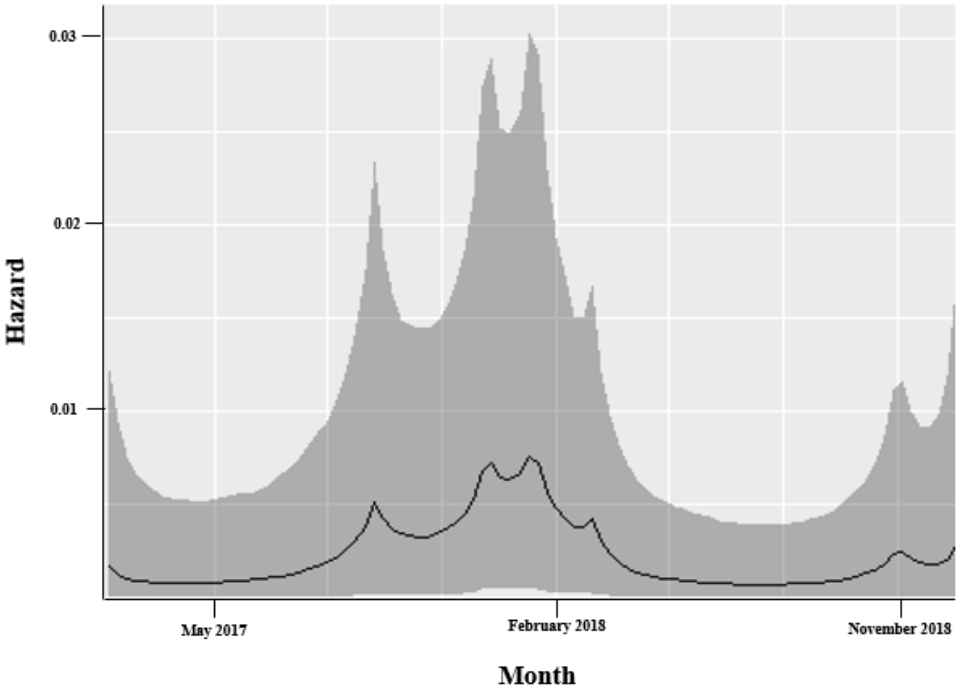
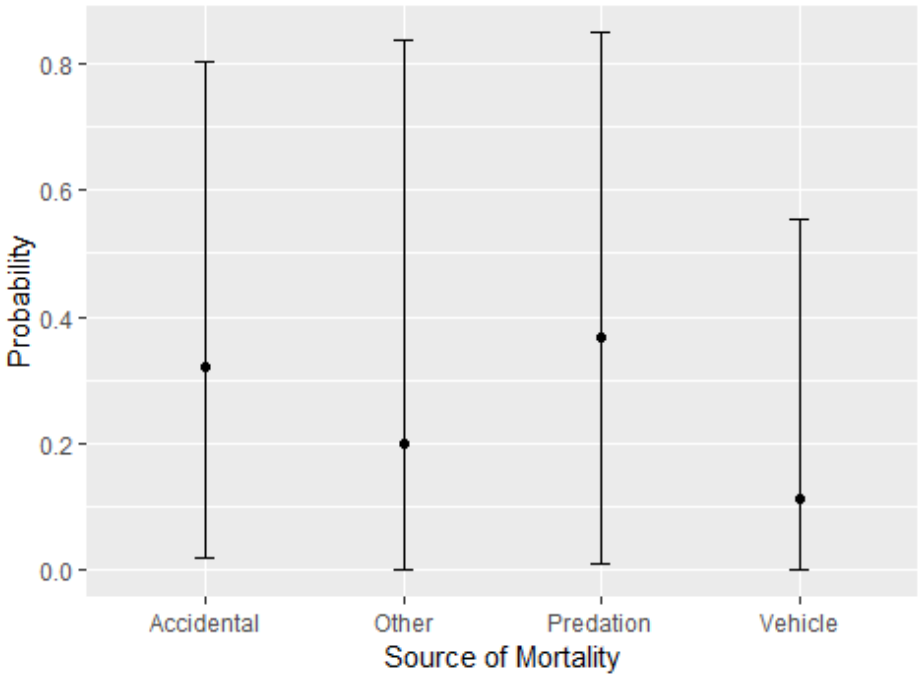


Figure 3. Estimated cause-specific mortality probabilities based on survival analysis. Panel [i] shows adult mortality probabilities and Panel [ii] shows lamb mortality probabilities with 95% credible intervals.

[i]



[ii]

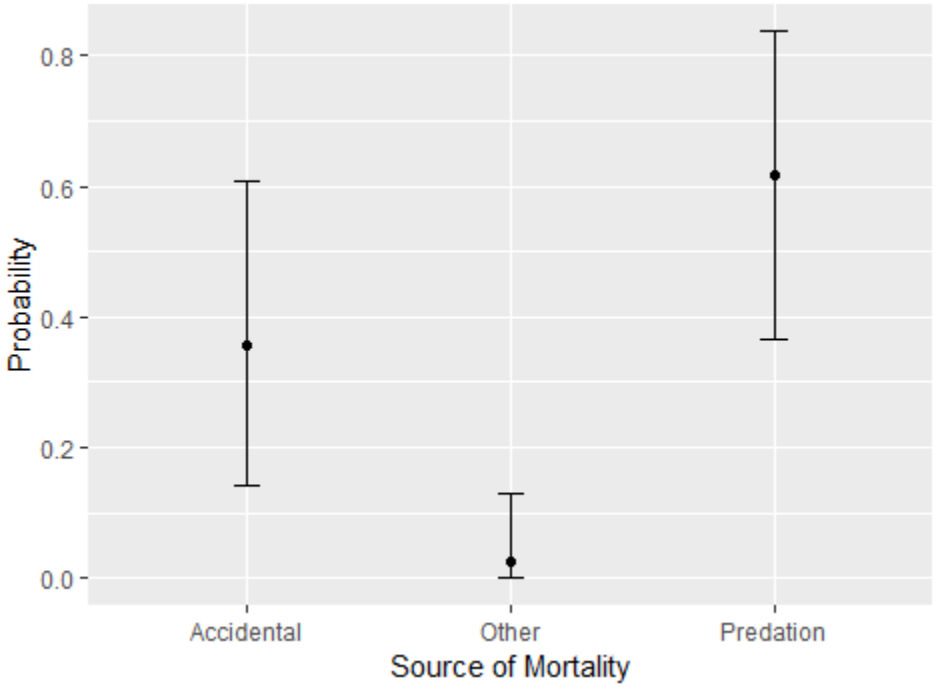


Figure 4. Overall log hazard for lamb bighorn sheep in Badlands National Park (γ). Plot is based on the model, $\ln(\Lambda_{i,j}) = \gamma + \beta_{capture\ weight} \times capture\ weight_i + \beta_{sex} \times sex_i + \beta_{year} \times year_i + \rho_j$, where $\ln(\Lambda_{i,j})$ is the unit log cumulative hazard for the i^{th} individual in the j^{th} day, $\beta_{capture\ weight}$ is the effect of capture weight with $capture\ weight_i$ representing weight of the i^{th} individual, β_{sex} is the effect of being male with sex_i representing whether the i^{th} individual was born male, β_{year} is the effect of year with $year_i$ being the effect of 2017, and ρ_j is the effect of a given age ($_j$) which is temporally smoothed via intrinsic conditional autoregressive (ICAR) random walk prior. Ninety-five percent credible intervals are shown in gray.

