Bighorn Sheep Ecology

An Integrated Science Project to Support Restoration and Management

2021 Final Report

MONTANA STATE UNIVERSITY

MONTANA FISH, WILDLIFE & PARKS

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Photo: Cindy Goeddel

EXECUTIVE SUMMARY

In 2013, MFWP and MSU initiated a collaborative 6-year research program designed to assess factors driving bighorn sheep population dynamics across Montana. The integrated study design entailed using standardized methods to investigate demographic rates, body condition and nutrition, respiratory pathogens, movements, habitat use, and population attributes across a diverse set of populations occupying a diverse set of landscapes. Similar designs have proven efficient at producing reliable and generalizable findings useful for management agencies. Accordingly, populations included in this research program were carefully selected by MFWP regional wildlife managers to capture varying respiratory disease histories, habitat types, management histories, as well as demographic performances. The 8 primary bighorn sheep populations incorporated into this study occupy portions of Deer Lodge, Fergus, Lewis & Clark, Madison, Missoula, Phillips, Sanders, Stillwater and Teton Counties, as well as the Flathead Indian Reservation. In recognition of the improved inference associated with incorporation of additional study populations, this research program also incorporated data from a companion MSU bighorn sheep study (Greater Yellowstone Area Mountain Ungulate Project), worked with the MFWP Wildlife Health Laboratory to incorporate data from additional populations captured for health monitoring purposes, and collaborated with Wyoming Game & Fish Department (WGF) to develop sampling methods that are comparable across states. There were five focal objectives of the integrated research project: 1) Describing Respiratory Pathogen Communities and Associated Population Performance, 2) Begin Development of a Health Panel for Bighorn Sheep, 3) Understanding Movements, Migratory Strategies, and Development of Habitat Models, 4) Evaluating the Genetic Attributes of Bighorn Sheep Populations, and 5) Understanding the Demographic Performance of Populations. This document represents the final report for this research project.

RESPIRATORY PATHOGEN COMMUNITIES & POPULATION PERFORMANCE

Respiratory disease has been a persistent problem for recovery of bighorn sheep in North America. The severity of respiratory disease epizootics has been variable, ranging from 30% to 90% mortality in affected populations. The epizootics often involve an extended phase where a high percentage of juveniles die from respiratory disease within four months of birth, however, the duration of this phase is also extremely variable, lasting from a single year to decades of poor recruitment. Our understanding of respiratory disease in bighorn sheep is limited, but a number of bacterial pathogens have been implicated as causing respiratory disease in bighorn sheep including *Mycoplasma ovipneumoniae* and several species within the Pasteurellaceae family. All bighorn sheep captured during management and research operations are tested for the presence of these pathogens, but interpretation of test results has been problematic as there has been no rigorous assessment of the reliability of the various diagnostic tests routinely employed.

We performed 2,093 standard culture-based Pasteurellaceae diagnostic tests and 768 PCR-based *M. ovipneumoniae* diagnostic tests on samples collected from approximately 470 bighorn sheep to gain insight into interpreting the pathogen data routinely collected by the agency. We found that PCR-based protocols improved detection of pathogens as compared to culture-based protocols. However, none of the diagnostic protocols were perfect, with estimates of the probability of detection when an animal was known to be infected ranging from approximately 30% to 70%, depending on the pathogen and protocol used. This finding means that the diagnostic reports of pathogens obtained from testing of captured animals are strongly biased unless detection rates of the diagnostic protocols are accounted for, as well as the intensity of sampling of both individuals and populations. To aid managers in interpreting pathogen test results we developed an app, readily available on the internet, that performs rigorous evaluations of pathogen tests, thus, producing more accurate results to inform decision-making. Using this improved interpretation of pathogen tests, we found that all bighorn sheep populations across Montana and Wyoming host 1 or more species of the Pasteurellaceae pathogens and approximately 80% of the herds tested also were infected with *M. ovipneumoniae*. Across the populations where *M. ovipneumoniae* was detected, mean lamb recruitment rates were lower, suggesting this pathogen plays an important role in respiratory disease. However, many populations where both *M. ovipneumoniae* and Pasteurellaceae were detected had average to good recruitment rates, indicating populations may be demographically robust while hosting all respiratory pathogens.

We concluded that the simple presence of any pathogen, or suite of pathogens, in a bighorn herd did not predict the demographic performance of the population and that multiple factors are likely interacting between the animals, the pathogens, and the environment to influence the expression of disease in infected bighorn populations. Understanding respiratory disease in bighorn sheep thus will remain a daunting challenge; however, there is strong evidence that some herds are resilient to infection with respiratory pathogens and can remain demographically robust, suggesting that strategies can be developed for successful management of bighorn sheep despite the near ubiquitous presence of the pathogens.

Development of a Health Panel for Bighorn Sheep

The quantity and quality of forage available to bighorn sheep on their ranges dictates their nutrition and body condition, which, in turn, influences survival and reproduction. Thus, there is a need to evaluate potential bottom-up (i.e., habitat) drivers of bighorn population dynamics. The evaluation of nutritional status across populations with varying demographic characteristics may provide insights as to the extent nutrition explains variation in demographic rates and to the potential associated of nutrition with expression of respiratory disease. Wildlife managers have limited tools to assess the nutrition and physiological state of bighorn sheep and other wild ungulates which limits our ability to understand links between habitat conditions and the health and demographic vigor of populations. The objective of this portion of our studies was to explore the potential of the emerging field of metabolomics to begin developing a tool to rigorously and quantitatively assess the physiological and nutritional state of bighorn sheep. Metabolomics is the study of metabolic intermediates and products of cellular metabolism, with the catalyst for this emerging technology the development of sophisticated new analytical machines that permit efficient and precise quantitative estimates of many 10s to 100s of biological molecules from less than 1 mL of serum or plasma. Metabolomics is a rapidly expanding research field that is being aggressively pursued in the fields of domestic animal production and human medicine and has great potential to aid wildlife managers.

To perform this study, we assembled a team of scientists with complimentary expertise from 3 additional academic departments on the MSU-Bozeman campus and gained access to the Nuclear Magnetic Resonance (NMR) Center in the Department of Chemistry and Biochemistry that provided access to the machines for performing the assays. This team refined sample processing and NMR protocols and built a library of biological molecules that could be accurately identified and quantified. NMR assays were completed on serum samples from 562 bighorn sheep captured from 14 wild bighorn sheep populations in Montana and Wyoming. In addition, we assayed serum samples from 2 captive bighorn sheep research facilities and a small experimental flock of domestic sheep that were kept on a maintenance diet. Combined these samples represented animals experiencing a gradient in dietary intake and degree and duration of starvation. The samples were split into 4 categories (high, moderate, low, and very low) to reflect this gradient in nutrition and the results of assays were analyzed using several machine-learning statistical techniques to determine how well the metabolomics data could discriminate among the nutritional categories. The results indicated strong discrimination among the nutrition categories with approximately 10 of the 57 biological molecules quantified contributing most of the information. These biomarkers are associated with a variety of physiological processes and are good candidates for incorporation into the development of a 'health panel' that could be economically produced from serum samples routinely collected from captured animals and readily interpreted by wildlife managers to understand the nutritional status, disease, and physiological stresses on bighorn sheep populations. We anticipate that such a health panel, if successfully developed, would likely have similar utility for other wild ungulates.

Movements, Migratory Strategies, & Habitat Models

Animal movements across landscapes and their use of habitats can directly influence vital rates and demographic performance. Understanding movement patterns and habitat use can, therefore, be valuable for informing management and restoration decisions, particularly given the diversity of management histories and landscapes occupied by bighorn sheep across Montana. We placed GPS collars on a total of 218 adult female bighorn sheep across the 8 study populations in Montana to acquire continuous fine spatial and temporal scale location data for each animal over approximately an 18-month period. This effort resulted in a total of 643,431 GPS locations, averaging about 71,492 locations per population. To gain additional insight on bighorn movement ecology, we also incorporated a large GPS data set from Wyoming bighorn herds in the Greater Yellowstone area for some of our studies.

The data demonstrated that both of the restored populations occupying prairie breaks landscapes in eastern Montana were non-migratory and 2 of the restored populations occupying mountainous landscapes in western Montana were also non-migratory. In contrast all 3 of the native Montana study populations occupying mountainous landscapes were seasonally migratory, occupying low elevation foothills in winter and high-elevation portions of the mountains in summer. Only 1 study population restored into a Montana mountain range was found to have developed seasonal migratory behaviors. We found little individual variation within the populations studied in Montana, that is, all or nearly all of the instrumented animals displayed the same seasonal movement behaviors. The data from the Wyoming herds provide insight on what may have been the traditional seasonal movement behaviors of bighorn sheep occupying the mountainous regions of Montana before overexploitation and disease resulted in extirpation or severe range restriction and reduction in numbers. The Wyoming herds occupy the rugged mountains along the eastern border of Yellowstone National Park, were never extirpated or augmented, and represent the largest (4000-5000 animals) continuously distributed populations remaining in the species continental range. Movement data collected from these animals demonstrate much greater individual variation in seasonal movements than the restored and augmented Montana herds studied, with animals within the same populations displaying a diversity of behaviors from non-migratory, to short-distance elevational seasonal migrations, to long-distance migrations over a number of drainages. The limited migratory diversity in restored and augmented populations may be an additional limiting factor to demographic performance and range expansion. Preserving native migrations and matching migratory patterns of source populations with local landscape attributes for restoring and augmenting populations may improve success of broad restoration efforts and increase the resiliency of populations to perturbations and increase resilience to contagious respiratory disease.

The movement data from the 4 Montana seasonally migratory populations also documented that many of the bighorn sheep performed rapid summer migrations from high elevation seasonal ranges back to specific areas on low elevation winter range, returning to summer range within a few days. Similar movements were documented in the Wyoming herds. Assessment of these localized areas visited by the Montana animals indicate that these movements are being made to natural and anthropogenic 'lick' sites to alleviate essential trace minerals deficiencies of natural forage on high-elevation summer ranges. Assays of lick site soils indicate that the primary trace minerals the animals are seeking are calcium, magnesium, and sodium. These licks sites are an important habitat attribute for bighorn sheep populations that are likely required to maintain animal health and population demographic vigor. Wildlife managers should consider mapping and inventorying mineral licks, both natural and anthropogenic, used by bighorn sheep and share this information with land management agencies to aid in conserving and maintaining important movement corridors. When reintroducing bighorn sheep to new areas, the consideration of establishing specially formulated artificial licks (i.e. salt blocks) where the bighorn sheep summer, could help ensure they have access to trace minerals. This would also eliminate the potential risk of animals making long forays in an unknown landscape and leaving the desired reintroduction area when searching for sources of trace minerals during periods of deficiencies.

The extensive movement data collected during our studies also provided an opportunity to develop habitat models for both the mountainous and prairie regions of the state using state-of-the-art analytical techniques. Prior to our studies the agency had no habitat models specifically for prairie environments and the habitat model currently used to inform management actions is based on location data collected during population inventory surveys which has a number of limitations. The models developed for mountainous landscapes revealed that there is significant unoccupied habitat adjacent to many of Montana's restored populations suggesting significant potential for intra-mountain restoration exists that could expand the distribution and abundance of populations to help secure their viability into the future. Our habitat models also provide managers with a useful tool for identifying future translocation sites in currently vacant mountain ranges that historically supported bighorn sheep if additional restoration efforts are desirable. Behavior-specific habitat modeling suggest that it may be worthwhile to consider future restoration experiments in appropriate montane environments of western Montana to attempt to establish diverse seasonal movement strategies by using multiple source populations with differing movement behaviors when establishing new populations. Habitat modeling in the prairie environments of eastern Montana indicate that considerable restoration opportunities also exist both within and outside of the recognized historic range of bighorn sheep in eastern Montana. Most of the currently unoccupied areas of eastern Montana with high restoration potential, however, exists within a mosaic of private and public lands that will require the development of collaborative partnerships between wildlife and land management agencies and private land owners.

GENETIC ATTRIBUTES OF BIGHORN SHEEP POPULATIONS

Genetic studies were added to the Montana bighorn sheep study project in 2016 as an integral component of a comprehensive research program to address potential limiting factors in bighorn sheep restoration, conservation, and management. For example, genetic consequences of inbreeding in small populations can impact recruitment, and local adaptations, and can influence translocation success. Comparing genetics of different bighorn sheep populations can also provide information to describe genetic connectivity and diversity of examined populations, as well as discover links between population demography and genetics. Genetic data may also serve to inform evaluation of genetic diversity in current or previously small populations, aid in selection of potential source populations for augmentation or reestablishment projects, determine what populations have low genetic diversity and might benefit from augmentation, and discover what populations are genetically unique.

The first objective of our genomics studies was to determine the optimal number of animals to genotype in each bighorn population as the literature provides little insight on this issue. This is an important question as sample size may impact genetic inference, as genetic uniqueness, genetic distance, and inbreeding could be assessed differently, depending on the sampling scheme and the total number of bighorn sheep evaluated. Determining an optimal sample size per herd also assures maximizing genetic insight at the most economical costs associated with genetic sample processing and analysis. To address this objective, we used modern genomics techniques that provided information on 6,155 to 33,289 genetic markers to genotype 30 individuals from each of 4 different populations that we predicted would differ in genetic characteristics due to population dissimilarities that included origin (native/reintroduced), population size, bottleneck history, degree of connectivity, and augmentation history. Simulations of different size samples using this dataset determined a sample size of 20-25 bighorn sheep per population is adequate for assessing the key genetic metrics of interest for addressing management questions. Based on this insight, we then genotyped samples from 17 bighorn herds, limiting the total number of samples genotyped per herd to 25 for those herds where a larger number of samples were available. A total of 511 bighorn sheep were genotyped, representing native and reintroduced populations that received 0-10 translocations.

Using this large data set we identified 5 unique genomic groups that could be characterized primarily based on geographic proximity and translocation history. These groups included 3 native population clusters including animals occupying eastern and northern portions of Glacier National Park, the 2 populations occupying the Madison Range in southwestern Montana, and the bighorn found in the Beartooth and adjacent Absaroka Range complex along the northern and eastern borders of Yellowstone National Park. A fourth unique genomic group was comprised of the native population occupying the Sun River region of Montana's Rocky Mountain Front and a complex of restored populations that originated from translocations from the Sun River herd or received significant augmentations from this native herd. The fifth unique genomic group was represented by the Wild Horse Island population established from translocations from multiple source populations and a restored population in northwestern Montana that was established from Wild Horse Island animals.

Given the unique signatures of the 5 distinct genomic groups identified in Montana, we could evaluate the success of 24 different translocation events where both populations were included in the study. Eight reintroduced populations with founding source data confirmed that the contemporary population originated from the founding animals. Fifteen of the translocations were augmentations, including 11 unique pairs of source and recipient populations. Four out of 11 augmentation pairs could not be assessed for genetic contribution, as the source population was the same as or genetically similar to the founding source. Of the remaining 7 source and recipient augmentation pairs, we detected the genetic signatures of 5 past augmentations that each consisted of 18 to 57 animals, indicating that the translocated animals survived and successfully contributed progeny to the recipient population. The 2 genetically undetected augmentations each consisted of 2 males, suggesting that the translocated animals dispersed, did not survive to reproduce, or were outcompeted for mating opportunities by resident animals. Literature addressing success of translocations provides evidence that it may be important to match the environmental conditions of the source population with that of the area they are translocated into, however, data from our study found that animals translocated from the Sun River population successfully founded populations in multiple ecological regions of the state including the arid prairie breaks habitats of eastern Montana. This result suggests that matching environmental conditions may not be required for populations to persist following reintroduction, at least across the range of environmental conditions realized in Montana.

Our assessment of relatedness among individuals within sampled populations indicated that this metric was below the suggested threshold of concern, suggesting that inbreeding in these populations was not high enough to negatively affect population vigor. We also did not observe a relationship between average relatedness among individuals within sampled populations and median juvenile survival at the population level, supporting the conclusion that inbreeding depression is not suppressing population growth in the examined populations. Analysis of potential predictors of relatedness among individuals within a population indicated that greater gene flow, from population connectivity and animals received in augmentations, were more important predictors of genetic diversity than historic minimum count and whether the population was native or restored.

DEMOGRAPHIC PERFORMANCE OF POPULATIONS

Accurate estimates of population size and demographic vital rates of wildlife populations are fundamental to guiding management actions because they elucidate demographic health and can help inform the prediction of future population dynamics. Population growth is explicitly described by several vital rates: adult survival, fecundity (pregnancy), juvenile survival, and in some populations immigration, and emigration. Reliable estimates of these vital rates allow for inferences regarding population growth or decline independently from the use of sequential population counts which are normally fraught with issues related to imperfect and variable animal detection rates during surveys. Knowledge of the relative contribution of different vital rates to dynamics of wildlife populations is imperative to identifying mechanistic drivers of population dynamics. Accordingly, accurate estimates of vital rates are fundamental for both effective research to gain ecological insight and for implementing management programs of wildlife populations. An important objective of the Montana bighorn sheep study was to collaborate with area biologists to estimate population size, adult female survival and pregnancy rates, and annual recruitment.

A total of 218 adult females from the 8 core Montana study populations were radio-collared and monitored for survival. In addition to these populations, we also incorporated data from the survival monitoring for instrumented female bighorn sheep in the Upper Yellowstone population complex, the Highland, and Spanish Peaks populations performed by area biologists. The pooled annual survival rates for the Petty Creek, Fergus, and Hilgard populations were high, ranging from 0.90 to 0.97. Pooled survival estimates for Lost Creek, Stillwater, Middle Missouri, and Highland populations were intermediate, ranging between 0.86 and 0.88. Pooled survival rates were relatively low for the Paradise, and Castle Reef herds, ranging between 0.80 to 0.82, suggesting adult females in these 2 populations experience mortality rates which can result in weaker overall demographic performance. While survival estimates for the Spanish Peaks and Upper Yellowstone populations were relatively low, the modest sample sizes resulted in considerable uncertainty in these estimates, indicating caution in interpreting the point estimates.