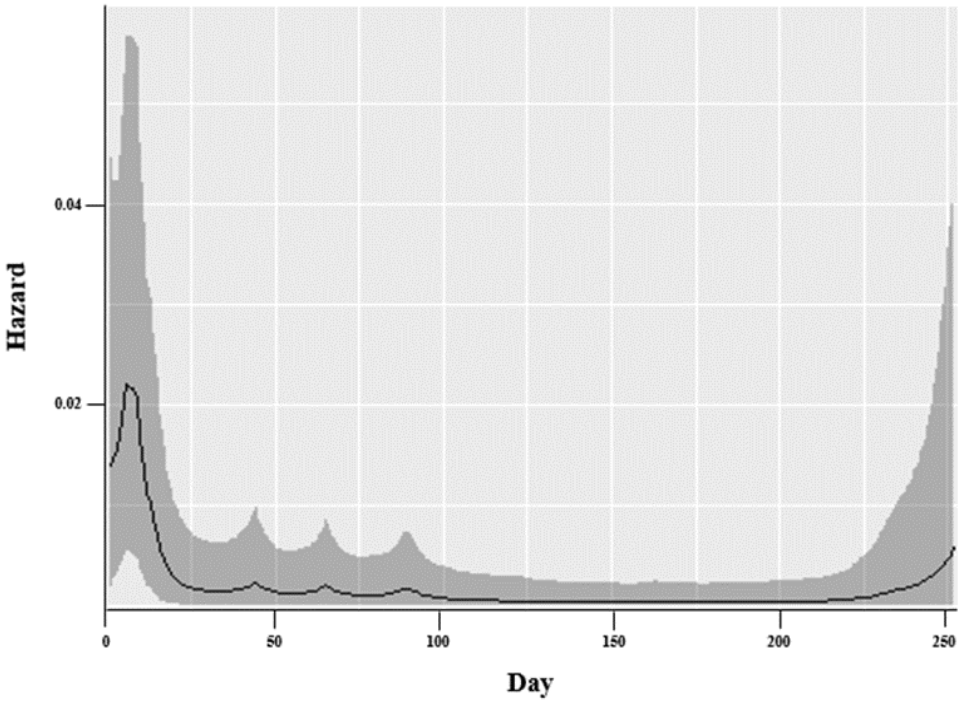
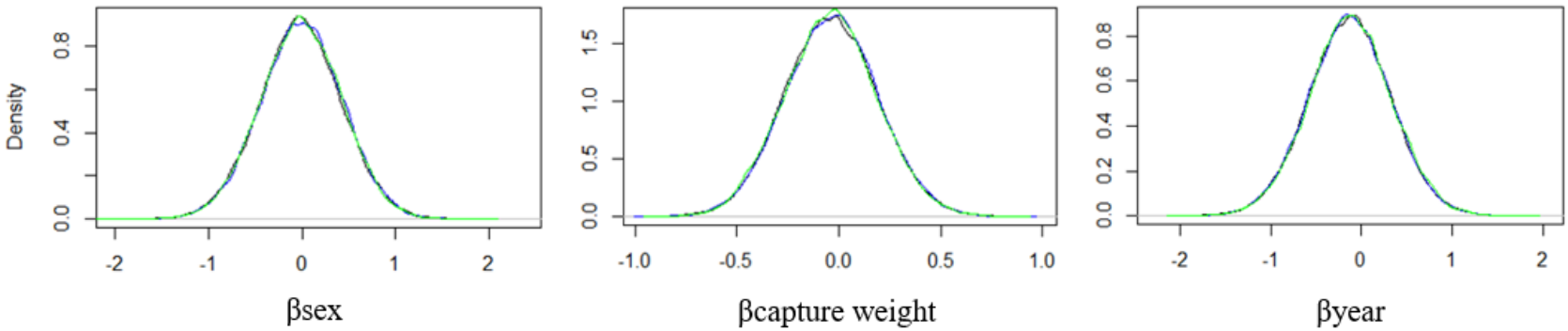


Figure 5. Posterior distributions of variables in the top-ranked lamb survival models. Each MCMC chain is denoted in a separate color (i.e., black, blue, green). Panel [i] shows posterior distributions of variables in the top ranked model: $\gamma + \beta sex + \beta capture\ weight + \beta year + \rho_j$ and Panel [ii] shows posterior distributions of variables in the 2nd ranked model: $\gamma + \beta herd + \rho_j$.

[i]



[ii]

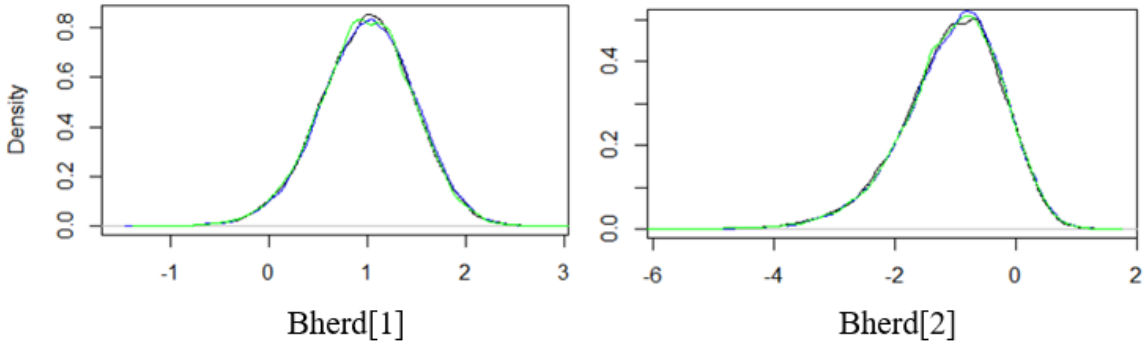
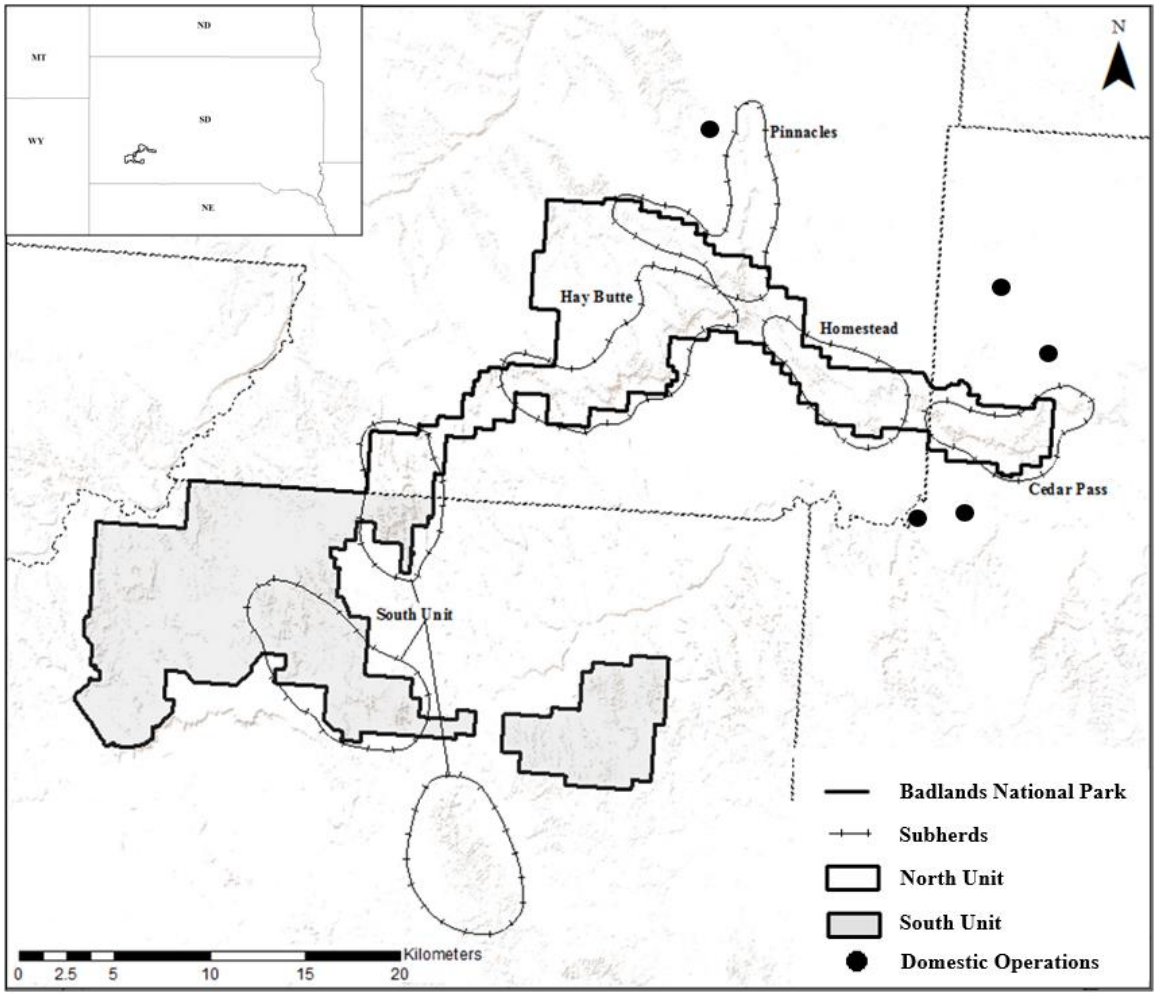


Figure 6. Bighorn sheep subherd ranges and documented domestic sheep and goat operations within 8-km of the North Unit of Badlands National Park, South Dakota, 2017-2019.



CHAPTER 2: GENETIC VARIATION AND STRUCTURE OF A REINTRODUCED BIGHORN SHEEP METAPOPULATION: EXPLORING OVER 3 DECADES IN BADLANDS NATIONAL PARK

This chapter is being prepared for publication and was coauthored by Daniel P. Walsh, E. Frances Cassirer, Thomas E. Besser, Eddie L. Childers, and Jonathan A. Jenks.