Pregnancy rates were determined via serum assays using blood collected from captured adult females and indicated that for most study populations pregnancy rates were very high, generally exceeding 0.90. These results corroborate findings from previous studies that bighorn sheep pregnancy rates are likely not an important factor limiting lamb recruitment. Despite the evidence for overall high pregnancy rates, some data indicated potentially lower pregnancy rates occurred in a few populations and in some years that could have the potential to dampen demographic performance of populations. The causes for the lower pregnancy rates are uncertain, but the most likely explanation is poor nutrition driven by years when weather conditions resulted in poor annual production of forages.

Recruitment rates were indexed by lamb:ewe ratios obtained by area biologists as part of their routine population monitoring surveys. As is typical for large ungulate populations, the age-sex classification surveys documented substantial annual variation in recruitment rates for all populations included in the statewide study. The 2 populations with the lowest recruitment rates experienced a pneumonia epizootic during the winter of 2010, however, lamb:ewe ratios in both of these populations improved (>0.30) in the latter years of the study, suggesting recruitment in both populations may be returning to more typical rates experienced in the populations prior to the disease events.

The observed variation in herd-specific vital rates (survival, pregnancy, recruitment) were incorporated into a population model that integrates with routine survey and classification data collected by management biologists. The model has important advantages for the informed management of populations of bighorn sheep, including the ability to incorporate the best-available information from other studies, the ability to estimate and understand the drivers of population growth rates, and the ability to assess sources of variation in vital rates. Model results indicated the majority of populations in this study had low growth rates, and we identified key relationships between lamb and adult ewe survival that may be limiting growth rates. The drivers of changes in population trajectories demonstrated substantial variation among populations such that it is difficult to generalize which vital rate should best be targeted for management interventions to enhance demographic vigor. The model results also demonstrated the substantial role that environmental conditions can play in driving lamb survival; however, the relationships between environmental covariates and lamb survival varied among populations. Combined, these results indicated that the dynamics of the diverse bighorn populations in Montana vary substantially among populations and understanding these dynamics will be dependent on the routine herd-specific monitoring performed by area biologists. Stronger ecological insight for informing management may be gained by considering enhancing monitoring protocols for at least some of the most important bighorn populations in the state.


Photo: Cindy Goeddel

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PROJECT BACKGROUND

THE HISTORY of bighorn sheep (*Ovis canadensis)* conservation shares many similarities with the conservation history of other North American ungulates, but is also quite distinctive. Similar to other ungulates, bighorn sheep existed in continuous and broadly distributed populations and likely numbered 1-2 million prior to colonization of western North America. Following settlement of western North America by Euro-Americans, bighorn sheep and other ungulate species experienced drastic reductions in numbers and extirpation from much of their former range, which prompted a dedicated restoration effort by wildlife management agencies throughout the 20th century. This effort was successful in recovering most ungulate species back from perilously low populations (Picton and Lonner 2008). Restoration efforts of most ungulates entailed regulating harvest, protecting habitat, and translocating animals to facilitate colonization of previously occupied habitat; a prescription that has been successful to the point that wildlife managers are now challenged by conflicts between broadly distributed and abundant wildlife populations and humans. However, such issues are rarely described as challenges for bighorn sheep management.

There are currently estimated to be approximately 80,000 wild bighorn sheep in North America, representing a four-fold increase compared to the beginning of restoration efforts, but still likely at least a ten-fold decrease from historic numbers (Buechner 1960, Toweill and Geist 1999). The total population of bighorn sheep in North America is the sum of hundreds of patchily distributed individual populations. In Montana, most populations are isolated and number less than 150 animals (Butler, Garrott and Rotella 2013) and this pattern has been described across their range (Berger 1990). This stands in contrast to the comparatively continuous distribution of other ungulates such as deer, elk and antelope. The most obvious factor hindering further bighorn sheep restoration is continued, widespread expression of respiratory disease. However, high predation rates, habitat loss, poor genetic diversity, and "unique factors" are also cited as factors limiting bighorn sheep populations (Festa-Bianchet *et al.* 2006, Hogg *et al.* 2006, Johnson *et al* 2010). Given multiple potential limiting factors, managers often face difficult decisions regarding bighorn sheep conservation with insufficient information on the drivers of demographic processes. The small size of many populations makes management decisions even more challenging by heightening the consequences of these decisions. However, there still exist numerous populations that, for unknown but presumably tangible reasons, are well distributed, robust, and require minimal management intervention. Thus, additional information regarding general bighorn sheep ecology would be useful for management agencies to have more confidence in predicting outcomes of different management actions.

As an initial start to establishing a statewide bighorn sheep research project, Montana Fish, Wildlife and Parks (MFWP) supported a six-month contract to Montana State University (MSU) during fiscal year 2012/2013 to consolidate all population-specific bighorn sheep classification data into a single standardized database and analyze these data to learn as much as possible from existing data routinely collected by area biologists (Butler, Garrott, and Rotella 2013). This effort revealed substantial variation in population size and annual recruitment rates (as indexed by lamb:ewe ratios) among populations as well as within each population through time, even after accounting for numerous weather metrics and respiratory disease epizootics. Further, the report's findings suggested population-specific responses of bighorn sheep recruitment to annual weather variability. Collectively, the report indicated there is much to be learned about the factors that drive bighorn sheep demographic rates and accordingly, much to be learned about potential management strategies that can be used to influence demographic rates in desirable ways.

In 2013, MFWP and MSU initiated a collaborative six-year research program designed to assess factors driving bighorn sheep population dynamics across Montana. The integrated study design (Figure 1) entailed using standardized methods to investigate demographic rates, body condition and nutrition, respiratory pathogens, movements, habitat use, and population attributes across a diverse set of populations occupying a diverse set of landscapes. Similar designs have proven efficient at producing reliable and generalizable findings useful for management agencies. In recognition of the improved inference associated with incorporation of additional study populations, this research program has done the following: incorporated data from a companion MSU bighorn sheep study (Greater Yellowstone Area Mountain Ungulate Project), worked with the MFWP Wildlife Health Laboratory to incorporate data from additional populations captured for health monitoring purposes, and collaborated with Wyoming Game & Fish Department (WGF) to develop sampling methods that are comparable across states. This project has greatly benefited from inclusion of these parties in the research project. This document

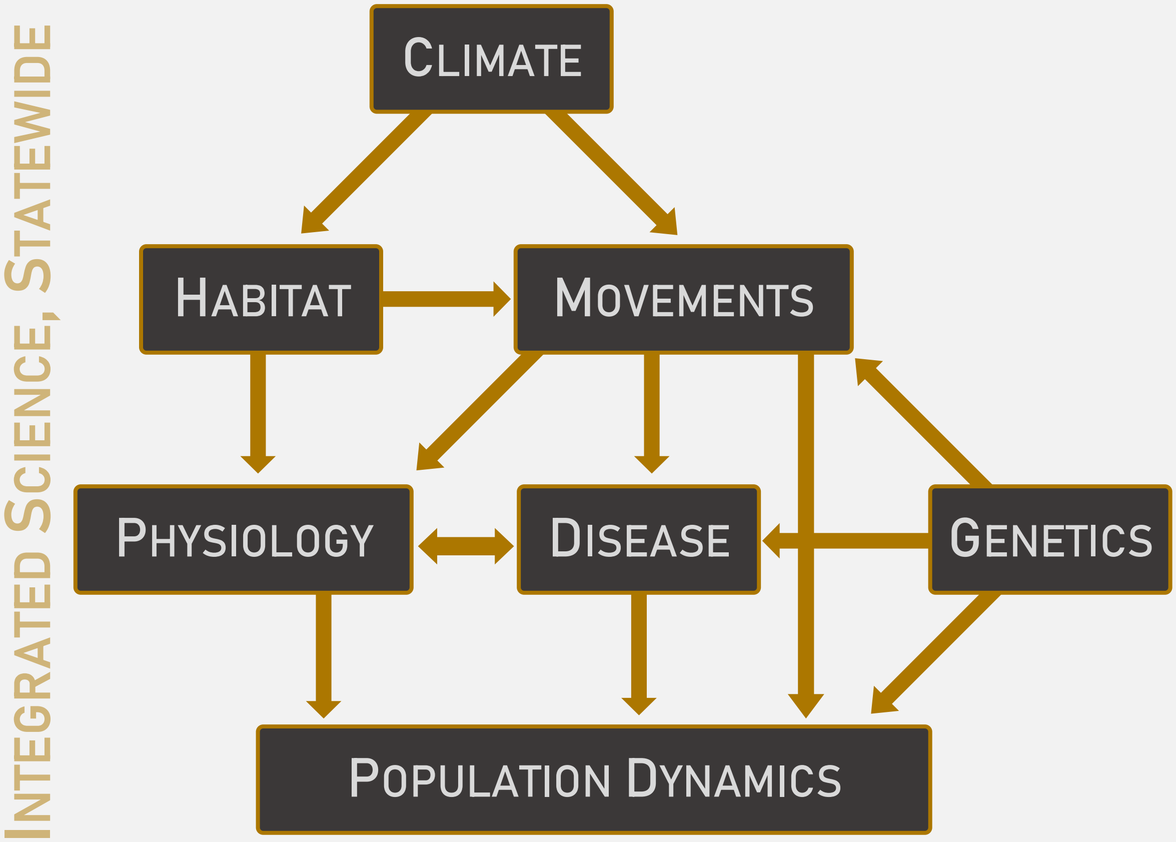


Figure 1 - The integrated study design of the Montana Bighorn Sheep Research program and the Greater Yellowstone Area Mountain Ungulate Project, which is led by the same core research team. Data from both research programs were combined where appropriate to provide stronger inference.

represents the final report for this research project. We provide a list of products completed by the project (in Chapter 7 and at the close of each respective chapter), including manuscripts currently in review or in press with appropriate scientific journals.

Research conducted under this grant was primarily focused within the range of 8 distinct bighorn sheep populations across varying ecological settings in Montana (Chapter 1: Figure 2). Bighorn sheep populations incorporated into this study occupy portions of Deer Lodge, Fergus, Lewis & Clark, Madison, Missoula, Phillips, Sanders, Stillwater and Teton Counties, as well as the Flathead Indian Reservation. Data were also incorporated from ancillary populations to strengthen biological insights and enhance the utility of the study to inform management across all populations within the state. Ancillary Montana bighorn sheep populations include Wild Horse Island, Glacier National Park, the Tendoy Mountains (HD 315), the Highlands (HD 340), Galton (HD 102) and the Spanish Peaks (HD 301). For some aspects of our studies we have also incorporated data from bighorn sheep populations in Wyoming, Idaho, and Colorado.

Photo: Cindy Goeddel

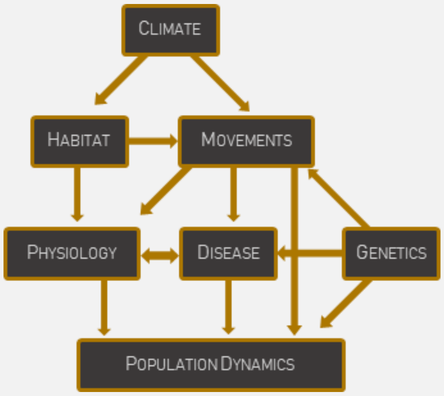
PROJECT OBJECTIVES

THE PRIMARY objectives of the project were to:

1) Complete capture, sampling, and instrumentation of bighorn sheep in all study populations (Chapter 1)
2) Assess respiratory pathogen communities and associations with demographic performance of bighorn sheep populations (Chapter 2)
3) Develop a health panel for bighorn sheep based on metabolomics (Chapter 3)
4) Analyze GPS data to evaluate bighorn sheep movement strategies and predict habitat (Chapter 4)
5) Evaluate sample size requirements for understanding genomics of bighorn sheep and assess the genomic consequences of translocations in Montana populations (Chapter 5)
6) Estimate demographic rates and evaluate correlates of demographic rates of each bighorn sheep population in the statewide study (Chapter 6)

CHAPTER 1

CAPTURE, SAMPLING, & INSTRUMENTATION



Objective Describe the capture, collection of biological samples, and radio collar instrumentation of adult bighorn sheep from 8 populations across Montana, and provide details on the background and sampling accomplishments for each population. The data obtained from the sampling and collaring formed the foundation of the research project and provided insight into the factors driving bighorn sheep population dynamics across the diverse populations and landscape settings in Montana.

AN IMPORTANT principle underlying this research program is that inferences obtained from research are most broadly applicable to wildlife management needs by addressing the same questions in multiple wildlife populations occupying different ecological settings. Accordingly, populations included in this research program were carefully selected by MFWP regional wildlife managers to capture varying respiratory disease histories, habitat types, management histories, as well as demographic performances.

All captures occurred during winter months from 2013 to 2018. Animals were captured using 3 different methods including helicopter net-gunning (performed by Quicksilver Air Inc.), drop-netting, and chemical immobilization using B.A.M. All capture and handling procedures followed protocols approved by the MSU Institutional Animal Care and Use Committee (permit nos. 2011-17, 2014-32, 2016-6, & 2017-29).

A series of measurements and samples were taken from each animal captured. Sex was determined based on genitalia and age was estimated using incisor eruption patterns (Hemming 1969). Age was classified as 0.5, 1.5, 2.5, and >3.5 years. Thirty-five mL of blood was drawn from the jugular vein. Nasal swabs, tonsil swabs, and fecal samples were also collected. Lactation of adult females was assessed by palpating the teats. Ultrasonography was used to measure subcutaneous rump fat thickness of adult females and body condition was also assessed using skeletal palpation methods (Stephenson et al. 2020). Additionally, weight and hind foot length (Zannèse et al. 2006, Garel et al. 2010) were measured for all adult females. A sample of up to 30 adult females in each population were instrumented with paired GPS/VHF radio-collars (Telonics TGW4400 [GPS] and MOD400 [VHF]). In some cases, individuals were instrumented with only a remote-upload GPS collar (Lotek LifeCycle Pro 330 on the Globalstar network and Telonics TGW4570-12 on the Iridium network) programmed to transmit locations and mortality alerts to researchers and managers every 2 days for approximately 5 years.

We completed capture, sampling, and instrumentation efforts in 2018 and met the broader study sampling objectives. Descriptions and sampling accomplishments of the 8 study

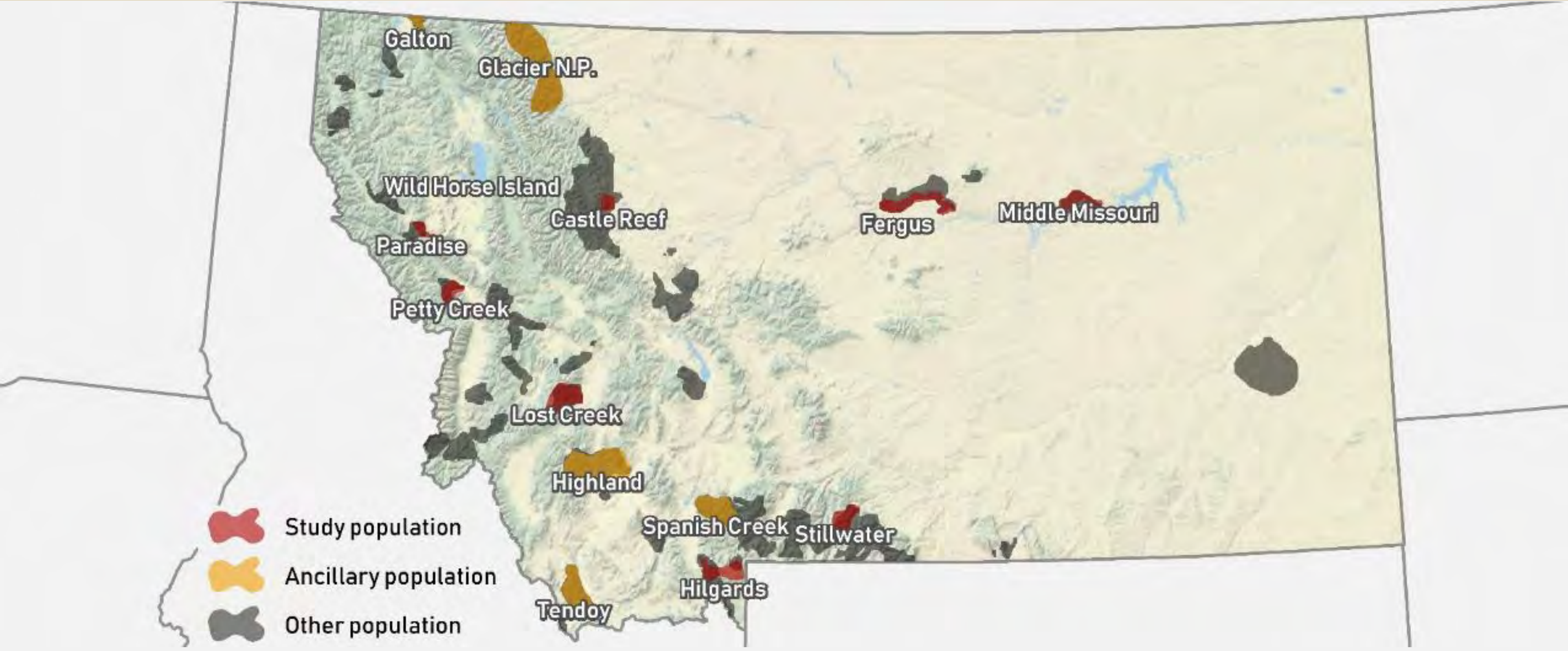


Figure 2 - Estimated distributions of the 8 study populations (red polygons) in the Montana bighorn sheep study. Ancillary bighorn sheep populations (yellow polygons) were sampled opportunistically as parts of other bighorn sheep studies, and other bighorn sheep populations (grey polygons) are the remaining bighorn sheep populations not part of this research effort.

populations, as relevant to the above characteristics, are outlined below (Figure 2, Table 1).

PARADISE

This population, also known as Perma-Paradise, is located in hunting district (HD) 124 of northwestern Montana in the Northwest Montane ecoregion. The population was established with a reintroduction of 14 animals from Wild Horse Island in 1979 and was augmented with 22 animals from Wild Horse Island in 2011. Across the past 5 years, the minimum count average was 262 animals. The population experiences moderate recruitment in most years, and is believed to be isolated from other bighorn sheep populations. There is no known history of respiratory disease in this population.

In December 2014, 30 adult females were captured and sampled, with 15 paired GPS/VHF collars deployed. The population was resampled in December 2016 where 30 animals were captured, with 10 paired GPS/VHF collars deployed.

PETTY CREEK

Also known as the Grave Creek Range population, this population is located in HD 203 of western Montana in the Northwest Montane ecoregion. The population was established with an initial reintroduction of 16 animals from the Sun River in 1968 and received a small augmentation in 1985. Across the past 5 years, the minimum count average was 141 animals. The population is thought to be isolated from other populations. The population typically experiences strong annual recruitment rates. There is no known history of respiratory disease in this population.

Following unsuccessful capture attempts in Winter 2014/2015, 17 adult females were captured and sampled in February 2016, with 15 paired GPS/VHF collars deployed. The

Table 1 - Sampling accomplishments in each study population, 2013 - 2018.

	BIGHORN SHEEP SAMPLED						RADIO-COLLARED EWES
	2013/ 2014	2014/ 2015	2015/ 2016	2016/ 2017	2017/ 2018	TOTAL	TOTAL COLLARED
Paradise		30	0	30	0	60	25
Petty Creek		0	17	0	21	38	24
Lost Creek		13	6	24	0	43	27
Hilgard*	29	50	35	31	32	177	32
Castle Reef		23	7	38	0	68	29
Fergus**		60	0	30	0	90	40
Stillwater		16	3	11	1	31	21
Middle Missouri			19	20	0	39	20
TOTAL	29	192	87	184	54	546	218

** Collar total does not include 5 rams captured 2012/2013 or 27 ewes collared as part of MFWP translocations. Increased number sampled and radio-collared resulted from collaboration with MFWP.*

*** Increased number sampled and radio-collared resulted from collaboration with the Hells Canyon Initiative.*

population was resampled in November 2017 where 21 animals were captured, with 9 paired GPS/VHF collars deployed.

HILGARD

Also known as the Taylor Hilgard population, this native population is located in HD 302 of southwestern Montana within the Mountain Foothills ecoregion. The population was augmented on 3 occasions during the late 1980s and early 1990s due to concerns over low numbers after a respiratory disease event in 1987. A 2nd major disease-related mortality event occurred in 1997, but the population experienced a robust recovery without management intervention. Across the past 5 years, the minimum count average was 185 animals. The population is believed to be isolated from other bighorn sheep populations and has strong annual recruitment in recent years.

Sampling and collaring of the Hilgard population was enhanced beyond the original research objectives. Just prior to the initiation of this study in winter 2011/12, MFWP instrumented 5 adult females and 5 mature rams with VHF collars that have been incorporated into the demographic studies. In addition to our research, capture and sampling of 29 animals in this population was completed during the winter of 2013/14, 52 animals were captured and translocated from the Hilgard population in winter 2014/2015, and data and samples that were contributed to the research program were collected from 50 of these animals.

Additional data from 2 supplementary translocations out of the Hilgard to adjacent winter ranges within the Madison Range in 2016 (35 animals) and 2018 (32 animals) was also incorporated into the study. Sampling goals were successfully achieved in 2016 and 10 adult females were instrumented with Iridium satellite-linked GPS collars. Additional animals were captured February 2017 and January 2018 to redeploy Iridium collars collected from earlier mortalities.

LOST CREEK

This population is located in HD 213 of southwestern Montana within the Mountain Foothills ecoregion. The population was established with a reintroduction in 1967 and was augmented in 1985. It is believed to be relatively isolated and traditionally has had high recruitment rates. The population has experienced 2 significant respiratory disease outbreaks, the most recent occurring in 2010. Across the past 5 years, the minimum count average was 58 animals.

In winter 2014/2015, 13 animals (12 adult females and 1 adult male) were captured and sampled. All females were fit with paired GPS/VHF collars, however, 2 of these animals died before winter 2015/2016. In December 2015, 5 adult females were captured, sampled, and instrumented with paired GPS/VHF collars. An additional adult female was captured and collared in March 2016, resulting in a total of 19 animals sampled and all 15 collars deployed. In December 2016, 24 additional animals were sampled and 9 adult females instrumented with paired GPS/VHF collars.

CASTLE REEF

This native population is located in HD 422 along the Rocky Mountain Front in the Prairie Mountain Foothills ecoregion of central Montana. The population received a single small augmentation in 1944 and experienced 3 respiratory disease outbreaks between 1924 and 1936, a 4th outbreak in 1984, and the most recent outbreak in 2010. Across the past 5 years, the minimum count average was 123 animals. The population is part of a metapopulation complex along the Rocky Mountain Front representing an aggregate total of approximately 380 animals. Recruitment was

historically moderate to high but dropped to low levels after the most recent respiratory disease event. From 2015-2018, recruitment has rebounded to moderate levels.

Twenty-three animals were captured and sampled winter 2014/2015, with 15 adult females instrumented with paired GPS/VHF collars and 1 with a VHF collar. An additional 3 animals were captured and sampled in December 2015 and 4 adult females were captured in March 2016 to redeploy 2 collars from animals that had died. In winter 2016/2017, 38 animals were captured and sampled, of which 10 adult females were fitted with Iridium-linked GPS collars and 1 with a paired GPS/VHF collar.

FERGUS

This restored population is located in HD 482 of east-central Montana on the south side of the Missouri River in the Prairie Breaks ecoregion. The population was established with a reintroduction in 1947, with 3 augmentations between 1959 and 1961, and the most recent augmentation occurring in 1980. This population consistently experiences high recruitment rates and is one of the largest populations in the state, with a minimum count averaged across 7 years (of which only 4 years of data was available) of 205 animals. There is free exchange of animals with the population (HD 680) on the north side of the Missouri River, creating a metapopulation approaching 1,000 animals with no known respiratory disease outbreaks since 1980.

Collaboration and coordination between MSU, MFWP, and the Hells Canyon Initiative (another collaborative bighorn sheep research program) allowed the Montana Bighorn Sheep Study to increase sampling effort in the Fergus population beyond project goals with minimal additional costs or effort. As a result of collaboration with the Hells Canyon Initiative, 15 additional VHF collars were deployed on adult females. This resulted in a total of 30 sampled animals in December 2014, of which 15 adult females were instrumented with paired GPS/VHF collars and 15 adult females were instrumented with VHF collars. In addition, concurrent with the research capture, 30 additional bighorn sheep were captured and translocated out of this population. Much of the same data and samples were collected from the translocated animals as were collected from the animals captured for the research project. Recapture sampling and instrumentation objectives were achieved in December 2016 with 30 captured animals, of which 10 were instrumented with paired GPS/VHF collars.

MIDDLE MISSOURI/LARB HILLS

This population is located in HD 622 of the Missouri River Breaks area in the Prairie-Breaks ecoregion of northeastern Montana. It was established with the reintroduction of 28 bighorn sheep in 1980. The population is composed of 2 distinct subpopulations thought to be linked by ram movement during the rutting season. The smaller portion of the population occupies typical Missouri River breaks habitat in the Mickey-Brandon Buttes area with the larger subpopulation occupying the Iron Stake Ridge/Larb Hills region distant from the breaks in prairie hills habitat. After establishment the population grew to >90 animals, but experienced an approximately 50% decline from 1997 to 2001. The cause of the decline was never determined, but disease and possibly poor nutrition were suspected. Since the die-off, the population has recovered and experiences high annual recruitment. Across the past 5 years, the average minimum count was 255 animals.

This population was included in the study in 2016 using surplus funds in order to enhance our understanding of bighorn sheep populations that utilize prairie habitat types. Only one population of this type (Fergus) was included in the original study plan despite the fact that some of the state's most robust bighorn sheep populations occupy prairie environments. The addition of the Middle Missouri/Larb hills population, along with Fergus population, provided the study with a dataset for the prairie habitats more comparable to the mountainous terrain associated with the other study populations.

Twenty animals were captured, sampled, and instrumented with paired GPS/VHF collars in December 2016. Prior to integration with this study, this population was sampled during the winter of 2015/2016 as part of the MFWP bighorn sheep disease monitoring program (n=19) and these sampling data were incorporated into various aspects of this project.

STILLWATER

This native population is located in HD 500 of south-central Montana within the Southern Mountains ecoregion. The population was augmented twice (1970, 1984). The population is believed to be small and relatively isolated and has moderate recruitment. Across the past 5 years, the minimum count was 73 animals. There are no known respiratory disease events in the population in recent times.

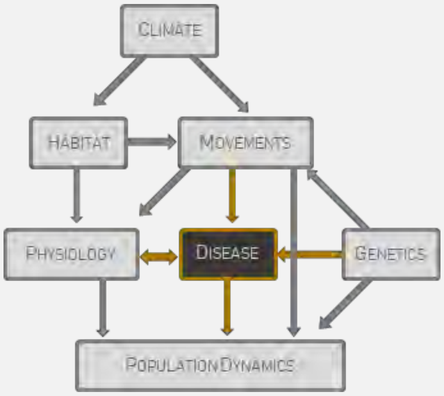
In winter 2014/2015, 16 adult females were captured and sampled, 15 of which were fit with paired GPS/VHF collars. In order to more closely reach the capture and sampling objective and redeploy a pair of GPS/VHF collars, which were originally deployed on an animal that died, 3 additional adult females were captured and sampled in December 2015 for a total of 19 animals sampled. Due to limited animal availability and logistical constraints associated with ground-based chemical immobilization, resampling goals were modified for the Stillwater population to capture and sample an additional 15 animals with 5 adult ewes fitted with paired GPS/VHF collars. In winter 2016/2017, 11 animals were sampled and all 5 pairs of GPS/VHF collars were successfully deployed. Two of these animals were subsequently recaptured to reprogram faulty collars, and an additional animal was captured in winter 2018 for genetic sampling.

Chapter Summary

- Captures occurred during winters 2013 – 2018 in 8 study populations across Montana, and all capture and sampling objectives were met for each population.
- 546 animals were sampled (i.e., blood draw, nasal swab, tonsil swab, fecal sample, lactation assessment, rump fat thickness, body condition, and skeletal measures).
- 218 animals were instrumented with GPS/VHF collars.

CHAPTER 2

RESPIRATORY PATHOGEN COMMUNITIES & POPULATION PERFORMANCE



Objectives Provide recommendations for sampling strategies that are needed to reliably characterize the presence of pathogens in populations. Assess respiratory pathogen communities in populations displaying a range of demographic performance to determine whether associations exist between pathogen communities hosted by each population and the population's demographic performance. Assess temporal variation in respiratory pathogen communities to understand how pathogen test results vary and how frequently populations should be tested for pathogens. Develop a tool to aid wildlife management agencies in interpreting respiratory pathogen test results.

RESPIRATORY DISEASE has been a persistent problem for recovery of bighorn sheep in North America. The severity of respiratory disease epizootics has been variable, ranging from 30% to 90% mortality in affected populations (Besser et al. 2013). The epizootics often involve an extended phase where a high percentage of juveniles die from respiratory disease within four months of birth, however, the duration of this phase is also extremely variable, lasting from a single year to decades of poor recruitment (Plowright et al. 2013). In numerous cases, local populations have gone extinct or have been depopulated after many years of chronically poor performance following respiratory disease epizootics (Montana Fish Wildlife and Parks 2010).

Anecdotal and experimental evidence suggests that domestic sheep (*Ovis aries*), and perhaps domestic goats (*Capra aegagrus hircus*), are likely the original source of the pathogen(s) responsible for respiratory disease in bighorn sheep. In captive animal experiments where bighorn sheep and domestic sheep were confined together for extended periods of time (up to 99 days), 98% of bighorn sheep developed respiratory disease and died (Besser et al. 2012a). While these experiments demonstrate the potential detrimental effects of commingling on bighorn sheep, the outcome of these experiments must be considered extreme as the domestic sheep and bighorn sheep were confined for extended periods of time in small enclosures. Commingling of free-roaming animals in rangeland and mountainous settings would likely be more ephemeral with less intensive than realized in the published commingling experiments, thus the high proportion of bighorn sheep that developed respiratory disease reported from these experiments should be interpreted with caution and may substantially overestimate the consequences of more ephemeral commingling events that would be expected in free-ranging animals.

Bacterial organisms belonging to the family Pasteurellaceae have long been implicated as important agents for respiratory disease in bighorn sheep, and recent experimental inoculation studies have shown that it is likely leukotoxigenic (lktA) Pasteurellaceae organisms, including strains of *Mannheimia haemolytica* and *Bibersteinia trehalosi*, which cause respiratory disease in captive bighorn sheep but not in domestic sheep (Dassanayake et al. 2009, 2010, 2013, Lawrence et al. 2010, Bavananthasivam et al. 2012). Epidemiologically, *Pasteurella multocida* has also been associated with bighorn sheep respiratory disease epizootics, though to a lesser degree (Besser et al. 2012b). Additionally, experimental and field evidence has emerged, providing strong evidence that the bacteria *Mycoplasma ovipneumoniae* plays an important role in causing respiratory disease epizootics in wild bighorn sheep populations (Besser et al. 2008, 2012a, 2012b) and that transmission of *M. ovipneumoniae* from asymptomatic domestic sheep to bighorn sheep is associated with development of respiratory disease in bighorn sheep (Besser et al. 2014).

The high mortality rate observed in bighorn sheep experimentally commingled with domestic sheep and goats represents, perhaps, the most consistent and repeatable finding related to respiratory disease in bighorn sheep. Accordingly, maintaining separation of wild bighorn sheep from domestic sheep and goats to avoid disease transmission is currently recognized as the primary tool management agencies use to reduce the probability of respiratory disease outbreaks (Brewer et al. 2014).

Although some proportion of epizootics have certainly been caused by introduction of novel pathogens into bighorn sheep populations, commonly referred to as a ‘spillover’ event (novel pathogen hypothesis), there are numerous examples of respiratory disease outbreaks in bighorn sheep populations where domestic sheep were not known to be in the vicinity (Festa-Bianchet 1988, Ryder et al. 1992, Edwards et al. 2010) and each of the pathogens which have been tied to bighorn sheep respiratory disease have also been detected in populations with little or no evidence of respiratory disease epizootics (Miller et al. 2011, 2012, Besser et al. 2013, H. Edwards unpublished data). These observations lead to an alternative hypothesis which posits that epizootics have also been triggered by pathogens already resident in a population (resident pathogen hypothesis), which turn virulent and/or increase in transmissibility under certain conditions and that carriage of these respiratory pathogens does not necessarily imply a diseased state for an individual or a population (Miller et al. 2012). Given the body of evidence that domestic sheep carry the pathogens responsible for bighorn sheep respiratory disease and transmit those pathogens to bighorns in captive studies, these “resident pathogens” in bighorn sheep populations likely originated from sympatric domestic sheep at some point since domestic sheep were introduced to western North America over a century ago. Distinguishing to what extent these alternative hypotheses (novel vs resident) explain respiratory disease expression would be a useful assessment because the management strategies to reduce disease expression caused by the 2 hypothesized mechanisms are very different.

Over the first 4 years of this statewide bighorn sheep research program, our efforts were focused on the development and implementation of rigorous assessments of respiratory pathogens communities hosted by bighorn sheep populations. This work is an extension of a collaboration between the MSU research team and personnel associated with the Wyoming Game and Fish Department’s Wildlife Health Laboratory as part of the Greater Yellowstone Area Mountain Ungulate Research Initiative that began in 2009. With the successful

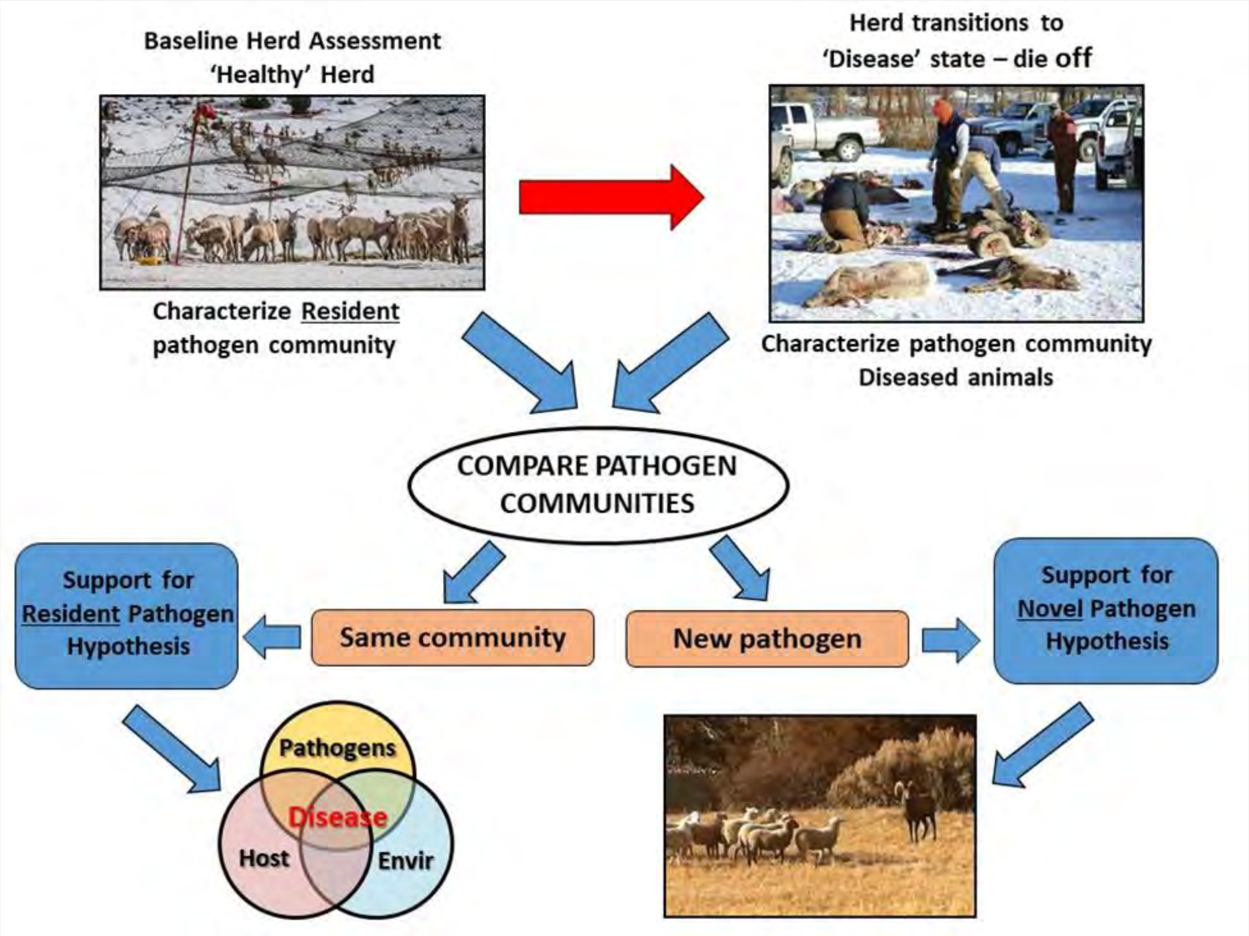


Figure 3 - A conceptual diagram of how rigorous assessments of the resident respiratory pathogen communities in bighorn sheep populations can help provide insight into competing ideas regarding the role of resident pathogens in epizootics (die-offs) as opposed to novel pathogens being introduced (spillover event) via interactions with domestic sheep and/or bighorn sheep from another population.

funding of the Montana bighorn project we extended our collaborations to include personnel associated with the MFWP Wildlife Health Laboratory and extend the geographic scope of the pathogen work to perform a regional assessment of the resident pathogens hosted by a sample of bighorn sheep populations throughout Montana and Wyoming.