ABSTRACT

Bighorn sheep (*Ovis canadensis*) populations often experience irregular periods of growth and declines. Consequently, bighorn sheep populations tend to be small and isolated exhibiting a fragmented distribution. Managing the viability of isolated populations often requires translocations to maintain genetic variability, improve fitness, and increase growth. Within the last century, bighorn sheep in the badlands ecosystem of western South Dakota have been subjected to complete extirpation, reintroduction, suspected disease related die-offs, genetic bottlenecking, and population augmentation. Subsequently, the population in Badlands National Park (BNP) appears to have recovered, but it was unknown how management actions had affected the current metapopulation. From 2017-2019, we conducted research on 5 subherds within two management units to determine genetic variation and population structuring and differentiation. Genetic analysis was conducted at 15 microsatellite loci from 75 individual samples. Overall genetic variation for the BNP population was consistent with other native and translocated populations of bighorn sheep across their range. We identified three genetically distinct clusters recognized as the three source herds used to establish and supplement the BNP population between 1967 and 2014. Our results indicate population structuring was clear at various degrees within the population, yet healthy genetic variation and sufficient gene flow between genetic clusters and subherds was occurring, avoiding the vulnerability of genetic drift/inbreeding commonly associated with isolated, small populations. Our results provide a baseline assessment of the effects of translocation management on an isolated metapopulation of bighorn sheep over the course of three decades.

Keywords: bighorn sheep, microsatellite, translocation, genetic variation, population structure, metapopulation.

INTRODUCTION

Bighorn sheep (*Ovis canadensis*) were historically one of the most abundant ungulates in North America. Populations were estimated to be in the millions, and were distributed across much of the western United States, portions of Mexico, and Canada (Buechner 1960, Geist 1971). Bighorn sheep are an ecologically fragile species that have experienced a population decline of two orders of magnitude since the settlement of western North America, leading to <20,000 individuals inhabiting one-third of their native range by 1960 (Buechner 1960).

A combination of environmental and demographic factors, such as unregulated hunting, domestic livestock grazing, introduced infectious agents, loss of genetic fitness, predation, and displacement from range and loss of migratory behavior are accredited with these large-scale declines (Douglas and Leslie 1999, Miller et al. 2012a). Due to these declines, many bighorn sheep populations were reduced and distribution highly fragmented (Singer et al. 2000). Maintaining the genetic variability of small, isolated populations is difficult; therefore, supplementing isolated populations via translocations from outbred sources is a common management tool to improve growth, distribution, and genetic variability of bighorn sheep populations (Singer et al. 2000, Ostermann et al. 2001, Hogg et al. 2006, Zimmerman 2008). However, translocation management can have negative results on a recipient population in the loss of locally adapted alleles and disrupting co-adapted gene complexes, potentially lead to outbreeding depression reducing the fitness of the population (Storfer 1999, Edmands 2007).

Audubon's bighorn sheep (*O.c. audubonii*; previously described as a subspecies of bighorn sheep now Rocky Mountain bighorn sheep [*O. c. canadensis*]) historically inhabited the badlands of the Yellowstone and Missouri rivers in eastern Montana, eastern Wyoming, western North Dakota and South Dakota, and northwestern Nebraska (Valdez and Krausman 1999, Wehausen and Ramey 2000). The badlands ecosystem (present day Badlands National Park [BNP], Pine Ridge Reservation, and Buffalo Gap National Grasslands) of western South Dakota sustained a bighorn sheep population until the species was extirpated in 1924 in Washabaugh (a.k.a., south Jackson) County, near the present day location of BNP (Figure 1) (Gross et al. 2000, Zimmerman 2008). The badlands ecosystem held no bighorn sheep from 1924 to 1964. In 1964, the National Park Service (NPS), in collaboration with the South Dakota Department of Game, Fish and Parks (SDGFP) and Colorado Parks and Wildlife, relocated 22 Rocky Mountain bighorn sheep from the Pikes Peak, Colorado source herd, into a 150 ha enclosure in BNP. After ~50% loss of the herd due to pneumonia-caused respiratory infections, the remaining 14 individuals were released into the park during late-summer of 1967 (Ramey et al. 2000).

Slow population growth was observed for the next decade and the population separated into two subherds (i.e., South Unit and North Unit) in 1981, with the majority of the population occupying the North Unit of the park (McCutchen 1980, Singer and Gudorf 1999). A second disease epizootic in 1982 was suspected to have been due to a respiratory infection and/or bluetongue virus outbreak that further inhibited population growth and reduced the North Unit to ~50 individuals (Ramey et al. 2000). Significant population growth occurred following this decline, and by 1988 the total estimated population for both subherds was ≈160 individuals. A third disease epizootic occurred in

the early 1990's with an estimated >50% loss occurred; this outbreak reduced the population to ~60 individuals by 1996 (National Park Service 1998, Ramey et al. 2000). After determining the population underwent a population bottleneck at founding and following decades of variable growth and decline attributed to the multiple suspected disease epizootics (Figure 2[a]), a mixed sex augmentation ($n > 30$) from an outbred, native population of Rocky Mountain bighorn sheep was recommended (Ramey et al. 2000). In September 2004, BNP, in conjunction with SDGFP and New Mexico Department of Game and Fish, relocated 23 bighorn sheep from Wheeler Peak, New Mexico to BNP to augment the genetic variation of the population in BNP (Zimmerman 2008). The augmentation proved to be successful, resulting in enhanced genetic variation, recruitment, and population health, and post-augmentation estimates indicated strong population growth (Zimmerman 2008). In January of 2014, SDGFP, Rocky Boy's Reservation, Montana, and the Oglala Sioux Tribe from Pine Ridge Reservation, South Dakota, captured and translocated 40 bighorn sheep from Montana to South Dakota. Twenty bighorn sheep were released at Cedar Butte in the South Unit of BNP in an attempt to supplement the existing subherd located in that unit (Parr 2015, South Dakota Department of Game, Fish and Parks 2018).

At the time of this study, the bighorn sheep metapopulation in BNP resided in two management units (North and South Unit) (Figure 1). This metapopulation structure consisted of 5 known subherds (Pinnacles, Cedar Pass, Hay Butte, Homestead, and South Unit) (Figure 1). The North Unit (Pinnacles, Cedar Pass, Hay Butte, and Homestead subherds) is managed by the National Park Service, and the South Unit (South Unit subherd) is managed by the Oglala Sioux Tribe of the Pine Ridge Reservation. To date,

the BNP metapopulation was established from three sources of bighorn sheep. The reintroduction of bighorn sheep in 1964 (Pikes Peak, Colorado, USA; n = 22), which established a population in both the North and South Unit by 1981 and is referred to as the resident herd within BNP, the supplementation effort in 2004 (Wheeler Peak, New Mexico, USA; n = 23) in the North Unit of BNP, and the supplementation effort in 2014 (Rocky Boy Reservation, Montana, USA; n = 20) in the South Unit of BNP.

The complexities and outcomes of translocations on recipient populations are lacking and not well understood. Particularly those regarding the spatial and temporal dynamics following multiple translocations in a metapopulation structure. Given the history of the BNP bighorn sheep population and the need to further understand how translocation management has formed and influenced the current metapopulation, our specific objectives were to: 1) determine contemporary genetic variation within management units and the overall population; 2) compare contemporary genetic variation estimates with those from 6 different time periods from BNP; 3) identify structuring within the population; and 4) assess population differentiation across management units and genetic clusters within the BNP metapopulation.

METHODS

Study Area

BNP is located in the White River badlands of southwest South Dakota in Pennington, Jackson, and Oglala Lakota counties. Our study area in BNP encompassed ~98,400 ha ranging in elevation from 700 to 1,000 m above mean sea level (Weedon 1999). The surrounding region with suitable bighorn sheep habitat (hereafter, the greater

badlands ecosystem) was composed of United States Forest Service (Buffalo Gap National Grasslands), Pine Ridge Reservation, and privately owned lands (Sweanor et al. 1995). The topography consisted of highly eroded, diverse cliffs, canyons, and spires over 100 m in height with steep gradients (0-71°) giving away to sod tables, crevasses, toadstools, and fragmented higher plains (Sweanor et al. 1995, Weedon 1999). Climate of the badlands was highly variable and unpredictable; total annual precipitation was 41 cm and mean annual temperature was 11°C (range: -27°C to 41°C) during 2017-2018 in Scenic, SD (National Oceanic and Atmospheric Administration 2019).