The 1st objective of the regional study was a rigorous assessment of the various diagnostic protocols used to characterize respiratory pathogens in bighorn sheep. Specifically, we focused on assessing detection probability of the numerous diagnostic protocols used to identify the suite of respiratory pathogens of interest in order to provide recommendations to management agencies for sampling strategies needed to reliably characterize presence of pathogens. Reliable characterization of pathogen communities establishes a level of baseline information so that when asymptomatic populations that have been previously sampled become affected by respiratory disease, the pathogen communities before and during/after an epizootic can be compared to assess whether novel pathogens were introduced between healthy and diseased states (Figure 3).

The 2nd objective was an assessment of respiratory pathogen communities in regional bighorn sheep populations displaying a range of demographic performance to determine whether there were any associations between certain pathogen communities hosted by each population and the population's demographic performance. Lack of associations would suggest that respiratory disease can be managed without the onerous and perhaps unattainable task of eradicating pathogens, and would provide indirect evidence that disease expression can be caused by pathogens already present in a population. The 3rd objective was to assess the temporal variation in respiratory pathogen communities to understand how pathogen test results vary and how frequently populations should be tested for pathogens. The 4th objective was to develop a tool to aid in the interpretation of respiratory pathogen test results from protocols that have imperfect detection rates.

ASSESSING PATHOGEN DETECTION PROBABILITY AND INSIGHTS FOR SAMPLING

A total of 2,093 Pasteurellaceae diagnostic tests were conducted for 476 bighorn sheep and a total of 768 *M. ovipneumoniae* diagnostic tests were conducted for 469 bighorn sheep. Results from this effort were published in the peer-reviewed literature (Butler et al. 2017, 2018, Paterson et al. 2020). An abbreviated summary of the results of this work follows.

Conclusions from Pathogen Detection Studies:

1) Diagnostic protocols for all Pasteurellaceae available from commercial laboratories are based on successfully culturing bacteria from swabs and identification of colonies on the culture plates. All diagnostic protocols depending on culture have relatively low estimated detection probabilities (<50%). Low detection probability of these protocols may be due in large part to diminished viability of targeted organisms during the process of delivery to the laboratory rather than sensitivity of the diagnostic test itself (Wild and Miller 1994, Safaee et al. 2006). Nevertheless, this is a limitation whenever samples must be shipped to a laboratory for culture tests.

2) The PCR-based diagnostics protocols for Pasteurellaceae available from the Wyoming Game and Fish Department Wildlife Health Laboratory uniformly detected pathogens at higher rates than the culture-based protocol with estimated detection probabilities for *Mannheimia* sp., *B. trehalosi*, and *P. multocida* of 95%, 96%, and 83%, respectively. Estimated detection probability for *M. haemolytica* (45%), however, was only slightly better than culture-based protocols. The Wyoming laboratory does not offer commercial assay services and, to our knowledge, the PCR-based diagnostics protocols for Pasteurellaceae are not currently available from fee-for-service laboratories.

3) The estimated detection probability of the commercially-available PCR-based diagnostic protocol for detecting *M. ovipneumoniae* from nasal swabs was substantially higher (70-75%) than the culture-based protocols for Pasteurellaceae, but still far from perfect with one in four negative test results likely in error. The consequences of ignoring this detection probability can be illustrated by the suggestion in the literature that 'carriers' can be identified as animals that have tested positive for *M. ovipneumoniae* on 2 consecutive sampling occasions. If a sample of 100 consistently infected animals were tested 2 times, only ~53% would test positive both times (100 x (0.73 x 0.73)), ~7% (100 x (0.27 x 0.27)) would not test positive either time, and ~40% would test positive for 1 of the 2 sampling events (100 - (53 + 7)).

4) Low detection probability of Pasteurellaceae pathogens using fee-for-service culture-based protocols makes simple assessment of species presence at the population-level unreliable when species are at low prevalence and populations are not intensively sampled.

Although these specific findings apply to live-sampling bighorn sheep by swabbing the nasal cavity or tonsillar crypts, incongruent findings among studies investigating pathogen communities present in pneumonic and healthy lungs from the same respiratory disease epizootics (Besser et al. 2012b, Shanthalingam et al. 2014) suggest that detection error affects these assessments as well. Thus, an assessment of detection probability applied to the sampling of lung tissues is warranted.

5) Naïve (not accounting for imperfect detection) prevalence estimates of Pasteurellaceae pathogens are strongly biased when culture-based diagnostic protocols are used, unless protocols are conducted multiple times per animal. Given poor detection power and biased prevalence estimates, any true associations between the presence of Pasteurellaceae organisms and historic or current respiratory disease in bighorn sheep would likely be unobservable using these protocols.

6) High detection probability for *M. ovipneumoniae* likely leads to more consistent detection and less biased naïve prevalence estimates in bighorn sheep populations where it is resident.

7) The imperfect estimated detection probabilities of commercially-available protocols for all pathogens suggest that prevalence of any pathogen is estimated with poor precision unless intensive sampling is employed (i.e., many animals are sampled and protocols are conducted multiple times per animal). Therefore, variability in observed pathogen prevalence among different populations or different years within a population could be explained by either sampling variation or true variation in prevalence. Without accounting for differences in detection probability and sampling effort, differences in true prevalence remain unknown.

Recommendations to Improve Characterization of Resident Pathogen Communities in Bighorn Sheep Populations:

1) Encourage commercial laboratories to adopt PCR-based diagnostics for all respiratory pathogens of interest to enhance detection probability over the uniformly low detection (<50%) of culture-based diagnostics.

2) When employing the commercially-available culture-based pathogen diagnostic tests (currently all Pasteurellaceae), collect and assess 2 or 3 tonsil swabs from each live-sampled animal.

3) The presence of *P. multocida* should be assessed using nasal swabs as this pathogen was seldom detected from tonsil swabs.

4) PCR-based diagnostics for detecting the leukotoxin gene (lktA) should be employed on swabs or cultures from swabs from a minimum of 3-5 animals sampled from each population.

5) The use of a single nasal swab to assess presence of *M. ovipneumoniae* with the commercially available PCR-based diagnostic test is likely adequate when the goal is to determine if this pathogen is present in the sampled population (given an adequate number of animals from the population are sampled). However, if the goal is to determine if the pathogen is present in the individual sampled (e.g. identification of purported 'carriers'), the estimated 73% detection probability is not adequate without employing multiple swabs.

6) Exposure of sampled animals to *M. ovipneumoniae* should also be assessed by submitting a small volume of serum from each animal for a commercially available (WADDL) ELISA test to detect antibodies against *M. ovipneumoniae*. This less expensive antibody test could be substituted for the more costly PCR swab diagnostic test; however, we found it was not uncommon for animals with a positive nasal swab test to have a negative ELISA serum

test. Nasal swabs also provide the opportunity for more detailed genetic assessment (strain-typing) that cannot be performed using serum samples and is necessary to document the introduction of novel strains in populations that already host *M. ovipneumoniae*.

7) Simulations suggest that 30 to 35 animals need to be sampled from a bighorn sheep population to reliably assess (>80% power) presence of Pasteurellaceae pathogens and *M. ovipneumoniae* using the commercially available diagnostic tests currently available.

8) When a pathogen of interest is not detected in a population, information on the number of animals sampled, number of swabs assessed per animal, and estimated detection probability of the diagnostic protocol should be used to estimate the probability that the pathogen was present in the population, but remained undetected.

9) If prevalence of a pathogen in a sampled population is of interest, the uncertainty associated with the point estimate (proportion of sampled animals with positive detection) should be quantified.

10) As new diagnostic protocols are developed for pathogens of interest a rigorous evaluation of the detection probability of the protocol should be undertaken with the results incorporated into interpretation of population- and individual-level evaluations of resident pathogen communities and pathogen prevalence estimates.

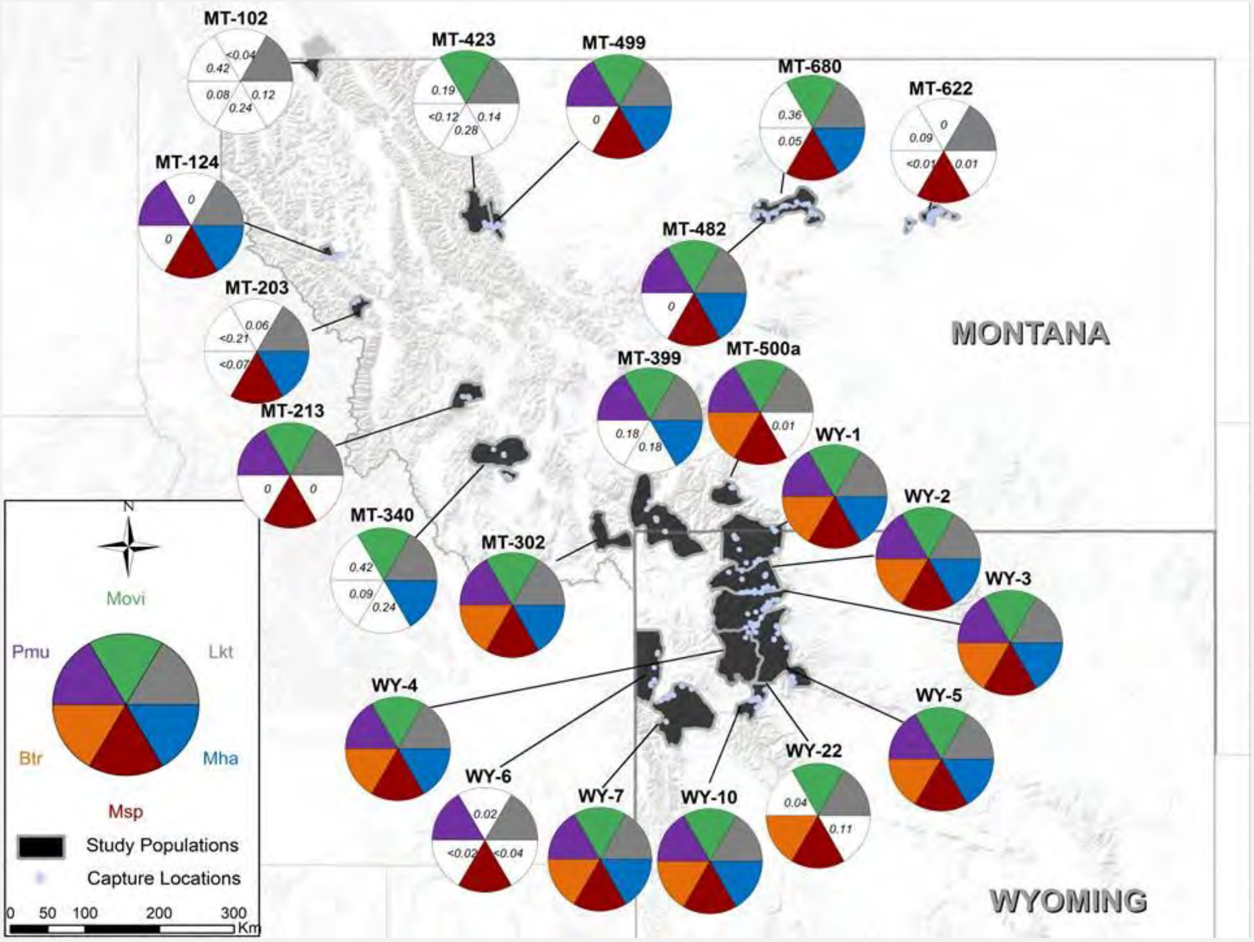


Figure 4 - Map of 22 bighorn sheep study populations and detected respiratory pathogen communities. All sections of the pie-charts are fixed to equal size and represent whether the respective pathogens were detected in the study population. The key for pathogen abbreviations are as follows: Movi= Mycoplasma ovipneumoniae, Mha = leukotoxigenic Mannheimia haemolytica/glucosida, Msp = leukotoxigenic Mannheimia spp., Btr = leukotoxigenic Bibersteinia trehalosi, Pmu = Pasteurella multocida. Where pathogens were not detected, the numbers in the unfilled section indicate the probability that the pathogens were present (assuming 10% prevalence) in the population. Figure reproduced from Butler et al. 2018 PLoS One.

CHARACTERIZING RESPIRATORY PATHOGEN COMMUNITIES & DEMOGRAPHIC ATTRIBUTES OF DIVERSE BIGHORN SHEEP POPULATIONS

We coordinated efforts across Montana and Wyoming to rigorously assess respiratory pathogen communities in a diverse set of bighorn sheep populations and then relate estimates of average recruitment and population characteristics to presence of Pasteurellaceae and *M. ovipneumoniae*. Our primary objectives were to assess the pervasiveness of respiratory pathogens in the study populations and assess whether presence of any specific pathogen or combination of pathogens was associated with differences in recruitment as measured by lamb:ewe ratios. We hypothesized little or no association between demographic performance and presence of suspected respiratory pathogens. Given the long history of domestic sheep grazing and the translocation of bighorn sheep to establish new populations and augment struggling populations, we also hypothesized that the respiratory pathogens would be resident in the majority of sampled populations. This research effort was completed and published in 2018 (Butler et al. 2018) and is summarized below.

Respiratory Pathogen Communities Resident in Sampled Bighorn Populations

We captured and live-sampled a total of 821 individual bighorn sheep (female: 724, male: 93, unknown: 14) from 22 populations in Montana and Wyoming between November and March 2012–2017 (Figure 4). Four of the 5 pathogenic agents were detected in >65% of the study populations. *M. ovipneumoniae* was detected in 17 of 22 (77%) study populations. Leukotoxigenic *M. haemolytica* was detected in 15 of 22 (68%) study populations and leukotoxigenic *Mannheimia* spp. was detected in 18 of 22 (82%) study populations. *P. multocida* was detected in 15 of 22 (68%) study populations, and leukotoxigenic *B. trehalosi* was detected in 10 of 22 (45%) study populations, including all but 1 Wyoming study population and 2 Montana populations that are adjacent to Wyoming. LktA was detected in all study populations and, therefore, all populations that hosted *M. ovipneumoniae* also hosted leukotoxigenic Pasteurellaceae. Eighty-eight percent of the 8,460 individual bighorn sheep estimated to exist in the study populations live in populations known to carry both *M. ovipneumoniae* and leukotoxigenic Pasteurellaceae.

The findings demonstrate that the majority of bighorn sheep populations occupying a variety of landscapes, including national parks and wilderness areas, host a diverse suite of bacterial pathogens associated with respiratory disease. It is not known how long the sampled populations have hosted these respiratory pathogens. Accordingly, it is not known the extent to which the current pervasiveness of these pathogens in the populations is the result of continued “spillover” events from domestic livestock or the result of past eras when domestic sheep were ubiquitous across bighorn sheep range. Regardless, these results highlight the substantial, landscape-level, challenges that wildlife agencies face when attempting to craft management strategies to reduce the occurrence of respiratory disease die-offs and advance bighorn sheep restoration.

Respiratory Pathogen Communities & Recruitment

Mean lamb:ewe ratios (i.e., recruitment) of study populations where any specific pathogen was detected ranged from <0.20 to >040. For each pathogen species, there were at least 4 populations that hosted it and had mean lamb:ewe ratios >0.30. There was evidence for an association between detection of *M. ovipneumoniae* and lamb:ewe ratios. In populations where *M. ovipneumoniae* was detected, the estimated mean lamb:ewe ratio was 0.27 and in populations where it was not detected the estimated mean lamb:ewe ratio was 0.39. There was no evidence for an association between detection of any of the other pathogen species and lamb:ewe ratios. Associations between presence of leukotoxigenic Pasteurellaceae in general and lamb:ewe ratios were not explored because leukotoxigenic Pasteurellaceae were detected in all study populations. Interactive effects of *M. ovipneumoniae* and leukotoxigenic Pasteurellaceae could not be explored because *M. ovipneumoniae* was never detected in the absence of leukotoxigenic Pasteurellaceae, however, recruitment data for populations where both *M. ovipneumoniae* and leukotoxigenic Pasteurellaceae were and were not detected are shown in Figure 5.

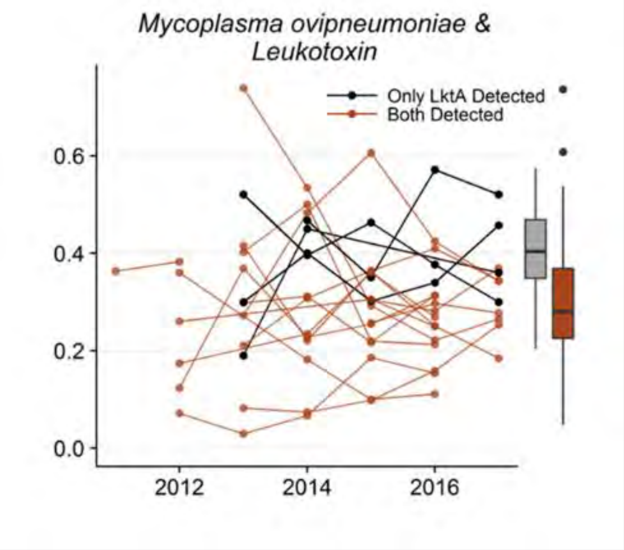


Figure 5 – Lamb:ewe ratios of 14 bighorn sheep populations in Montana and Wyoming where both M. ovipneumoniae and leukotoxigenic Pasteurellaceae were detected and where only leukotoxigenic Pasteurellaceae was detected.

Although both *M. ovipneumoniae* and leukotoxigenic Pasteurellaceae were detected in most (n=16) study populations, these populations often showed no demographic signs of respiratory disease. Over half of the populations where these pathogens were detected met population objectives and had average lamb:ewe ratios greater than 0.20 (threshold for "healthy" recruitment defined by the Western Association of Fish and Wildlife Agencies), and 6 had average lamb:ewe ratios greater than 0.30. Generally, this group of populations included those with the lowest and among the highest population sizes and average recruitment rates. The number of populations found to host *M. ovipneumoniae* and leukotoxigenic Pasteurellaceae and the variation in demographic performance among these populations resulted in the paradoxical finding that, although average demographic performance in this group of populations was lower than where *M. ovipneumoniae* was not detected, most populations that were considered to be increasing or have average recruitment rates greater than 0.30 were ones that carried both *M. ovipneumoniae* and leukotoxigenic Pasteurellaceae. This pattern suggests that bighorn sheep populations may be demographically robust while hosting all respiratory pathogens that have been tied to respiratory disease. However, the significance of this pattern hinges on whether the collection of study populations here is representative of bighorn sheep populations as a whole and the drivers of the variation in demographic performance of populations hosting apparently similar pathogen communities. Although the study populations were not randomly selected, they were chosen to capture a wide range of variability in population attributes in order to maximize the generalizability of the findings.

There are numerous plausible hypotheses to explain the observed variation in demographic performance. The strong demographic performance of some populations hosting *M. ovipneumoniae* and leukotoxigenic Pasteurellaceae could be explained by the presence of less virulent pathogen strains which the available diagnostic tests are unable to distinguish. Differences in virulence could be inherent in the various pathogen strains or attenuated after years of persistence in bighorn sheep populations. Variation in demographic performance could also be explained by differences in prevalence of *M. ovipneumoniae* or leukotoxigenic Pasteurellaceae, however, given currently available protocols, this parameter is likely estimated with poor precision in the face of imperfect detection probability, particularly for Pasteurellaceae. Given variable population-management histories and over a century of exposure to domestic sheep experienced by some populations, natural selection may also have produced increased disease resilience in some populations. High adult and juvenile mortality rates associated with respiratory disease suggest potential for strong selective pressure for physiological or behavioral adaptations against respiratory disease so long as surviving individuals continue to be exposed to the causative agent, traits associated with survival are heritable, and sufficient genetic variability exists. An equally plausible explanation for the variation of demographic rates, and presumably disease expression, may be dictated by interactions between the resident pathogens, the physiological attributes of the host, and the environment (the classic epidemiologic triad), which is the tradition model of infectious disease causation (Figure 3). It is also plausible that much of the variation among populations is driven by other well-documented factors that influence vital rates of wild ungulates such as predation and weather events.

A Tool to Aid Wildlife Management Agencies in Interpreting Respiratory Pathogen Test Results

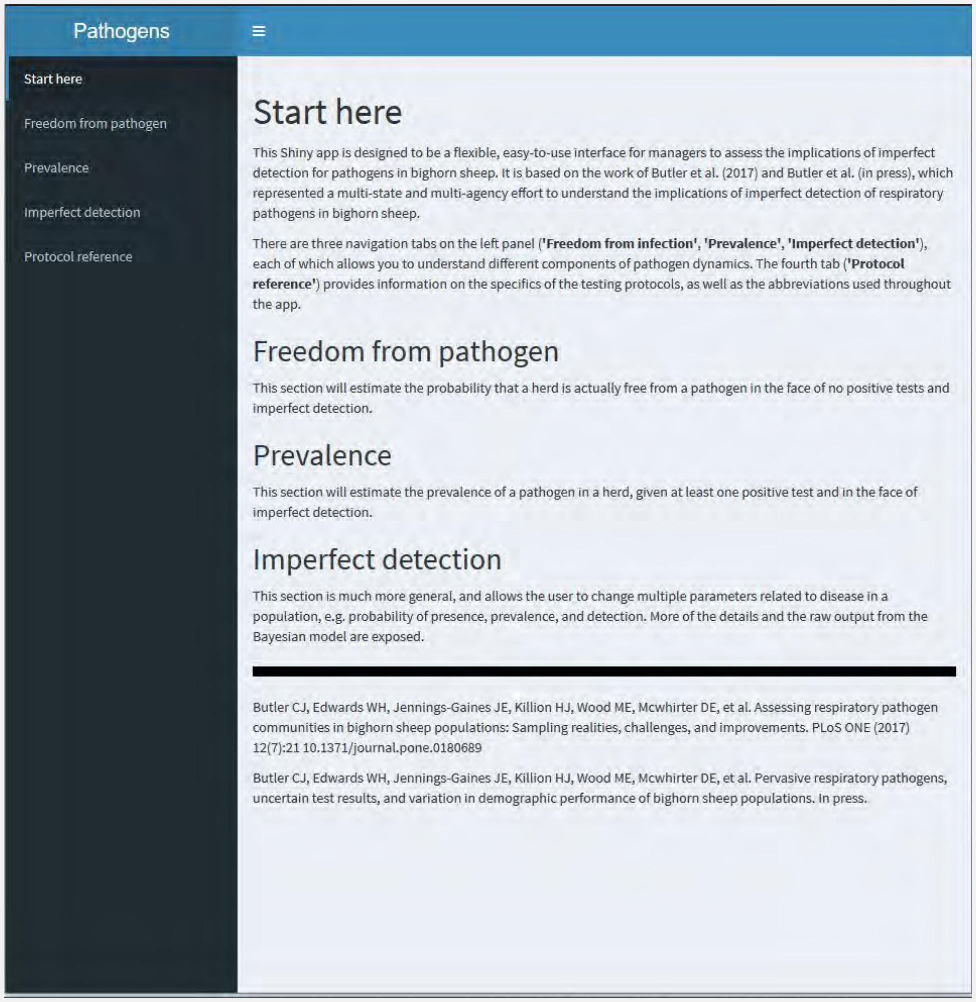
The primary data collected by managers to inform translocation and augmentation decisions, as well as evaluating risk of disease die-offs, is the sampling of animals to determine the presence and prevalence of respiratory pathogens. In addition, nearly all of the hypotheses and tentative explanations posed by research biologists and wildlife health professionals that appear in the literature related to pathogens responsible for disease and the disease process can be traced back to interpretations of results of pathogen sampling and interpretations of those data. Every disease-related word in the bighorn literature that is commonly used to describe ideas about the disease process (spillover, carrier, shedder, disease fade out, prevalence, etc.) is based on interpreting pathogen test results and, to date, such results have been interpreted with no consideration of uncertainty in test results arising from imperfect detection of pathogens and sampling of populations. Essentially, test results have been interpreted as if they reflected 'truth' (or, perfect detection), that is, whether a specific pathogen is present in an individual or not. Given the results of our evaluations of the diagnostic protocols used for bighorn sheep pathogen surveys, it is clear that failing to consider uncertainty in pathogen testing inhibits our ability to understand the disease and formulate effective management actions to mitigate disease risk and enhance restoration, conservation, and management of bighorn sheep throughout North America.

We developed protocol-specific estimates of detection probability for nearly all the standard diagnostic protocols used to assess the suite of pathogens associated with respiratory disease in bighorn sheep to provide the information required to perform rigorous evaluations of pathogen testing results that account for imperfect detection and variation in sampling. We used a flexible Bayesian framework to incorporate our knowledge of pathogen-specific detection probabilities and account for a diverse set of sampling scenarios, in order to gain insight into pathogen prevalence and/or presence within a population. Given positive test results, the models estimate the true prevalence of a pathogen in a population and provide appropriate confidence limits (Paterson et al. 2020). Perhaps most importantly, the models can also provide an estimate of the probably that a pathogen is present in a population when sampling failed to detect the pathogen. This software was used when reporting the results of our regional assessment of resident pathogen communities in bighorn sheep populations throughout Montana and Wyoming (Figure 4) and is now being used by MFWP's Wildlife Health Lab personnel to interpret the pathogen test results for the ongoing health assessments of all bighorn sheep populations in the state.

The analytical procedure to accomplish this rigorous interpretation of pathogen testing results is not trivial and requires advanced statistical training to execute. Thus, in order to assure that the broader community can benefit from the results of our work, we developed an easy-to-use web-based tool to assist in the rigorous interpretation of population-level respiratory pathogen assessments that specifically accounts for imperfect detection of diagnostic protocols as well as the intensity of the sampling performed in each population (Figure 6). Using test results and controlling parameters related to sampling design and detection probabilities, this application allows users to estimate the probability of pathogen presence when it was not detected in a given sampling event (Figure 7), or prevalence in the event of at least 1 positive test (Figure 8). Furthermore, it informs sampling design by allowing users to determine the sample size and number of replicate tests per individual that are required to achieve a specified confidence in the probability of pathogen presence. Overall, this work has produced a practical, readily-accessible, and easily-used tool that will allow managers to more accurately assess the probability and uncertainty of pathogen presence/absence in wild populations. The web-app is currently available (see link below), and a manuscript describing the app is published (Paterson et al. 2020).

Visit the web-app:

https://quantitativebiology.shinyapps.io/pathogens/



Start here

This Shiny app is designed to be a flexible, easy-to-use interface for managers to assess the implications of imperfect detection for pathogens in bighorn sheep. It is based on the work of Butler et al. (2017) and Butler et al. (in press), which represented a multi-state and multi-agency effort to understand the implications of imperfect detection of respiratory pathogens in bighorn sheep.

There are three navigation tabs on the left panel (**'Freedom from infection'**, **'Prevalence'**, **'Imperfect detection'**), each of which allows you to understand different components of pathogen dynamics. The fourth tab (**'Protocol reference'**) provides information on the specifics of the testing protocols, as well as the abbreviations used throughout the app.

Freedom from pathogen

This section will estimate the probability that a herd is actually free from a pathogen in the face of no positive tests and imperfect detection.

Prevalence

This section will estimate the prevalence of a pathogen in a herd, given at least one positive test and in the face of imperfect detection.

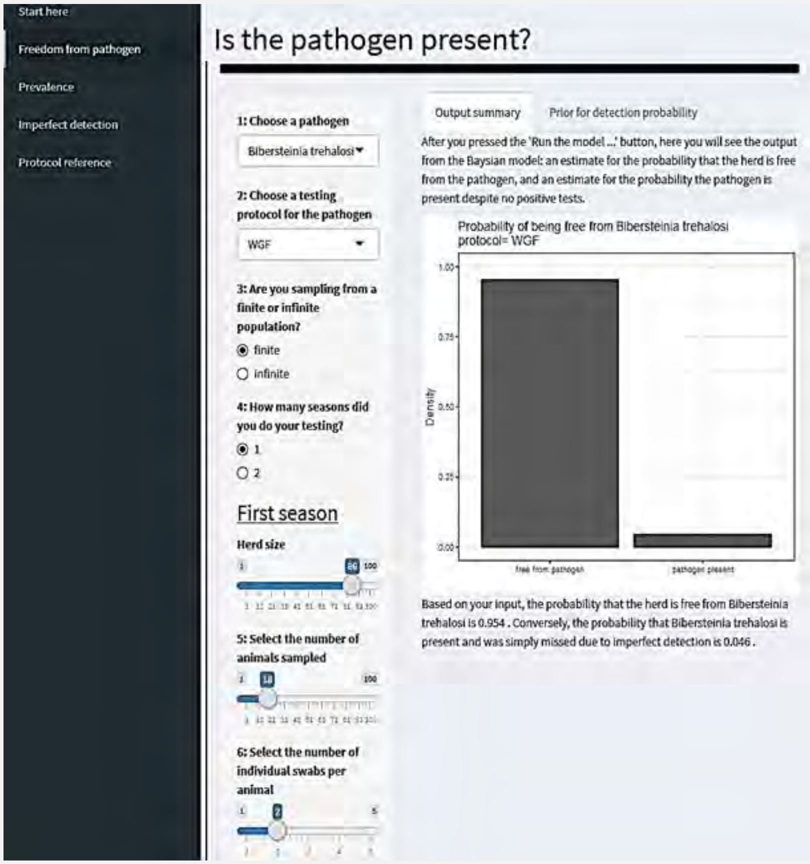
Imperfect detection

This section is much more general, and allows the user to change multiple parameters related to disease in a population, e.g. probability of presence, prevalence, and detection. More of the details and the raw output from the Bayesian model are exposed.

Butler CJ, Edwards WH, Jennings-Gaines JE, Killion HJ, Wood ME, Mcwhirter DE, et al. Assessing respiratory pathogen communities in bighorn sheep populations: Sampling realities, challenges, and improvements. PLoS ONE (2017) 12(7):21 10.1371/journal.pone.0180689

Butler CJ, Edwards WH, Jennings-Gaines JE, Killion HJ, Wood ME, Mcwhirter DE, et al. Pervasive respiratory pathogens, uncertain test results, and variation in demographic performance of bighorn sheep populations. In press.

Figure 6 – A screenshot of the opening page of a web-based software application that provides an easy-to-use interface for managers to enter sampling information and pathogen testing results and obtain a rigorous analysis of the results that incorporates the estimates of detection probability of common pathogen diagnostic protocols as well as the number of animals sampled from a population.



Is the pathogen present?

1: Choose a pathogen
Bibersteinia trehalosi

2: Choose a testing protocol for the pathogen
WGF

3: Are you sampling from a finite or infinite population?
● finite
○ infinite

4: How many seasons did you do your testing?
● 1
○ 2

First season

Herd size
86

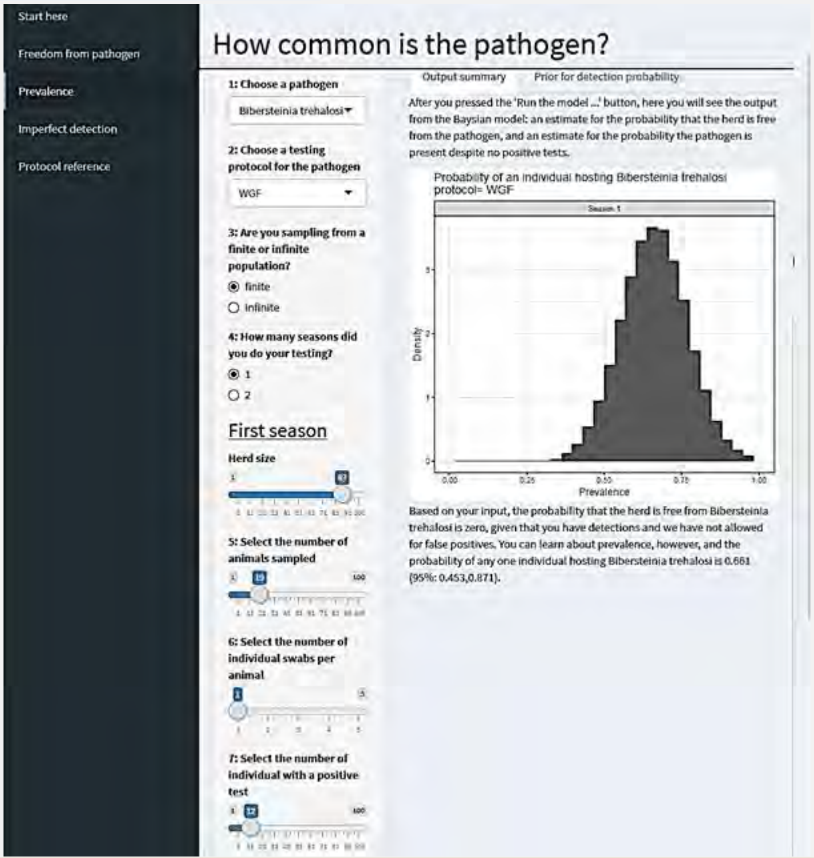
5: Select the number of animals sampled
18

6: Select the number of individual swabs per animal
2

After you pressed the 'Run the model ...' button, here you will see the output from the Baysian model: an estimate for the probability that the herd is free from the pathogen, and an estimate for the probability the pathogen is present despite no positive tests.

Based on your input, the probability that the herd is free from Bibersteinia trehalosi is 0.954 . Conversely, the probability that Bibersteinia trehalosi is present and was simply missed due to imperfect detection is 0.046 .

Figure 7 – A screenshot of the page that prompts the user to enter the sampling information for a pathogen that was not detected and the results of estimating the probability that the pathogen is present in the population.



How common is the pathogen?