The badlands are primarily a mixed-grass prairie ecosystem dominated by buffalograss (*Buchloe dactyloides*), western wheatgrass (*Pascopyrum smithii*), and prickly pear cactus (*Opuntia polyacantha*), with limited tree and brush species of Rocky Mountain juniper (*Juniperus scopulorum*) and eastern red cedar (*J. virginiana*) in draws and vegetated slopes (Weedon 1999). Other ungulates in the study area included bison (*Bison bison*), mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), and pronghorn (*Antilocapra americana*), along with additional herbivore competition from black-tailed prairie dogs (*Cynomys ludovicianus*). Potential predators of bighorn sheep in BNP include coyotes (*Canis latrans*), bobcats (*Lynx rufus*), golden eagles (*Aquila chrysaetus*), and mountain lions (*Puma concolor*). Mountain lion presence and impact on bighorn sheep in BNP was limited, with rare sightings and sign generally attributed to dispersing individuals from the Black Hills of South Dakota (Thompson and Jenks 2010).

Capture Methods

We captured adult ewes and rams via aerial net-gunning (Jacques et al. 2009) from a helicopter (Hells Canyon Helicopters, Lewiston, ID, USA; Quicksilver Air, Inc., Fairbanks, AK, USA; and Helicopter Wildlife Services, Austin, TX, USA) in March 2017, February 2018, and February 2019. We estimated ewe age based on tooth eruption (Krausman and Bowyer 2003) and ram age based on horn annuli (Geist 1966). All captured individuals were fitted with either very high frequency (VHF) or global positioning system (GPS) collars manufactured by Advanced Telemetry Systems (ATS; Isanti, MN, USA). The South Dakota State University Institutional Animal Care and Use Committee approved all capture and handling procedures (Approval number 17-003A).

Genetic Analysis

We collected whole blood via jugular venipuncture and transferred it to Whatman FTA Cards (GE Healthcare Life Sciences; Chicago, IL, USA) for genetic analysis. Nuclear DNA was extracted at the National Genomics Center for Wildlife and Fish Conservation, United States Forest Service, Rocky Mountain Research Station (Missoula, Montana, USA) using the QIAGEN DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) according to manufacturer's instructions for tissue and blood. DNA samples were amplified with PCR and standard microsatellite typing procedures at 15 (8 neutral loci, 7 loci in genes of known function) polymorphic loci: MAF36, MAF48, FCB304, AE16, HH62, MAF209, MAF33, FCB266 (Forbes and Hogg 1999), KRT2 (McLaren et al. 1997), KERA (J.F. Maddox unpublished), SOMA (Lucy et al. 1998), ADCYAP1 (Wood and Phua 1993), TCRG4 (Diez-Tascón et al. 2002), MMP9 (Maddox 2001) and OLADRBps (Blattman and Beh 1992). The reaction volume (10 μl) contained 1.0μL DNA, 1x reaction buffer (*Applied Biosystems*), 2.0 mM $MgCl_2$, 200μM of each dNTP,

1μM reverse primer, 1μM dye-labeled forward primer, 1.5 mg/ml BSA, and 1U *Taq* polymerase (*Applied Biosystems*). The PCR profile was 94°C/5 min ([94°C/1 min, 55°C/1 min, 72°C/30s] x 45 cycles). The resultant products were visualized on a LI-COR DNA analyzer (LI-COR Biotechnology). Data were error checked using program Dropout (McKelvey and Schwartz 2005), GenAlEx (Peakall and Smouse 2006, Peakall and Smouse 2012), and Microchecker (Van Oosterhout et al. 2004).

We used the multilocus genotype data to assess the overall genetic variation and population structure of the bighorn sheep population in BNP. We calculated observed (H_O) and expected (H_E) heterozygosity, allelic diversity (A), effective alleles (A_E), and tested for deviations from Hardy-Weinberg equilibrium (HWE) using GenAlEx 6.5 (Peakall and Smouse 2006, Peakall and Smouse 2012). We evaluated population structure of individuals sampled between 2017-2019 using STRUCTURE 2.3.4 (Pritchard et al. 2000). STRUCTURE utilizes a Bayesian model-based clustering method with allele frequency data to investigate population structure from individual genotypes (Pritchard et al. 2000). We assumed individuals had mixed ancestry (admixture model) and correlated allele frequencies while excluding geographic information in the analysis (Juarez et al. 2016, Love Stowell et al. 2020). The admixture model utilized an initial value of 1.0 for alpha and a uniform prior for alpha with a maximum value of 10.0 and standard deviation of 0.025. We set both burn-in periods to 10,000 and evaluated 1 to 7 possible genetic clusters (*K*) with three iterations at 100,000 reps. To check for evidence of non-convergence, we plotted the alpha and likelihood values alongside the number of iterations for each run of *K*. For selecting the appropriate number of genetic clusters (*K*), we assessed the maximal value of L(*K*) or the log likelihood of the data given *K*

(Pritchard et al. 2000). In addition to assessing L(*K*) for determining genetic clusters, we assessed a statistic based on the second order rate of change of L(*K*) between successive *K* values (Δ*K*) (Evanno et al. 2005). Utilizing both L(*K*) and (Δ*K*), visualized using STRUCTURE HARVESTER web version 0.6.94, we inferred the most probable number of genetic clusters (Earl 2012). F_{ST} was calculated to estimate population differentiation between the management units (i.e., North Unit and South Unit) with GenAlEx and between the identified genetic clusters (*K*) from STRUCTURE analysis. F_{ST} values <0.05 relate to low genetic differentiation, 0.05-0.15 moderate genetic differentiation, and >0.15 significant genetic differentiation (Hartl et al. 1997, Frankham et al. 2002).

RESULTS

We successfully genotyped 75 bighorn sheep from BNP at 8 neutral and 7 adaptive microsatellite loci (Table 1). We compared genetic variation in two management units (i.e., North Unit and South Unit) and the overall population at BNP (Table 2). Significant deviations from HWE included two loci from the South Unit and 5 loci from the overall analysis (Table 3). However, these deviations were likely the result of hierarchical subdivision (i.e., Wahlund effect) due to the recent 2014 translocation effort in the South Unit; therefore, we retained all loci for analyses. The North Unit of BNP had no deviations from HWE when analyzed separately. We documented slightly higher genetic variation in the North Unit than in the South Unit based on 5 different metrics (Table 2). We compared contemporary genetic variation results with research from Zimmerman (2008), who evaluated genetic variation from 6 previous time periods between 1925 – 2006 within the BNP bighorn sheep population (Figure 2[b]; Table 4). Prior to the translocation event in 2004, the BNP population showed a gradual decline in

population size and a decrease in levels of observed/expected heterozygosity (Ramey et al. 2000, Zimmerman 2008) (Figure 2; Table 4). Following the translocation event in 2004, the BNP population increased in population size and had higher levels of observed and expected heterozygosity (Zimmerman 2008) (Figure 2; Table 4).

STRUCTURE analysis for the BNP population resulted in similar maximal values of L(K) for K = 3 (L[K] = -2796.50), K = 4 (L[K] = -2780.03), and K = 5 (L[K] = -2811.37) (Figure 3). With L(K) similar among three genetic K's, we compared ΔK values between likelihoods for K and selected K = 3 with a ΔK = 977.26 as the best supported number of genetic clusters (Figure 3), when using the admixture model and excluding geographic information. Using K = 3, we compared the genetic ancestry between the 5 subherds throughout the BNP metapopulation determining the degree of similarity of individuals and subherds to each cluster (Figure 4; Table 5). We recognize the three genetic clusters identified by the STRUCTURE analysis as the three sources of bighorn sheep introduced into BNP via reintroduction in 1964 (source population= Pikes Peak, CO; n = 22) and the supplemental translocations in 2004 (source population = Wheeler Peak, NM; n = 23) and 2014 (source population = Rocky Boy Reservation, MT; n = 20). Based on these assemblages, the genetic structuring between subherds spatially and across time appear to align with the identified clusters from the STRUCTURE analysis. A majority of sampled individuals' degree of similarity to genetic clusters was assigned to "Cluster 1" and "Cluster 3" (Figure 4; Table 5). Within the North Unit, individuals from Pinnacles subherd (0.663 [SE = 0.076]) and Homestead subherd (0.845 [SE = 0.107]) had a majority of degree of similarity with "Cluster 1" (Figure 4; Table 5). Individuals from Cedar Pass subherd (0.879 [SE = 0.056]) and Hay Butte subherd (0.583

[SE = 0.101]) have a majority of degree of similarity with “Cluster 3” (Figure 4; Table 5). The South Unit subherd was separated into two classifications for evaluating the degree of similarity to genetic clusters. One classification included all the individuals sampled from the South Unit subherd. The second classification was from only the individuals born in BNP, removing the 6 individuals that were part of the original 2014 translocation. Based on these two classifications, all the individuals in the South Unit subherd had comparable degrees of similarity between “Cluster 2” (0.436 [SE = 0.102]) and “Cluster 3” (0.490 [SE = 0.099]) (Table 5). With the removal of the 6 individuals that were part of the 2014 translocation, the degree of similarity to genetic clusters shifted to a majority in “Cluster 3” (0.671 [SE = 0.105]) and a reduction in similarity in “Cluster 2” (0.230 [SE = 0.099]) (Table 5). The overall breakdown of similarity of all the individuals sampled in the population (n = 75) to the three genetic clusters identified were: “Cluster 1” = 0.445 (SE = 0.050), “Cluster 2” = 0.141 (SE = 0.037), and “Cluster 3” = 0.416 (SE = 0.049) (Figure 4; Table 5).