1: Choose a pathogen
Bibersteinia trehalosi

2: Choose a testing protocol for the pathogen
WGF

3: Are you sampling from a finite or infinite population?
● finite
○ infinite

4: How many seasons did you do your testing?
● 1
○ 2

First season

Herd size

5: Select the number of animals sampled
19

6: Select the number of individual swabs per animal
1

7: Select the number of individual with a positive test
12

After you pressed the 'Run the model ...' button, here you will see the output from the Baysian model: an estimate for the probability that the herd is free from the pathogen, and an estimate for the probability the pathogen is present despite no positive tests.

Based on your input, the probability that the herd is free from Bibersteinia trehalosi is zero, given that you have detections and we have not allowed for false positives. You can learn about prevalence, however, and the probability of any one individual hosting Bibersteinia trehalosi is 0.661 (95%: 0.453,0.871).

Figure 8 – A screenshot of the page that prompts the user to enter the sampling information and test results for a pathogen that was detected and the results of estimating the prevalence of the pathogen in the population.

TEMPORAL VARIATION IN RESPIRATORY PATHOGENS & DEMOGRAPHIC ATTRIBUTES OF A BIGHORN SHEEP POPULATION

Respiratory disease (pneumonia) is a major impediment to bighorn sheep restoration (Singer et al. 2000b, Cassirer et al. 2018) and is recognized as a polymicrobial disease with a number of pathogens associated with expression of disease. The primary focus of management actions over the past 2 decades to reduce the impact of respiratory disease on bighorn sheep populations has been the establishment of policies to reduce the potential for all-age die-offs that have been attributed to the transmission of novel pathogens or pathogen variants from domestic sheep and goats (i.e., spillover events) or contact with other infected bighorn populations (Brewer et al. 2014). These policies emphasize physical separation of bighorn sheep populations from domestic sheep and goats and other bighorn populations. The recent revelation that most bighorn populations host a suite of respiratory pathogens suggests the epizootics involving all-age die offs may also be caused by resident pathogens when specific conditions are experienced (Butler et al. 2018). What proportion of all-age die-offs experienced in the past were due to novel versus resident pathogens and what conditions may be required for resident pathogens to cause a disease event is currently unknown and presents a major challenge to gaining insight that can inform additional management strategies to minimize the expression of resident pathogens as disease.

While all-age die-offs are the most obvious and dramatic impacts of respiratory pathogens, an equally important consequence of chronic infection is persistent poor lamb survival subsequent to an epizootic event that can inhibit population recovery (Cassirer et al. 2013, Smith et al. 2014). Exposed adults can develop protective immunity but remain infected and thus become carriers of the respiratory pathogens (Cassirer et al. 2013). Evidence suggests that immunity, however is not transferred from ewes to lambs (Plowright et al. 2013) and that lambs are vulnerable to infection following birth (Besser et al. 2013). Thus, infected ewes can pass pathogens to their lambs soon after birth and infected lambs can subsequently become carriers, passing the pathogens to other lambs in nursery groups that form several weeks after lambing. This dynamic can then lead to high levels of lamb mortality, peaking when lambs are 1 to 4 months old (Cassirer et al. 2013, Smith et al. 2014). Poor recruitment due to lamb pneumonia can last from 3-5 years to decades (Cassirer et al. 2013, Manlove et al. 2016). In some cases where populations experience persistent infection, management agencies have attempted to identify chronically infected individuals and remove them from the population (Garwood et al. 2020), and in some cases, agencies have extirpated entire populations with a goal of subsequently reestablishing a new population free of infection by respiratory pathogens (e.g., Tendoys population in Montana).

Gaining a better understanding of pathogen dynamics and, more broadly, the association between prevalence of pathogens and demographic performance of infected populations, is requisite in order to evaluate the potential for developing management interventions to promote resilience in infected bighorn populations. Here we present the results of an intensive 5-year study of the Hilgard bighorn population that occupies the southern portion of the Madison Range in southwestern Montana. This herd experienced multiple pneumonia-related epizootics and substantial annual variation in lamb recruitment, presents evidence of persistent, but varying levels of pneumonia in animals observed on the winter range, and yet has demonstrated robust population growth over the decade preceding the study, with 130-150 animals counted on winter range in the years immediately prior to the initiation of this study (Montana Fish Wildlife & Parks 2013).

The primary objectives of the study were to quantify annual variability in prevalence of respiratory pathogens and assess correlations between estimates of annual prevalence of pathogens and estimates of annual recruitment and lamb survival. Based on the current understanding of the disease process in infected bighorn populations, we expected the higher the proportion of infected ewes in the population the higher the proportion of lambs born that year that would be exposed to the pathogens and succumb to respiratory disease in the first 5 months of life. Thus, after accounting for annual variation in pregnancy and adult ewe survival rates, we expected to find positive correlations between estimated annual variation in prevalence of at least 1 pathogen and estimated recruitment and lamb survival. We expected little to no correlation between estimated annual pathogen prevalence and pregnancy rates or adult ewe survival rates as the pathogens are not known to impact pregnancy and adults are expected to have immunity to resident pathogens that have likely been circulating in this population for many decades (Cassirer et al. 2013, Plowright et al. 2013, Manlove et al. 2016).

Animal Capture

In each of the 5 years of the study (2014-2018) we used a drop net to capture animals in the Hilgard population for sampling and radio collaring. A total of 182 animals were captured and sampled over the 5-year study with the number of animals sampled annually ranging from 29 to 55. Sampled animals included 120 ewes, 46 rams, and 27 lambs. Variable numbers of adult females (≥1.5 yr old) were instrumented with GPS collars equipped with mortality sensors each year, which combined with 5 VHF-collared ewes that existed in the population at the start of the study, resulted in 20-31 instrumented ewes available annually for estimating survival and for mark-resight surveys. Three of the annual captures also involved translocating between 22-52 animals per event to new areas north of traditional winter range within the Madison Range (Montana Fish Wildlife & Parks 2013).

Temporal Variation in Pathogen Communities

We collected biological samples from all animals captured each year for the detection of 5 bacterial respiratory pathogens. We targeted *Mycoplasma ovipneumoniae* and 4 species in the Pasteurellaceae family including *Mannheimia haemolytica*, *Bibersteinia trehalosi*, *Mannheimia* species, and *Pasteurella multocida*. Sampling included the collection of 1-4 swabs of the tonsillar crypts and 1-2 swabs of the nasal cavity as well as a blood sample to harvest serum. We used multiple diagnostic protocols to detect the presence of each pathogen as described in Chapter 2 and Butler et al. (2017, 2018). We used a flexible, hierarchical Bayesian approach to estimate the prevalence of each pathogen in each year, in order to accommodate the multiple testing cohorts. We used informative priors for the probability of detection for each pathogen-protocol combination. Prior work estimated these probabilities of detection using an occupancy-modeling approach (Butler et al. 2017, Butler et al. 2018), and the mean and variance of these estimates was used to moment match the parameters of a Beta distribution that was then used as the prior for detection probabilities in our model.

We conducted a total of 779 pathogen diagnostic assays on 180 animals to evaluate temporal variation in the prevalence of the 5 pathogens. The PCR tests detected the presence of *Mycoplasma ovipneumoniae* in the population in all years of the study and in 32%, 32%, and 84% of the tested ewes, rams, and lambs, respectively. In addition, the serology test indicated 46%, 34%, and 64% of the tested ewes, rams, and lambs, respectively, had been exposed to *M. ovipneumoniae*. The suite of diagnostic assays employed to detect Pasteurellaceae pathogens revealed that 3 of the pathogens were detected in the population in all years of the study and 1 pathogen was rarely detected. *Mannheimia haemolytica* was detected in 20%, 29%, and 12% of the tested ewes, rams, and lambs, respectively. *Mannheimia* spp, was detected in 22%, 29%, and 12% of the tested

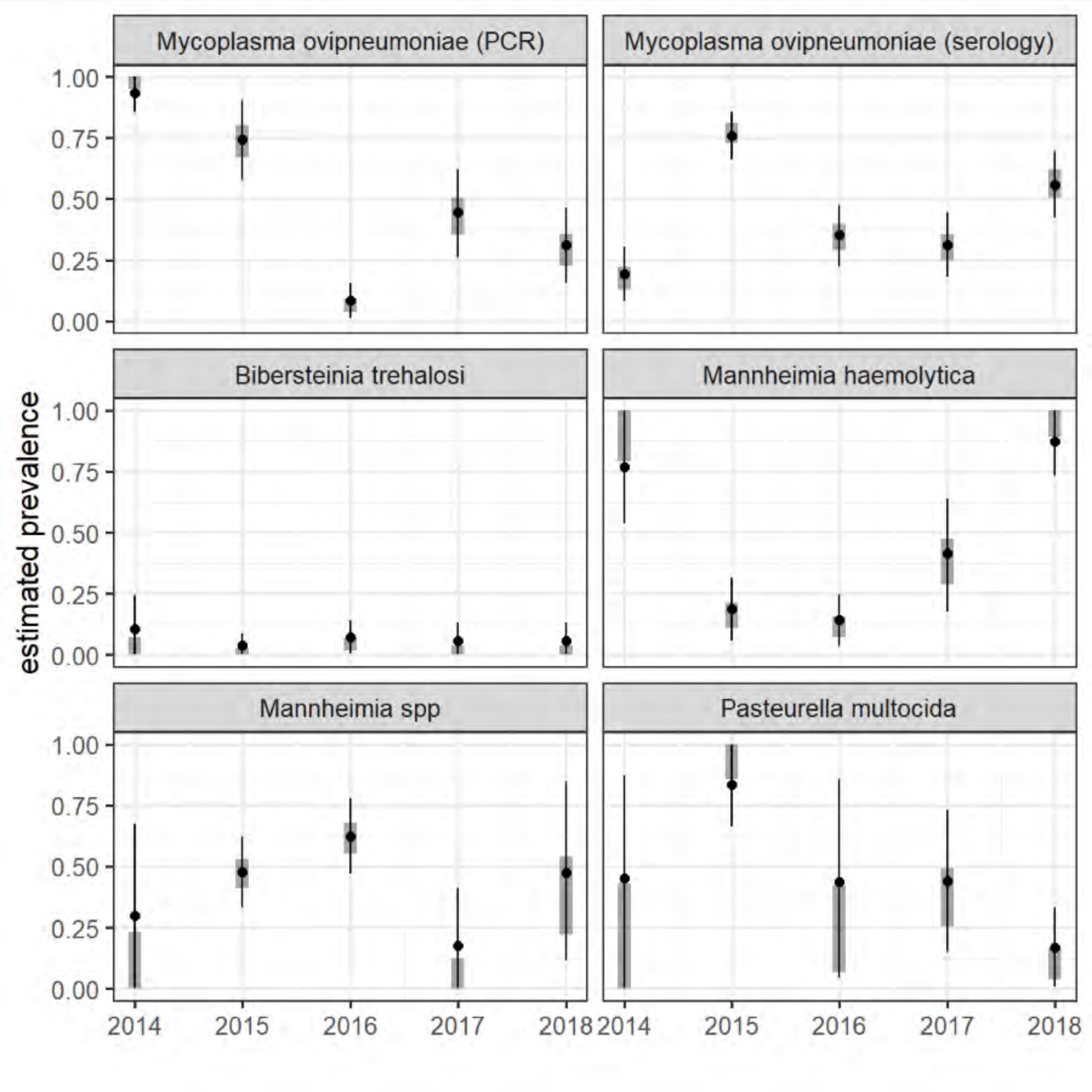


Figure 9 – Temporal variation in estimated prevalence of respiratory pathogens in the Taylor-Hilgard bighorn sheep population in southwestern Montana during 2014-2018. For each estimate, the black dot represents the mean, the thin line represents the 90% highest posterior density interval (HPDI), and the light gray box represents the 50% HPDI.

ewes, rams, and lambs, respectively. *Bibersteinia trehalosi* was detected in 0%, 0%, and 4% of the tested ewes, rams, and lambs, respectively. *Pasteurella multocida* was detected in 10%, 8%, and 36% of the tested ewes, rams, and lambs, respectively.

We found substantial among-year variation in prevalence for 4 of the 5 pathogens (Figure 9). The estimated prevalence of *M. ovipneumoniae* (PCR) ranged from a low of 0.09 in 2015-2016 (90% CI = 0.01 – 0.16) to a high of 0.94 in 2013-2014 (90% CI = 0.86 – 1.00). Using serology assays to detect exposure to *M. ovipneumoniae*, we estimated the proportion of animals recently exposed to *M. ovipneumoniae*. ranged from a low of 0.19 in 2013-2014 (90% CI = 0.08 = 0.30) to a high of 0.76 in 2014-2015 (90% CI = 0.66 – 0.86). Notably, the temporal patterns of prevalence for *M. ovipneumoniae* displayed little agreement between the two tests, e.g., the highest estimated prevalence using the PCR test (2013-2014: 0.94, 90% CI = 0.86 – 1.00) corresponded to the lowest estimated prevalence using serology (2013-2014: 0.19, 90% CI = 0.08 – 0.30). The estimated prevalence of *Mannheimia haemolytica*, Mannheimia spp., and *Pasteurella multocida* demonstrated among-year variation similar to that seen for *M. ovipneumoniae*: *Mannheimia haemolytica* ranged from 0.14 (90% CI = 0.04 - 0.24) to 0.77 (90% = 0.54 - 1.00), Mannheimia spp. ranged from 0.18 (90% CI = 0.00 – 0.14) to 0.62 (90% CI = 0.47 – 0.77), and *Pasteurella multocida* ranged from 0.17 (90% CI = 0.01 – 0.33) to 0.83 (90% CI = 0.66 – 1.00). In contrast, the estimated prevalence of *Bibersteinia trehalosi* showed little among-year variation (range: from 0.04 (90% CI = 0.00 – 0.09) to 0.11 (90% CI = 0.00 – 0.24)) and was consistently estimated to be lower than all other pathogens (Figure 9).

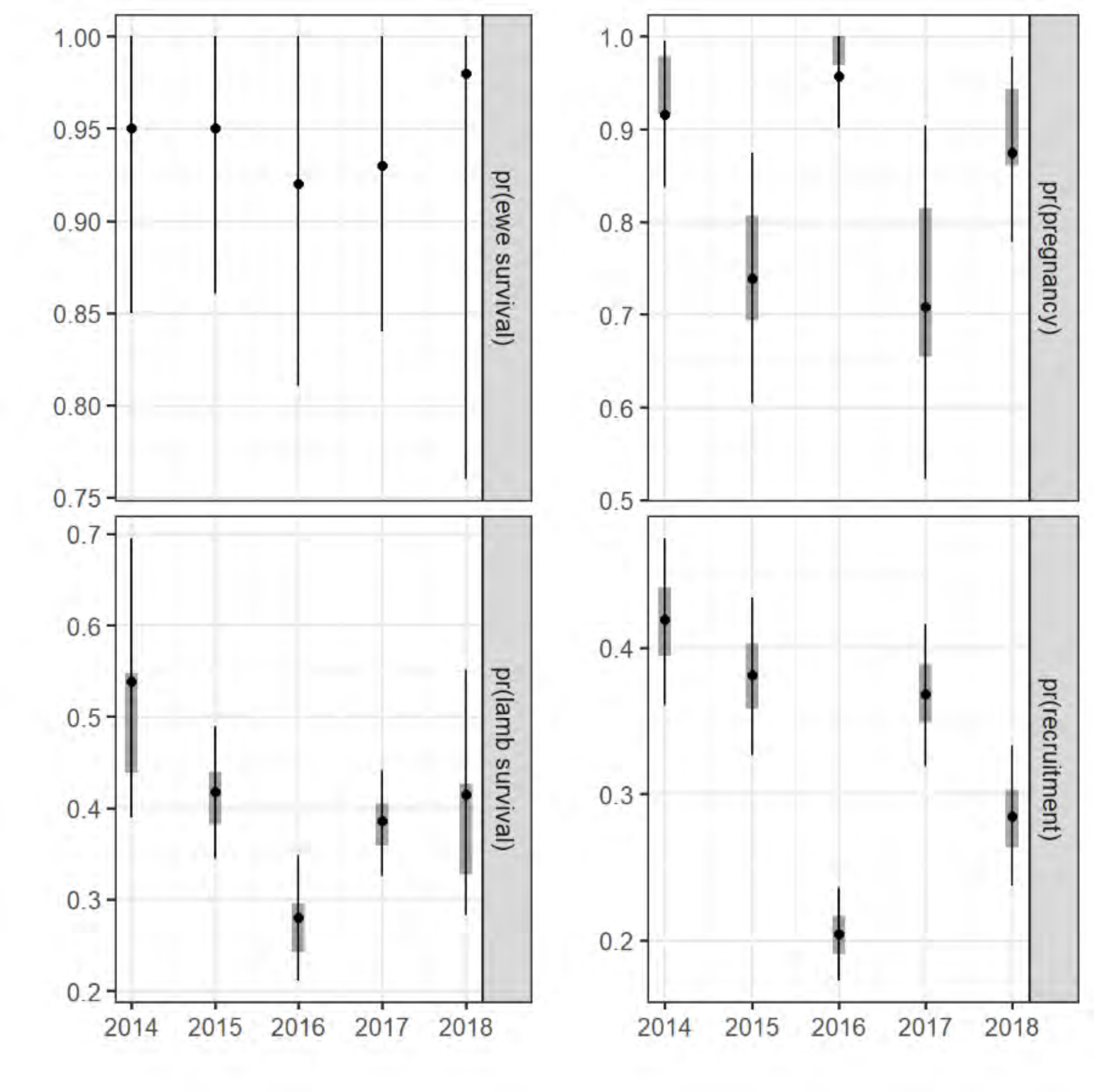


Figure 10 – Temporal variation in estimated vital rates of the Taylor-Hilgard bighorn sheep population in southwestern Montana during 2014-2018. For each estimate, the black dot represents the mean. The thin line represents the 95% confidence interval for ewe survival estimates and for the remaining vital rates the thin line represents the 90% highest posterior density interval (HPDI), and the light gray box represents the 50% HPDI.