We estimated the amount of gene flow or population differentiation between management units and between identified genetic clusters within BNP. Using GenAlEx, we calculated a F_{ST} value of 0.04 with ≈ 6.0 migrants occurring per generation between the North and South Units, indicating low genetic differentiation and high gene flow (Table 6). F_{ST} values calculated between the STRUCTURE analysis’ genetic clusters were 0.07 between “Cluster 1” and “Cluster 3”, 0.15 between “Cluster 2” and “Cluster 3”, and 0.20 between “Cluster 1” and “Cluster 2” (Table 6).

DISCUSSION

We examined how nearly three decades of translocation management, variable population growth and decline, and a genetic bottleneck had affected genetic variation and population structuring of a metapopulation of bighorn sheep in BNP. The importance of genetic variation in maintaining population viability is essential where populations are small, have undergone bottlenecks, or are isolated (Fitzsimmons et al. 1997). We recorded levels of genetic variation that were consistent with native and translocated populations of bighorn sheep across their range, including two neighboring, reintroduced populations in western South Dakota (Parr et al. 2016, Gille et al. 2019, Werdel et al. 2019, Love Stowell et al. 2020). However, this comparison across studies is difficult because different loci were sampled in each study except for Parr et al. (2016). Our results suggest that genetic variation is not a current population limiting factor for the BNP bighorn sheep population.

We used measurements of heterozygosity and allelic diversity due to their standard and frequent use for evaluating genetic variation of populations (FitzSimmons et al. 1995, Whittaker et al. 2004, Miller et al. 2012b). Higher diversity among alleles better prepares individuals and populations to adapt to local environments and/or demographic stochasticity, meanwhile estimating heterozygosity provides a reflective evaluation of recent breeding activity (Whittaker et al. 2004). Decreases in allelic diversity typically occur faster than that of heterozygosity; however, both are likely to occur following a severe decline in population size (Nei et al. 1975, Leberg 1992). Therefore, decreases in allelic diversity and heterozygosity may strongly affect the overall genetic variation of a population. Ramey et al. (2000) and Zimmerman (2008) both found low and declining levels of heterozygosity and allelic diversity along with a decreasing population size in

BNP prior to a successful translocation effort in 2004 (Table 4, Figure 2). Post translocation estimates from Zimmerman (2008) detected restored levels of allelic diversity (3.11) and high observed heterozygosity (0.83) of the first generation offspring (Table 4). This fluctuation in genetic variation estimates between pre and post translocation were likely credited to the isolate breaking effect (i.e., the mixing of two previously isolated, distinct populations), which may have adverse effects in the form of outbreeding. Outbreeding can further affect newly augmented populations by altering their ability to adapt to the local environment and lower the overall fitness of the population (Gutiérrez-Espeleta et al. 2000, Tallmon et al. 2004). Zimmerman (2008) recommended monitoring of growth rates and genetic composition of subsequent generations to determine if outbreeding or a genetic rescue was the result of the 2004 translocation. Our estimates from 2017-2019, which includes the translocation of 2014, represent a positive response to the translocations with increased overall population size and levels of genetic variation consistent with healthy native and translocated populations of bighorn sheep (Table 4; Figure 2). Hogg et al. (2006) documented similar results in the National Bison Range bighorn sheep population in Montana when translocation efforts were used to increase genetic variation and subsequently improved the fitness at the individual and population levels. Following recommendations from Zimmerman (2008), our results provided a continuation of the population's response to the 2004 and 2014 translocation efforts in restoring and maintaining genetic variation while improving fitness and growth.

Differences between genetic variation estimates were evident among the two management units (i.e., North and South Units) in BNP (Table 2). The subherds within

each management unit were spatially separated by >20 km and both have had translocations within the last two decades (Figure 1). Overall, the North Unit's estimates were higher across each variable measured for genetic variation than the South Unit's estimates (Table 2). The South Unit was a large area of habitat with current and historically low densities of bighorn sheep. Social interactions between resident and translocated individuals occur more frequently in populations with higher densities; therefore, low densities can have negative effects on the success of translocation efforts (Sarrazin and Barbault 1996). We hypothesize that the lower genetic variation within the South Unit, despite a recent 2014 translocation, was the product of low densities, the large range of suitable habitat, and the lack of socializing and genetic exchange between the resident population and the individuals of the 2014 translocation. The significant lack of socializing and intermixing of resident and augmented adult females within the first 3 years following a translocation has been documented in multiple populations of bighorn sheep (Roy and Irby 1994, Robinson et al. 2019). The difference in genetic variation between management units in BNP was likely the result of the limited timeframe since the 2014 translocation and the population spatial structure of the South Unit subherd. Additionally, the South Unit had deviations from HWE at two loci, when analyzed separately from the North Unit, and 5 loci deviations across both management units (Table 3). These deviations within the South Unit and the overall analysis were likely the result of hierarchical subdivision (i.e., Wahlund effect) among the individuals sampled for the analysis (Malaney et al. 2015). For example, 6 of the 22 individuals included in the analysis from the South Unit were the original individuals translocated in 2014 (Table 1). As a result, there remains a genetic disconnect between the individuals in the South

Unit subherd, but admixture will likely occur creating genetic similarity in subsequent generations.

Translocation management has been a consistent tool in the effort to increase population size, genetic variation, connectivity, and distribution of bighorn sheep in the greater BNP ecosystem. The result of this management being the formation of genetic clustering that reflects a combination of the geography, founding source herds, and generations passed since translocations. Identifying how these management actions have affected genetic structure and connectivity is essential to the long-term management of wildlife populations (Storfer et al. 2007, Segelbacher et al. 2010). There is limited understanding of the dynamics on how multiple translocations utilizing multiple source herds can influence and affect the population structuring of an isolated population of bighorn sheep. Since the initial reintroduction of bighorn sheep in 1964, the translocation in 2004, and the latest translocation in 2014, no assessment (until present) had been conducted to understand the composition of the 5 subherds in the BNP metapopulation and how translocations interacted.

We determined genetic structuring (K = 3) was consistent with the three source herds used to establish and supplement the BNP metapopulation in 1964, 2004, and 2014. We further suggest and assign specific genetic clusters to the individual events (i.e., 1964 reintroduction, 2004 translocation, and 2014 translocation) conducted over the last 60 years. We speculate that “Cluster 1” corresponds with the 2004 translocation effort (Table 5; Figure 4: denoted in red), “Cluster 2” corresponds with the 2014 translocation effort (Table 5; Figure 4: denoted in green), and “Cluster 3” corresponds with the 1964 reintroduction effort (Table 5; Figure 4: denoted in blue). Given the release location and

time elapsed since the management event (Figure 1), evidence exists in support of the assigned clusters to the reintroduction and translocation events. The 2004 translocation ("Cluster 1") was released in the Pinnacles subherd, and throughout the last two decades we have documented frequent exchange between the Pinnacles and the Homestead subherds (Figure 1). Additionally, the degree of similarity of individuals in the Pinnacles and Homestead subherds was largely assigned to "Cluster 1" and limited presence of "Cluster 1" was found in the South Unit subherd (Table 5; Figure 4), but limited samples were collected within the Homestead subherd potentially affecting the degree of similarity of the subherd to genetic clusters. The 2014 translocation ("Cluster 2") release site was in the South Unit of BNP and limited time has elapsed; therefore, concentrating most of the degree of similarity of individuals from "Cluster 2" to primarily the South Unit subherd (Table 5; Figure 4). The 1964 reintroduction ("Cluster 3") had the largest distribution of degree of similarity among individuals across subherds and has also had the most generations pass to encompass a larger distribution within BNP (Table 5; Figure 4). Prior to the 2004 and 2014 translocations, the bighorn sheep from the 1964 reintroduction were primarily concentrated in the Hay Butte subherd with low densities making up the South Unit and Pinnacle subherds. Additionally, the 1964 reintroduced bighorn sheep were used to establish the Cedar Pass subherd through a separate internal translocation of individuals from established subherds within BNP to suitable habitat that is now the Cedar Pass subherd range (Zimmerman 2008). However, the limited sample size for the Cedar Pass subherd may have affected the degree of similarity of the subherd to genetic clusters.

Evaluating the effects of translocations on recipient herds is often complicated through the use of multiple source herds, the subsequent social interactions among resident and translocated individuals, and the number of generations that have elapsed between translocation events and sampling (Singer et al. 2000, Olson et al. 2012;2013, Jahner et al. 2019, Robinson et al. 2019, Love Stowell et al. 2020). Our results provide a limited but important timeline on how translocated individuals from multiple sources intermixed genetically in a metapopulation structure. Translocations can have both detrimental and beneficial effects on genetic diversity and population structuring in highly managed, isolated wildlife populations (Gille et al. 2019). Buchalski et al. (2015) evaluated the population structuring in a well-established population of bighorn sheep and found populations were distinct in genetic structuring following discernable geographic boundaries. Love Stowell et al. (2020) found the most genetically distinct herds were the most geographically distant herds. Our results represent how genetic structuring can form in the absence of geographic boundaries and distances, but rather through limited social interactions among resident and translocated individuals in low density subherds occupying large areas of suitable habitat (e.g., “Cluster 2” [2014 translocation] versus “Cluster 3” [1964 reintroduction]). Our findings also show how less genetic structuring was prominent among subherds that had more generations elapse between translocation and genetic sampling (e.g., “Cluster 1” [2004 translocation] versus “Cluster 3” [1964 reintroduction]).

Genetic clustering was clear at various degrees within the BNP subherds, yet sufficient gene flow between genetic clusters and subherds was occurring (Table 6). A single migrant per generation, among idealized populations, is sufficient to prevent

complete population differentiation regardless of the size of the population (Wright 1969, Frankham et al. 2002). Dispersal and migration, coupled with sub-structured populations, helps maintain genetic diversity and gene flow avoiding the vulnerability of localized stochastic events due to genetic drift/inbreeding. (Bleich et al. 1990). The extent of migration and dispersal events between populations is often difficult to document, but is commonly associated with ram movements during the breeding season or in response to high densities (Schwartz et al. 1986, Borg et al. 2017). We have observed large scale dispersals (>200 km) of both sexes of yearling bighorn sheep out of BNP along with inter-subherd movements of rams likely contributing to the gene flow during the breeding season. BNP is an isolated population with no known dispersals into the population from neighboring bighorn sheep populations (e.g., Custer State Park, SD; Rapid and Spring Creek, SD; Pine Ridge, NE; Fort Robinson, NE).

F_{ST} values are commonly used to describe population differentiation with values 0.00 to 0.05 indicating little genetic differentiation, 0.5 to 0.15 indicating moderate genetic differentiation, and >0.15 indicating significant genetic differentiation between populations (Wright 1978, Hartl et al. 1997, Balloux and Lugon-Moulin 2002, Frankham et al. 2002). Based on F_{ST} values, we found evidence of interbreeding and genetic exchange between the North and South Units with ≈6.0 migrants/generation ($F_{ST=}$ 0.04) (Table 6). Zimmerman (2008) evaluated F_{ST} values between the North and South Units prior to the 2004 and 2014 translocations and found higher values of interbreeding with 11.7 migrants/generation ($F_{ST=}$ 0.01). Higher F_{ST} values prior to 2004 were likely due to the BNP population being comprised of one source of bighorn sheep at that time. Following 2004, two additional translocations resulted in three sources of bighorn sheep

within the BNP, which likely increased the F_{ST} values between management units we observed. F_{ST} values between the three genetic clusters were consistent with the degree of similarity between individuals from each cluster (Table 6; Figure 4). The more diverse a cluster across sampled individuals, the greater the gene flow that we observed for that cluster.

Genetic variation estimated at 15 microsatellite loci from 75 individuals was high across each subherd and management unit indicating no current limitations on the genetic health and fitness within the BNP metapopulation. The negative effects associated with an isolated, small population of bighorn sheep in the form of inbreeding appear to have been avoided through translocation management. In response to combining multiple distinct populations, the challenges of outbreeding depression following two translocations does not appear to be affecting the current population. Additionally, the estimated levels of genetic variation within BNP were equivalent to other native and translocated bighorn sheep populations. The identified genetic structuring within the population was consistent with the previous reintroduction in 1964, the translocation efforts of 2004 and 2014, and the three source herds used in those actions. The documented genetic clustering provides an understanding of how interactions between resident and translocated individuals unfold temporally and spatially following multiple translocations in an isolated, metapopulation structure. Although genetic clusters are apparent at various degrees among management units and subherds, sufficient gene flow was documented; however, continuous monitoring should be explored of subsequent generations with particular focus on the South Unit subherd where genetic clustering and higher population differentiation was evident.

With the lack of dispersals into the population, increasing dispersals out of the population, and the isolated nature of the population, the genetic methods in this study have further value by offering a resource to managers to identify and assign the source of lone dispersing or wandering individuals and a means of potential management action for addressing them (e.g., lethal removal, translocating/reducing high density subherds). Our research also provides a baseline of the current genetic status and population structuring of the BNP metapopulation, but due to the current absence of genetic connectivity to outside populations, we recommend future monitoring to detect shifts in genetic variation, population decline, and loss of population and individual fitness (i.e., genetic drift/inbreeding).

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Table 1. Samples from Badlands National Park bighorn sheep collected 2017-2019 included in genetic variation and STRUCTURE analyses.

Subherd	Management Unit	*n*	Sex		Age Class Distribution				
			Male	Female	<1	1+	2+	3+	4+
Cedar Pass	North Unit	2	0	2	0	0	0	0	2
Homestead	North Unit	9	1	8	0	1	2	1	5
Pinnacles	North Unit	27	0	27	0	1	7	4	15
Hay Butte	North Unit	15	4	11	0	1	1	2	11
South Unit[a]	South Unit	22	8	14	3	0	4	4	11
Total	**--**	**75**	**13**	**62**	**3**	**3**	**14**	**11**	**44**

[a] Includes 6 individuals that were translocated from Rocky's Boy Reservation in the 2014 translocation.

Table 2. Genetic Variation measured in bighorn sheep at 15 microsatellite loci (column names) in the North Unit (*n* = 53), South Unit (*n* = 22), and both Units combined (*n* = 75) in Badlands National Park, 2017-2019. A = number of alleles per locus (allelic diversity), A_E = number of effective alleles per locus, H_O = observed heterozygosity, and H_E = expected heterozygosity.

North Unit

	Mean	SE	MAF36	MAF48	FCB304	AE16	HH62	MAF209	MAF33	FCB266	KRT2	KERA	SOMA	ADCYAP1	TCRG4	MMP9	OLADRBps
A	**5.27**	0.42	5.00	5.00	4.00	7.00	6.00	6.00	4.00	4.00	5.00	6.00	3.00	3.00	7.00	9.00	5.00
A_E	**3.48**	0.34	3.25	4.27	2.88	5.34	2.50	3.27	2.13	1.67	2.80	4.13	1.91	2.68	4.71	6.09	4.62
H_O	**0.68**	0.04	0.70	0.79	0.62	0.89	0.53	0.75	0.58	0.38	0.64	0.77	0.43	0.64	0.75	0.91	0.77
H_E	**0.67**	0.03	0.69	0.77	0.65	0.81	0.60	0.69	0.53	0.40	0.64	0.76	0.48	0.63	0.79	0.84	0.78

South Unit

	Mean	SE	MAF36	MAF48	FCB304	AE16	HH62	MAF209	MAF33	FCB266	KRT2	KERA	SOMA	ADCYAP1	TCRG4	MMP9	OLADRBps
A	**5.00**	0.44	5.00	4.00	4.00	8.00	6.00	7.00	4.00	2.00	5.00	5.00	3.00	3.00	6.00	6.00	7.00
A_E	**3.33**	0.30	3.15	2.38	2.23	5.56	3.12	3.95	3.63	1.25	3.15	3.83	2.29	2.19	4.25	4.82	4.23
H_O	**0.58**	0.04	0.45	0.45	0.50	0.86	0.55	0.73	0.64	0.23	0.59	0.73	0.41	0.55	0.68	0.73	0.68
H_E	**0.66**	0.04	0.68	0.58	0.55	0.82	0.68	0.75	0.72	0.20	0.68	0.74	0.56	0.54	0.76	0.79	0.76

Overall

	Mean	SE	MAF36	MAF48	FCB304	AE16	HH62	MAF209	MAF33	FCB266	KRT2	KERA	SOMA	ADCYAP1	TCRG4	MMP9	OLADRBps
A	**5.80**	0.54	6.00	5.00	4.00	10.00	7.00	7.00	4.00	4.00	5.00	6.00	3.00	3.00	7.00	9.00	7.00
A_E	**3.67**	0.34	3.62	4.32	2.91	5.67	2.94	3.89	2.65	1.53	2.96	4.11	2.02	2.67	4.77	6.07	4.88
H_O	**0.65**	0.04	0.63	0.69	0.59	0.88	0.53	0.75	0.60	0.33	0.63	0.76	0.43	0.61	0.73	0.85	0.75
H_E	**0.69**	0.03	0.72	0.77	0.66	0.82	0.66	0.74	0.62	0.35	0.66	0.76	0.50	0.63	0.79	0.84	0.80

Table 3. Gene loci within the bighorn sheep population at Badlands National Park that deviated from Hardy-Weinberg equilibrium.