Estimating Pregnancy Rates

Annual pregnancy rates were estimated based on 2 assays of sera from 100 captured reproductive age (≥1.5 yr old) females with the number of animals sampled each of the 5 years ranging from 13 to 26. We assayed for pregnancy-specific protein B (PSPB) using an enzyme-linked immunosorbent assay (ELISA) kit that is 98% accurate at predicting pregnancy in bighorn sheep at ≥30 days after conception (Drew et al. 2001). Because some bighorn sheep captures occurred in mid-December, within 30 days of the breeding season, we also assayed sera from these animals for progesterone (P4) concentrations using an ELISA kit validated for sheep serum. We assumed any animals captured in mid-December with PSPB and P4 values indicating estrous cycling or early stage pregnancy to be pregnant or would become pregnant that year. We excluded yearlings from estimates of annual pregnancy rates as they tend to have lower pregnancy rates than older females (Proffitt et al. in press). We used a Bayesian model to estimate pregnancy rates in each year, pregnancy_t, by modeling the number of pregnant ewes using a Binomial distribution:

$$\text{No. of pregnant ewes}_t \sim \text{Binomial}(\text{pregnancy}_t, \text{N}_t),$$

where N_t was sample size in year t. We used vague priors for pregnancy rate in each year. Estimated pregnancy rates varied among years from a low of 0.71 (90% CI = 0.53 – 0.90) in 2016-2017 to a high of 0.96 (90% CI = 0.90 – 1.00) in 2015-2016 (Figure 10, Table 2).

Estimating Adult Ewe Survival Rates

We instrumented a total of 41 individual adult ewes with VHF and GPS radio-collars equipped with mortality sensors, with 20-31 collared animals monitored for survival annually. Ground-based monitoring of VHF signals was conducted at irregular intervals throughout the year with more frequent monitoring when animals were on winter range. GPS collars provided relative exact dates of death when data were retrieved from store-on-board memory or downloaded for satellite-linked collars. All mortalities were investigated to retrieve collars; however, mortalities were not investigated quickly enough to confidently determine cause of death for most animals. Over 126 animal years of monitoring we documented 11 mortalities. Suspected causes of mortalities included predation (n = 2), avalanche (n = 2), vehicle collision (n = 2), legal harvest (n = 1), and unknown (n = 4).

Annual survival rates were estimated in Program MARK using a known-fate analysis conducted via the nest-survival module (Rotella et al. 2004), which is appropriate for telemetry data collected according to an irregular schedule and where each animal's fate is known but the exact dates for mortality events are not all known. The model estimated a unique survival rate for each season. Seasons were defined as winter (December through May) and summer (June through November). We derived seasonal survival rates by raising estimated daily survival rates (DSR) for each season to the number of days in each season (estimated survival rate for winter = $\widehat{DSR}_{winter}^{182.5}$; estimated survival rate for summer = $\widehat{DSR}_{summer}^{182.5}$. The seasonal survival rates were then multiplied together to obtain estimates of annual survival. We used the delta method to derive measures of uncertainty for seasonal and annual rates. Estimated survival rates were generally high varying from a low of 0.88 in 2017-2018 (95% CI = 0.76 – 1.00) to a high of 0.95 in 2013-14 (95% CI = 0.85 – 1.00) and 2014-2015 (95% CI = 0.86 – 1.00) (Figure 10, Table 2).

Estimating Adult Ewe and Lamb Numbers, Recruitment, & Lamb Survival Rates

During each winter of the study from mid-November to approximately mid-February we conducted ground-based mark-resight surveys of the winter range. Attempts were made to conduct surveys of the primary winter range at approximately 2 week intervals, however, weather and logistic limitations resulted in irregular intervals between surveys and differing numbers of surveys each year. The bighorn sheep winter range is adjacent to a major highway, making animals readily observable from 10 to 500 m with any combination of a 60x spotting scope, 10x binoculars, or with the unaided eye. At these close distances, collars were readily visible. Observers counted the total number of bighorn sheep seen, and categorized each animal as either an adult male, adult female, lamb, or unknown sex-age class. Observers also noted whether adult ewes were radio collared, uncollared, or whether presence of a collar was uncertain. We performed mortality checks to ensure we knew how many marked animals were available to be detected during each ground survey and we did not use telemetry during surveys to aid in locating animals. We attempted to maintain consistent methodology for all surveys by providing 2 to 3 days training for all personnel that participated in the surveys.

The abundance of ewes and lambs on the winter range was estimated using a Bayesian version of a hierarchical model for mark-resight data (White 1996) which accounted for the known number of ewes that died or were translocated in each year. We then estimated the probability of recruitment in year t, recruitment_t, from the lamb surveys by modeling the number of lambs seen using a Binomial distribution:

$$\text{lambs}_{s,t} \sim \text{Binomial}(\text{recruitment}_t, \text{Ewes}_{s,t}),$$

Table 2 – The estimated demographic attributes of the Taylor-Hilgard bighorn populations over five consecutive years when the population was intensively monitored via annual captures, biological sampling, monitoring of radio-collared animals, and replicate mark-recapture surveys.

	2013-14	2014-15	2015-16	2016-17	2017-18
No. radio-collared animals	19-20	17-23	20-25	20-31	28
No. animals translocated	0	52	22	0	23
No. mark-resight surveys	9	5	7	8	4
Adult female population					
Estimate	141	129	103	104	105
Conf. interval	136-145	124-135	97-108	96-110	92-115
Lamb population					
Estimate	59	49	21	38	30
Conf. interval	51-67	42-57	18-24	33-44	24-36
Adult female & lamb population					
Estimate	200	179	124	142	135
Conf. interval	189-211	169-190	116-132	132-153	119-150
Lamb:ewe ratio					
Estimate	0.42	0.38	0.20	0.37	0.28
Conf. interval	0.36-0.48	0.33-0.44	0.17-0.23	0.32-0.42	0.24-0.33
Lamb survival					
Estimate	0.53	0.42	0.28	0.39	0.41
Conf. interval	0.39-0.68	0.34-0.49	0.21-0.34	0.33-0.44	0.28-0.54
Adult ewe survival[1]					
No. marked	20	23	25	31	28
Estimate	0.95	0.95	0.92	0.93	0.88
Conf. interval	0.85-1.00	0.86-1.00	0.81-1.00	0.84-1.00	0.76-1.00
Adult ewe pregnancy[2]					
No. sampled	23	26	22	13	16
Estimate	0.92	0.74	0.96	0.71	0.87
Conf. interval	0.84-1.00	0.61-0.88	0.90-1.00	0.53-0.90	0.78-0.98

[1] *≥ 1.5-year-old*

[2] *yearlings excluded from sample*

where $\text{Ewes}_{s,t}$ was the number of ewes seen on survey *s* in year *t*. We then estimated the total number of lambs in year *t* as the product of the estimated total number of ewes in year *t* Total ewes_t and the estimated probability of recruitment in year *t* recruitment_t. Finally, we used the information from estimated pregnancy rates to factor the probability of recruitment in year *t* into the constituent probabilities of conception and lamb survival to estimate the probability of lamb survival and get a more complete picture of variation in vital rates. We used independent, vague priors for each of the probabilities of detection, probabilities of pregnancy, and probabilities of lamb survival. No pregnancy information was available in the 2012-2013 season, so we modeled this pregnancy rate using the mean and variance of the estimated pregnancy rates (2013-2014 to 2017-2018) to parameterize a normal distribution.

A total of 4 to 9 mark-resight surveys were conducted each year. Coupled with a relatively high number of radio-collared ewes surveyed, this resulted in precise estimates of the adult female population abundance each year (Table 2). After accounting for the translocation of animals each year (minimum = 0, maximum = 52 translocations), abundance of adult females ranged from a low of 103 (90% CI = 97-108) in 2015-2016, the year following the largest translocation, to a high of 141 (90% CI = 136-145) in 2013-2014. The estimated probability of recruitment (indexed by lamb:ewe ratios) ranged from 0.20 (90% CI = 0.17 – 0.23) in 2015-2016 to 0.42 (90% CI = 0.36-0.48) in 2013-2014 (Table 2, Figure 10). We then applied the estimated probability of recruitment in each year to the estimated total number of ewes from the mark-resight model, which translated into variation in the number of lambs from 21 (90% CI = 18-24) in 2015-2016 to 59 (90% CI = 51-67) in 2013-2014 (Table 2). Lamb survival rates estimated by decomposing the estimated probability of recruitment into the probability of pregnancy and lamb survival showed substantial annual variation from a low of 0.28 (90% CI = 0.21 – 0.24) in 2015-2016 to a high of 0.53 (90% CI = 0.39 – 0.68) in 2013-14 (Figure 10, Table 2). Notably, the temporal pattern for pregnancy rates is different than that for lamb survival such that the year with the highest estimated pregnancy rate of 0.96 (2015-2016: 90% CI = 0.90 – 1.00) corresponded to the year with the lowest lamb survival rate of 0.28 (90% CI = 0.21 – 0.34).

Evaluating Correlations Between Annual Pathogen Prevalence & Lamb Survival Rates

In order to rigorously assess the relationship between estimated pathogen prevalence and the estimated probabilities of lamb survival we needed to account for the uncertainty in both estimates. We used the year-specific mean and variance of the estimated pathogen prevalence and lamb survival rates to moment-match a Beta distribution to each estimate. We then generated 1000 replicated data sets by drawing values of the response (1 random draw for lamb survival in each year *t*) and prevalence (one random draw for each pathogen *p* in each year *t*). For each replicated data set, we ran a beta regression to assess the strength of evidence for a relationship between the estimated prevalence of each pathogen and estimated lamb survival in each year. We assigned vague priors to the parameters underlying the regression. The approximate posterior distributions for the coefficient describing the relationship between estimated prevalence for each pathogen and estimated lamb survival, were then combined across all 1000 replicated data sets and used for inference. After incorporating the uncertainty in estimates of pathogen prevalence and lamb survival rates, we found no evidence for a relationship between the 2 for any pathogen (Figure 11). For each pathogen, the 90% credible interval for the coefficient for the slope term that described the linear relationship between pathogen prevalence and lamb survival on the logit scale overlapped 0.

Conclusions

Our study intensively sampled a bighorn sheep population for 5 consecutive years and

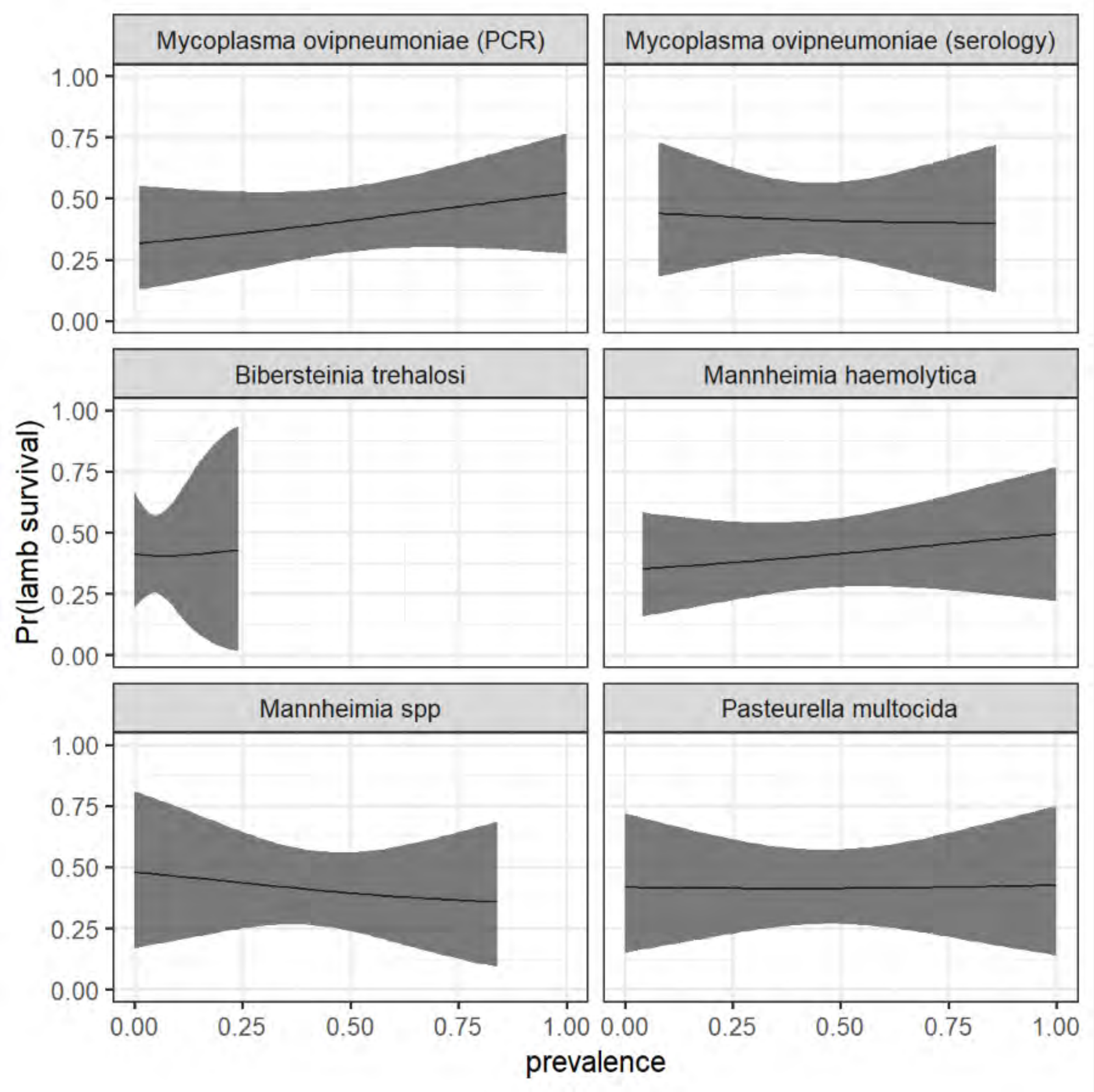


Figure 11 – Association between the probability of lamb survival and prevalence of pathogens for the Taylor-Hilgard bighorn sheep population during 2014-2018. The line shows the mean predicted relationship and the gray interval depicts the 90% CI.

characterized temporal variation in apparent prevalence of respiratory pathogens associated with pneumonia while accounting for both sampling variation and imperfect detection of pathogen diagnostic protocols. This rigorous assessment demonstrated that the full suite of respiratory pathogens associated with pneumonia were resident and circulating within the Hilgard bighorn sheep population. The apparent prevalence of these respiratory pathogens, including *M. ovipneumoniae*, varied substantially from year to year and showed no associations with variability in lamb survival rates. During the 5-year sampling period, the highest estimated prevalence of *M. ovipneumoniae* based on PCR testing was 0.94 and occurred in 2013-2014, the year with the highest estimated lamb survival (0.53). During this year, with nearly all adult ewes sampled testing positive for the presence of *M. ovipneumoniae* bacteria in their nasal cavities, it is reasonable to assume lambs in most, if not all, lamb-ewe groups were routinely exposed to *M. ovipneumoniae*, yet lamb survival was high compared to other reported estimates (Paterson et al. in review, Festa-Bianchet 1988, Smith et al. 2014), including this population during years with lower *M. ovipneumoniae* prevalence. This result was contrary to predictions that populations persistently infected with *M. ovipneumoniae* experience high rates of pneumonia-induced lamb mortality between 4 and 14 weeks of age (Cassirer and Sinclair 2007, Smith et al. 2014, Cassirer et al. 2018). Although supported in other bighorn and domestic sheep studies, our results are not consistent with the paradigm that persistently infected bighorn sheep populations experience poor lamb survival (Cassirer et al. 2018, Manlove et al. 2019).

Additionally, while only a modest number of lambs were sampled annually for pathogens our results provide additional evidence that challenges the current ideas regarding the relationship between *M. ovipneumoniae* and lamb mortality. Of the 27 lambs captured and tested during this study 84% were PCR positive for *M. ovipneumoniae* infection, 64% tested positive for exposure to *M. ovipneumoniae* based on the serum antibody test, and only 1 lamb tested negative for both the *M. ovipneumoniae* PCR and serum antibody tests. Thus, these results suggest that *M. ovipneumoniae* was transmitted to nearly all lambs in this herd by early winter when they were captured and tested, despite the high annual variation in estimated *M. ovipneumoniae* prevalence in the adult ewe population. So while the evidence suggests that nearly all lambs were infection with *M. ovipneumoniae* during this study our annual estimates of lamb recruitment and survival were high in 4 of the 5 years of the study (Figure 10, Table 2). High lamb infection rates were also reported for the intensively-studied Lostine herd in eastern Oregon that has a history of respiratory disease in both adults and lambs similar to the Hilgard herd. During a 4-year study, that involved annual captures and pathogen testing when animals were on winter range, Plowright et al. (2017) reported that 24 of 27 lambs were PCR positive for *M. ovipneumoniae* (Figure 4b in Plowright et al. 2017). These results from lambs, combined with similar evidence for adults, and general pathogen testing of bighorn herds throughout the species' range (Cassirer et al. 2017, Butler et al. 2018, and studies reported here) collectively suggests that the presence of *M. ovipneumoniae*, or any of the other respiratory pathogens associated with pneumonia in individual bighorn sheep or herds, does not necessarily equate to disease and a consequent reduction in an individual's or herd's demographic performance. Infection with pathogens clearly is requisite for respiratory disease and hence, the potential for disease to be a limiting factor, but our results suggest pathogen presence is not sufficient in itself for disease expression.