	Locus	DF	ChiSq	P-value
North Unit	--	--	--	--
South Unit	MAF33	6	12.933	0.044
	KRT2	3	13.530	0.004
Overall	MAF48	10	31.801	0.000
	HH62	21	58.583	0.000
	TCRG4-BV62	10	20.407	0.026
	KRT2	3	10.821	0.013
	FCB266	21	36.455	0.019

Table 4. Genetic variation and population trend of bighorn sheep in Badlands National Park compared across 7 time periods. N = number of samples, A = number of alleles per locus (allelic diversity), A_E = number of effective alleles per locus, H_O = observed heterozygosity, and H_E = expected heterozygosity. Mean with standard error parenthetically.

Year	Status of Population	N	A	A_E	H_O	H_E	Source
<1925	N/A	3	1.75 (0.25)	2.00 (0.28)	0.32	0.39 (0.06)	Zimmerman 2008
1992	Decline	26	2.23 (0.15)	4.20 (0.33)	0.51	0.52 (0.03)	Ramey et al. 2000 & Zimmerman 2008
1996	Decline	14	2.10 (0.14)	3.20 (0.20)	0.54	0.49 (0.04)	Ramey et al. 2000 & Zimmerman 2008
1998	Decline	14	2.03 (0.13)	3.20 (0.28)	0.5	0.47 (0.04)	Ramey et al. 2000 & Zimmerman 2008
2004	Steady (pre translocation)	3	1.66 (0.08)	2.20 (0.15)	0.47	0.37 (0.04)	Zimmerman 2008
2006	Steady (post translocation)	12	3.11 (0.22)	4.40 (0.29)	0.83	0.65 (0.03)	Zimmerman 2008
2017-2019	Increase	75	5.80 (0.54)	3.67 (0.34)	0.65 (0.04)	0.69 (0.03)	Current study

Table 5. STRUCTURE analysis by subherd, management unit, number of samples (*n*), and degree of similarity of each genetic cluster.

Subherd	Management Unit	*n*	Degree of Similarity to Genetic Clusters		
			Cluster 1	Cluster 2	Cluster 3
Cedar Pass	North Unit	2	0.006 (SE = 0.000)	0.116 (SE = 0.057)	0.879 (SE = 0.056)
Homestead	North Unit	9	0.845 (SE = 0.107)	0.013 (SE = 0.006)	0.142 (SE = 0.107)
Pinnacles	North Unit	27	0.663 (SE = 0.076)	0.016 (SE = 0.005)	0.320 (SE = 0.076)
Hay Butte	North Unit	15	0.405 (SE = 0.100)	0.012 (SE = 0.002)	0.583 (SE = 0.101)
South Unit	South Unit	22	0.080 (SE = 0.042)	0.436 (SE = 0.102)	0.490 (SE = 0.099)
South Unit[a]	South Unit	16	0.107 (SE = 0.057)	0.230 (SE = 0.099)	0.671 (SE = 0.105)
Overall Total	--	75	**0.445 (SE = 0.050)**	**0.141 (SE = 0.037)**	**0.416 (SE = 0.049)**

[a] Only resident individuals (born in BNP) excluding 6 individuals from Rocky Boy Reservation, Montana part of the 2014 translocation effort.

Table 6. F_{ST} values inferred by STRUCTURE and GenAlEx analysis. STRUCTURE analysis F_{ST} values between genotype population structures (K = 3) in Badlands National Park. GenAlEx analysis F_{ST} values between management units (North Unit and South Unit) in Badlands National Park.

STRUCTURE (F_{st})				**GenAlEx (F_{st})**		
	"Cluster 1"	"Cluster 2"	"Cluster 3"		North Unit	South Unit
"Cluster 1"	0.00	--	--	North Unit	0.00	--
"Cluster 2"	0.20	0.00	--	South Unit	0.04	0.00
"Cluster 3"	0.07	0.15	0.00			

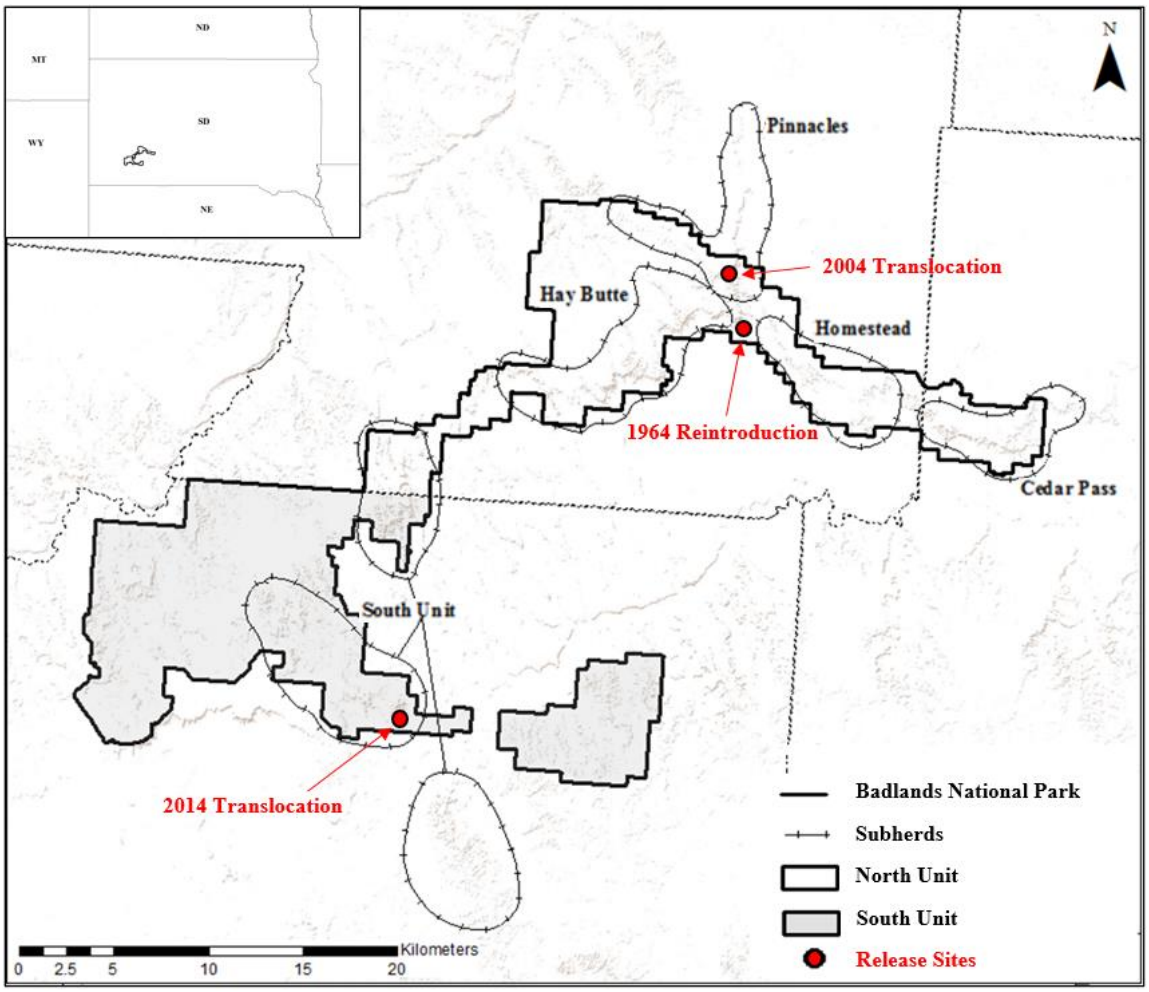
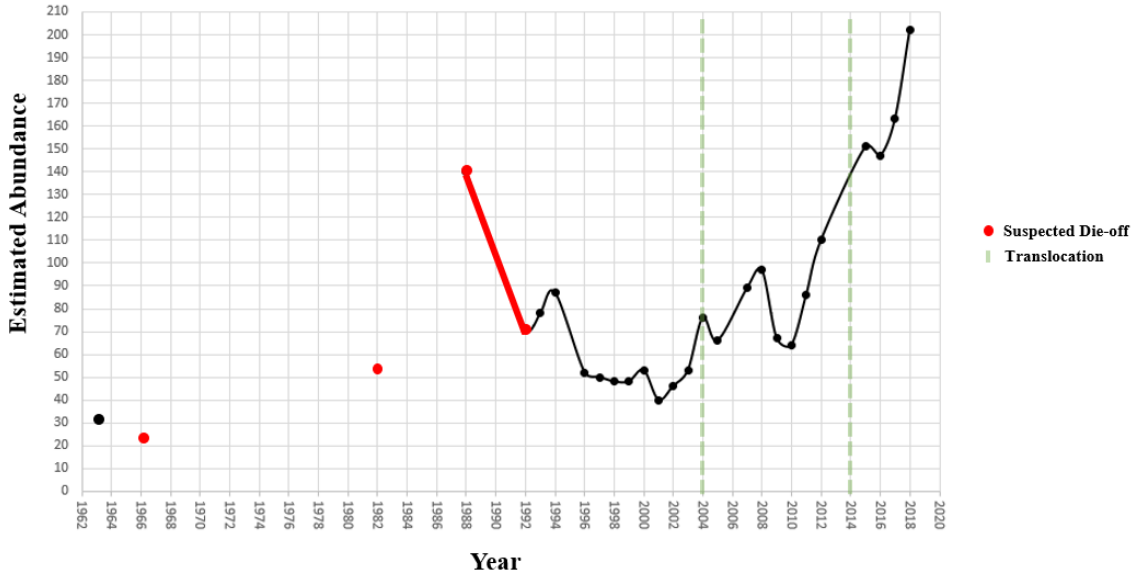


Figure 1. Badlands National Park bighorn sheep study area with delineated North and South Units, reintroduction and translocation release sites, and subherd range delineation in western South Dakota, USA, 2017-2019.

[a]



[b]

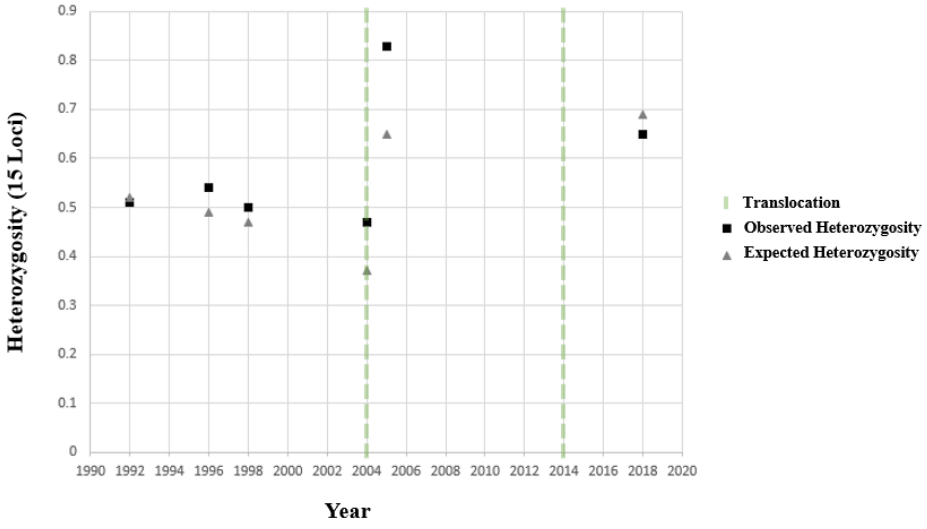


Figure 2. Estimated population size, trend, and heterozygosity of the Badlands National Park bighorn sheep population. Panel [a] population size and trend estimated from minimum survey between initial reintroduction in 1964 and 2018. Panel [b] observed and expected heterozygosity at 6 sampling points 1992-2018.

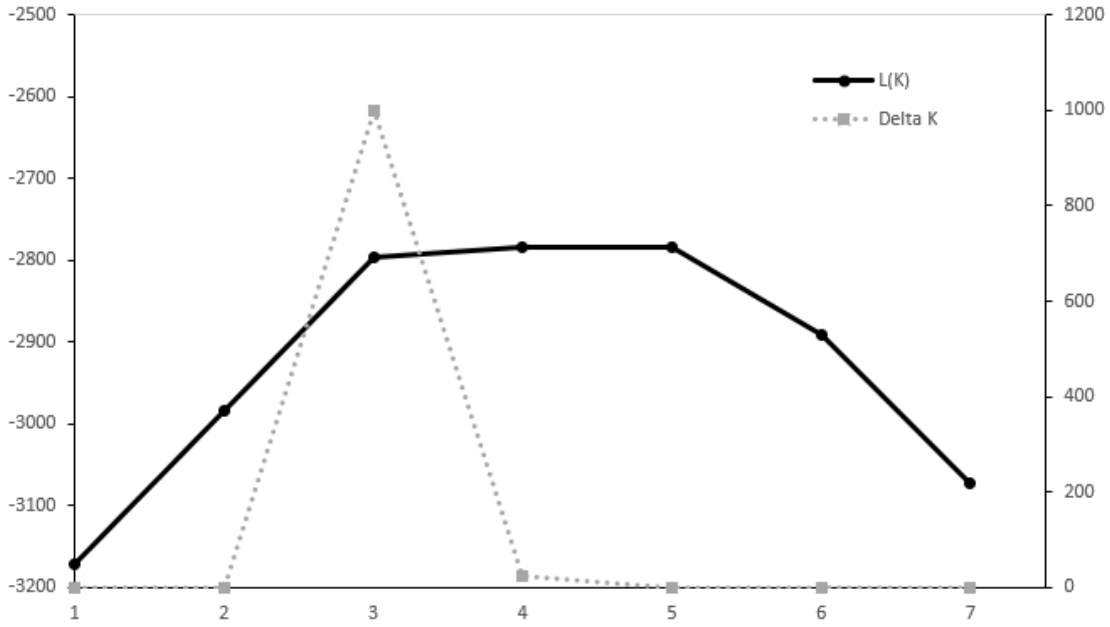


Figure 3. The most probable number of genetic clusters within the Badlands National Park bighorn sheep population using the maximal log likelihood value [L(*K*)] and second order rate of change (Δ*K*).

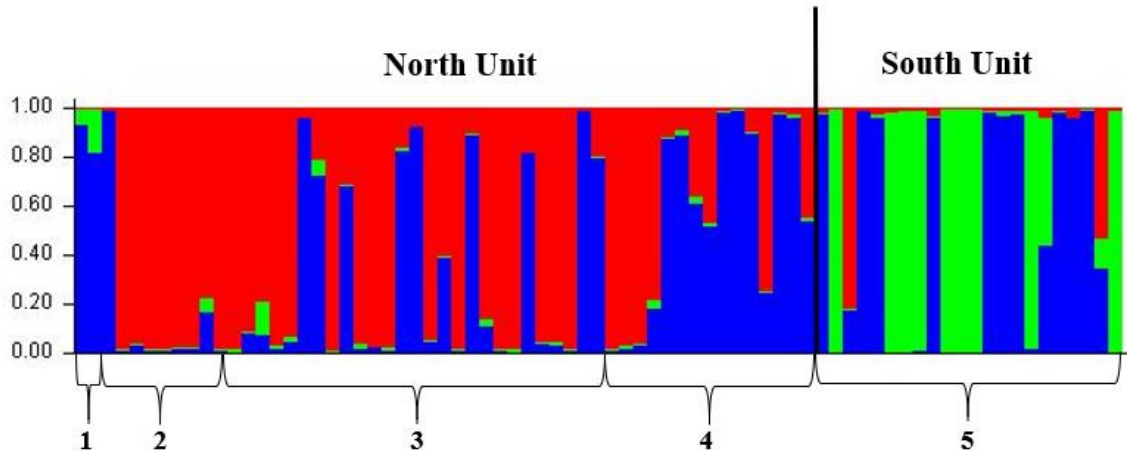


Figure 4. Population structure of the bighorn sheep population in Badlands National Park based on STRUCTURE analysis, *K* = 3. Management units (North Unit and South Unit) are divided by a vertical black line and subherds within both management units are numbered 1-5 (1 = Cedar Pass, 2 = Homestead, 3 = Pinnacles, 4 = Hay Butte, 5 = South Unit). Each individual (*n* = 75) is represented by a single column, where the color(s) of the column represent degree of similarity to each genetic cluster and translocation effort (1964 reintroduction effort = blue, 2004 translocation = red, 2014 translocation = green).