The data collected in this study, the other studies described in this chapter, as well as the broader literature related to bighorn sheep pneumonia consistently demonstrates wide variation in the vital rates and demographic performance of individuals and populations infected with respiratory pathogens. This heterogeneity impedes our ability to understand the disease process in bighorn sheep which is requisite for developing both proactive and reactive management strategies and interventions that can effectively reduce the frequency and severity of disease events to enhance bighorn sheep conservation and restoration. Clearly, multiple factors are interacting to influence the expression of disease, an idea captured in the traditional model of disease causation known as the epidemiological triad, the interactions between the pathogen, host, and environment. Factors hypothesized to be involved in the disease process and contributing to variable responses of bighorn populations to infection include interactions among multiple respiratory pathogens (Besser et al. 2008, 2013, Wood et al. 2017), pathogen dose or virulence (Hobbs and Miller 1992, Cassirer et al. 2013), previous exposure of hosts (Cassirer et al. 2013), heterogeneity in host immunity (Hobbs and Miller 1992), and genetics of the host populations (Plowright et al. 2017) and pathogens (Besser et al. 2012b, Cassirer et al. 2017). Additional hypothesized factors include spatial structuring and contact rates of infected animals both within and among populations (Hobbs and Miller 1992, Cassirer et al. 2013, Manlove et al. 2014, Butler et al. 2018), stress (Plowright et al. 2013), sinus tumors (Fox et al. 2015), population size (Sells et al. 2015), and age structure (Plowright et al. 2017). Given this long list of potential interacting factors that may be involved in disease expression in bighorn sheep, the scientific and management community faces a daunting challenge in gaining a better understanding of respiratory disease in bighorn sheep.

Despite our lack of understanding of the disease process in bighorn sheep, evidence suggests many infected populations are demographically robust (Butler et al. 2018, Paterson et al. in review, this chapter), suggesting some level of resilience to the pathogens. The Hilgard herd provides just 1 example of this resilience. The herd experienced 2 catastrophic die-offs associated with respiratory disease and recovered after the second die-off without direct management intervention. All the pathogens associated with pneumonia in bighorn sheep are circulating in the population, often at relatively high prevalence. Clinical signs of respiratory disease are frequently observed in individual animals on winter range and confirmed with occasional necropsies. The herd also experiences years of poor fall lamb recruitment that may suggest occasional bouts of lamb pneumonia when on summer range. Yet overall lamb and adult survival rates are good and population counts indicate a long period of increasing numbers such that the population attained sufficient size to serve as a source population for within range transplant operations. Why this population experienced a very different history than herds such as the Tendoys and Highland populations that failed to recover from pneumonia epizootics that occurred decades ago is uncertain. We suggest understanding of disease expression in bighorn sheep will require more intensive studies that include evaluations of a host of other factors that may be involved beyond the information on the presence and apparent prevalence of resident respiratory pathogens. While there is a strong focus on disease as the primary limiting factor for bighorn sheep populations, like other ungulates, bighorn sheep are exposed to a number of other ecological factors such as predation, weather, habitat alternations, and climate trends that may be important drivers of demographic performance that should also be considered in population management.

Chapter Summary

- The majority of bighorn sheep populations across Montana and Wyoming host a diverse suite of pathogens that have been implicated as causing respiratory disease (i.e., *Mycoplasma ovipneumoniae* and Pasteurellaceae family bacteria that include *Mannheimia haemolytica*, *Bibersteinia trehalosi*, and *Pastuerella multocida*).
- PCR-based protocols improved, often substantially, detection probability estimates of pathogens as compared to culture-based protocols, demonstrating that prevalence estimates for culture-based protocols are strongly biased when detection rates are not accounted for and require intensive sampling to achieve higher precision.
- In populations where *M. ovipneumoniae* was detected, recruitment rates were lower. However, populations where both *M. ovipneumoniae* and leukotoxigenic Pasteurellaceae were detected had average or increasing recruitment rates, indicating populations may be demographically robust while hosting all respiratory pathogens.
- By using an app (https://quantitativebiology.shinyapps.io/pathogens/) developed to incorporate detection probability estimates and temporal variation in sampling of all standard diagnostic protocols used to assess respiratory pathogens, managers are provided with a tool to perform rigorous evaluations of pathogen testing results that will produce more accurate results to inform decision-making.
- Multiple factors are likely interacting to influence the expression of disease in infected bighorn populations and remains a daunting challenge to understand, however, there is strong evidence that some herds are resilient to infection with respiratory pathogens and can remain demographically robust.

Peer-Reviewed Science Products

Butler, C., W. Edwards, J. Jennings-Gaines, H. Killion, D. McWhirter, M. Wood, J. T. Paterson, K. Proffitt, E. Almberg, J. Ramsey, P. White, J. Rotella, & R. Garrott. 2017. Assessing respiratory pathogen communities in bighorn sheep populations: sampling realities, challenges, and improvements. PLoS One. https://doi.org/10.1371/journal.pone.0180689.

Butler, C., W. Edwards, J. T. Paterson, K. Proffitt, J. Jennings-Gaines, H. Killion, M. Wood, J. Ramsey, E. Almberg, S. Dewey, D. McWhirter, A. Courtemanch, P. White, J. Rotella, & R. Garrott. 2018. Respiratory pathogens and their association with population performance in Montana and Wyoming bighorn sheep populations. PLoS One. https://doi.org/10.1371/journal.pone.0207780.

Paterson, J. T., C. Butler, R. Garrott, & K. Proffitt. 2020. How sure are you? A web-based application to confront imperfect and uncertain detection of respiratory pathogens in bighorn sheep. PLoS One.

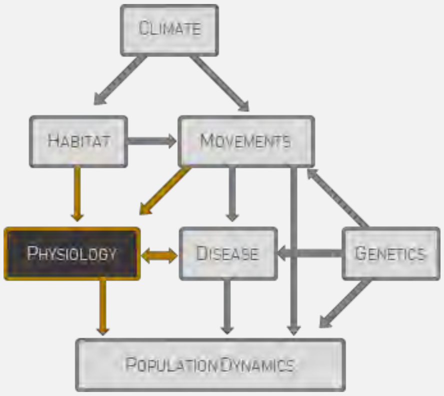
https://doi.org/10.1371/journal.pone.0237309.
https://quantitativebiology.shinyapps.io/pathogens/

Peer-Reviewed Science Products (continued)

Garrott, R., J. Cunningham, J. T. Paterson, C. Butler, B. Lowrey, H. Edwards, J. Rotella, K. Proffitt, & E. Lula. In preparation. Temporal variation in respiratory pathogens and demographic attributes of a bighorn sheep population. PLoS One.

CHAPTER 3

DEVELOPING A HEALTH PANEL FOR BIGHORN SHEEP



Objective Describe and explore advanced serum assay technologies useful for understanding the health and physiological status of bighorn sheep. Discuss the next steps for developing a health panel for bighorn sheep by identifying informative metabolites for indexing dietary intake and physiological processes within bighorn sheep.

THE QUANTITY and quality of forage available to ungulates on their ranges dictates their nutrition and body condition, which, in turn, influences survival and reproduction (Keech et al. 2000, Cook et al. 2004, 2013, Bender et al. 2008, Parker et al. 2009). Recent work in the Pacific Northwest suggests widespread occurrence of inadequate summer nutrition that limits adult fat accretion, pregnancy rates, and calf and yearling growth rates in elk (Cook et al. 2013), with similar limitations expected for other wild ungulates as well. These results highlight the need to evaluate potential bottom-up (i.e., habitat) drivers of ungulate population dynamics. The evaluation of nutritional status across populations with varying demographic characteristics may provide insights as to the extent nutrition explains variation in demographic rates and may also be associated with expression of respiratory disease. This research project assessed body condition and nutrition using two distinct, but integrated, methods. Body condition of adult females was assessed using field-based measurements including body morphometrics, ultrasonography, and traditional body condition scoring, while physiological and nutritional condition were assessed using state-of-the-art serum-based assays. In this report section, we will focus on our research efforts to develop and evaluate physiological assessment of animal condition and health based on serum assays.

METABOLOMICS

Studying global metabolism is known as metabolomics, or the study of metabolic intermediates and products of cellular metabolism. Metabolomics is a rapidly expanding research field because it can explain the functional nutritional and health states of an animal and is currently being applied in human and domestic livestock research to study a variety of physiological processes including disease and feed efficiency trials. The catalyst for this emerging technology is the development of sophisticated new analytical machines and the integration of multiple assay technologies that permit efficient and precise quantitative estimates of many 10s to 100s of biological molecules from less than 1 mL of serum or plasma, producing a rich dataset associated

with a myriad of physiological processes. Traditional serum assays, in contrast, require performing individual, labor-intensive, and costly 'bench-top' assay procedures for each biological molecule of interest.

To pursue the potential utility of metabolomics to contribute to our ecological understanding and management of bighorn sheep and other wild ungulates, we assembled a team of scientists with complimentary expertise from three additional academic departments on the MSU-Bozeman campus. Dr. Valerie Copie (faculty) operates the Nuclear Magnetic Resonance (NMR) Center in the Department of Chemistry and Biochemistry and provided access to the machines for performing the assays and contributing technical expertise associated with NMR technology. Dr. Jim Berardinelli (faculty) and Rashelle Lambert (recent M.S. student), are animal physiologists in the Animal and Range Sciences Department and are responsible for the development of all sample assay protocols and are our experts in interpreting assay results with respect to physiological processes. Because NMR technology generates large and complex datasets that require specialized statistical expertise in machine learning techniques in order to extract biological insight, we recruited Drs. Mark Greenwood (faculty) and Jennifer Weeding (recent Ph.D. student) in the Department of Mathematical Sciences to our team to lead the statistical analyses.

The goal of this collaboration was to identify a suite of metabolites and metabolic hormones that can be used to assess nutrition, body condition, and disease status of bighorn sheep using the same assay and analytical techniques that are being aggressively pursued in the fields of domestic animal production and human medicine. We aspire, as an end product from this work, the development of a 'health panel' of biological molecules that can be economically and rigorously quantified from a small volume of serum and that can be readily interpreted by wildlife managers to understand the nutritional status, disease, and physiological stresses on bighorn sheep populations. We anticipate that such a health panel, if successfully developed, would likely have similar utility for other wild ungulates.

DEVELOPING A BIGHORN SHEEP METABOLOMICS DATA SET

Over the past 4 years, we have assayed 562 serum samples collected during captures of 14 wild bighorn sheep populations in Montana and Wyoming in conjunction with the Montana Statewide Bighorn Sheep Research Project and the GYA Mountain Ungulate Research Project. Most of the Montana samples came from animals in the Castle Reef, Fergus, Lost Creek, Paradise, and Hilgard populations, with most Wyoming samples originating from animals in the Absaroka, Dubois, and Jackson populations. In addition, we assayed serum samples from 2 captive bighorn sheep research facilities in Colorado and South Dakota and also included samples from a small experimental flock of domestic sheep (Rambouillet) used by Rashell Lambert for her thesis research. These samples represented animals suspected of experiencing a range of physiological conditions including gradients in dietary intake, degree and duration of starvation, and transitions from a healthy to a disease (pneumonia) state.

Sample processing and NMR assay techniques have been refined over the course of the development of this project, gradually building the library of biological molecules we can accurately identify and precisely quantify from 32 to 78. In addition to these NMR-based molecules, we also performed traditional assays to quantify non-esterified fatty acids (NEFA) and total protein (TP) because they cannot be identified using NMR. NEFA is a metabolite associated with an animal's available energy reserves, where high NEFA concentrations reflect the mobilization of fat. TP concentrations reflect dehydration status and presence of acute infections.

EVALUATING ANALYTICAL TECHNIQUES FOR THE METABOLOMICS DATA SET

Extracting biological insight from such a large and complex dataset is challenging and is approached using statistical learning techniques, with no clear guidelines yet on which analytical tools are best for particular datasets. So, while the successful development of our large metabolomics dataset represents a major accomplishment in this emerging field, we were also challenged with exploring the most appropriate analytical tools to pursue our goal of developing a health panel for bighorn sheep. We still have considerable work to perform before we understand what we can accomplish with this research effort, but we can illustrate the approach we are taking with an example of an ongoing analysis of 518 samples where our goal is to discriminate the metabolic profiles of 4 subsets of samples from animals that we assume were experiencing differing levels of dietary intake at the time the blood samples were collected.

Captive animals provided 2 categories of dietary intake at or above daily maintenance requirements. We have 15 samples from captive bighorn sheep from a Colorado research facility that were fed a ration exceeding daily energetic requirements that was labeled 'high' dietary intake. Samples from a flock of 31 domestic sheep that were receiving a ration that just meant daily energetic requirements was labeled 'moderate' dietary intake. All samples from wild bighorn sheep were obtained between December and mid-March when dietary intake was assumed to be sub-maintenance due to senescence of plants on native range and limited access to forage due to snowpack. A total of 367 samples from wild bighorn sheep captured using helicopter net-gun techniques were labeled 'very low' dietary intake. Additionally, 105 wild bighorn samples originated from animals that were captured under baited drop-nets. The dietary intake from range forage for these animals was modestly augmented for appropriately 2 weeks prior to

Table 3 - A comparison of 3 analytical techniques (boosting, random forest, and partial least squares-discriminant analysis) to discriminate among 4 categories of dietary intake (high, moderate, low, very low) based on the metabolic profiles of 518 wild bighorn sheep and a small flock of domestic sheep.

BOOSTING	REFERENCE			
PREDICTION	HIGH	MODERATE	LOW	VERY LOW
HIGH	3	0	0	0
MODERATE	0	13	0	0
LOW	0	0	39	0
VERY LOW	5	0	14	184

92.3% correct classification

RANDOM FOREST	REFERENCE			
PREDICTION	HIGH	MODERATE	LOW	VERY LOW
HIGH	4	0	0	0
MODERATE	0	13	0	0
LOW	1	0	35	0
VERY LOW	3	0	17	172

91.4% correct classification

PLS-DA	REFERENCE			
PREDICTION	HIGH	MODERATE	LOW	VERY LOW
HIGH	4	0	0	1
MODERATE	0	13	0	0
LOW	0	0	37	5
VERY LOW	4	0	15	166

89.8% correct classification

capture due to the daily baiting of the drop-net sites so we assume these animals had an overall higher daily dietary intake than the net-gunned bighorn sheep and, hence, these samples were labeled 'low'. The samples from each group were split evenly into a training dataset that was used with various analytical procedures to build models to discriminate among the 4 dietary intake groups and a training dataset that was used to validate the models. We incorporated 52 metabolites that were detected in all samples into this analysis. In addition, we included 5 specific metabolite ratios commonly considered in physiological assessments into the dataset resulting in a metabolic profile of 57 potential predictors of dietary intake.

We evaluated 3 statistical methods that each employs a different analytical technique for determining the suite of metabolites that best discriminate the 4 categories of dietary intake, known as variables of importance. The most common statistical method employed in metabolomics is partial least squares-discriminate analysis (PLS-DA) which measures variable importance based on the weighted sums of the absolute regression coefficients. We also evaluated 2 extensions of classification tree techniques, random forests, and boosting, which are similar to regression tree techniques except classification trees predict a qualitative response rather than a quantitative one (James et al. 2017). Results of the dietary intake analyses suggest that all three analytical techniques produced comparable results with excellent discrimination among 3 of the 4 dietary intake categories (Table 3). All of the models did poorly at predicting the high dietary intake animals, but we suspect the poor discrimination for this category was likely due to the small number of samples (n = 7) in the training dataset.

IDENTIFYING INFORMATIVE METABOLITES FOR INDEXING DIETARY INTAKE

The key outcome of this metabolomics analysis is the identification of the most important metabolites (biomarkers) for discriminating among the 4 categories of dietary intake. Figure 12 presents the relative influence of the 15 top-ranked metabolites and metabolite ratios from the boosting model that provided the best discrimination among the 4 categories of dietary intake. These results suggest that approximately 10 of the 57 potential predictors evaluated contribute most of the information that results in the strong discrimination among the categories of dietary intake. These biomarkers that provide good discrimination among the categories of dietary intake are associated with a variety of physiological processes which is only the initial step in the development of the envisioned 'health panel.'

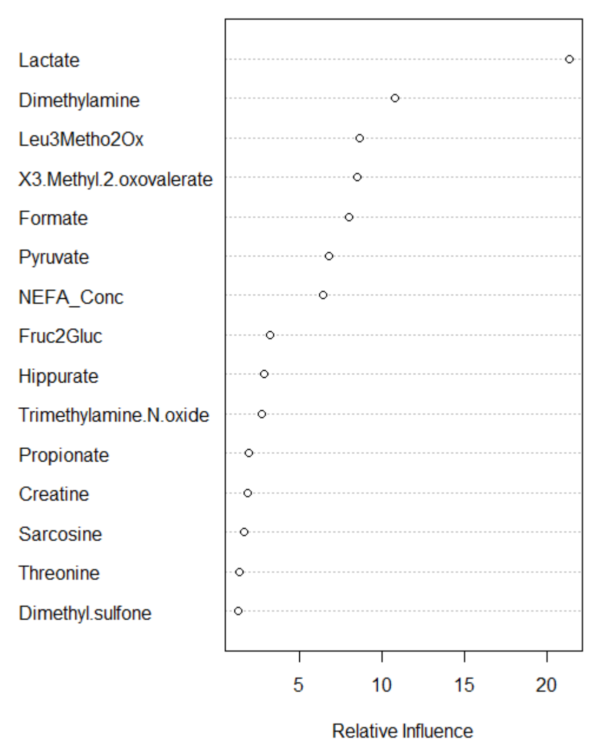


Figure 12 - The relative influence of the top 15 metabolites and metabolite ratios from the boosting model that provided the best discrimination among the 4 categories of dietary intake.

The ultimate goal of this work is to identify specific biomarkers for each physiological process of interest and interpret the quantitative values of the biomarkers with respect to that process. Figure 13 provides a visualization of the distribution of the quantitative values for a sample of the biomarkers identified as top predictors, demonstrating the potential of moving from qualitative to quantitative assessments.

In the future, we will complete additional analyses of our metabolomics dataset including assessments of gradients of nutritional deprivation based on expected degrees of depletion of body fat and lean body mass for subsets of wild bighorn sheep sampled early, mid, and late winter. In addition, we will be conducting analyses of a unique dataset containing metabolite profiles for 122 serum samples from a captive population of bighorn housed in South Dakota. These animals were repeatedly sampled over more than a year during which time most animals transitioned from a healthy state to various stages and severities of pneumonia, with most animals ultimately dying and a few recovering. For most of these samples, there is an associated value recorded that is an index of clinical signs of disease at the time each sample was collected. These data will be used to evaluate potential respiratory disease biomarkers.

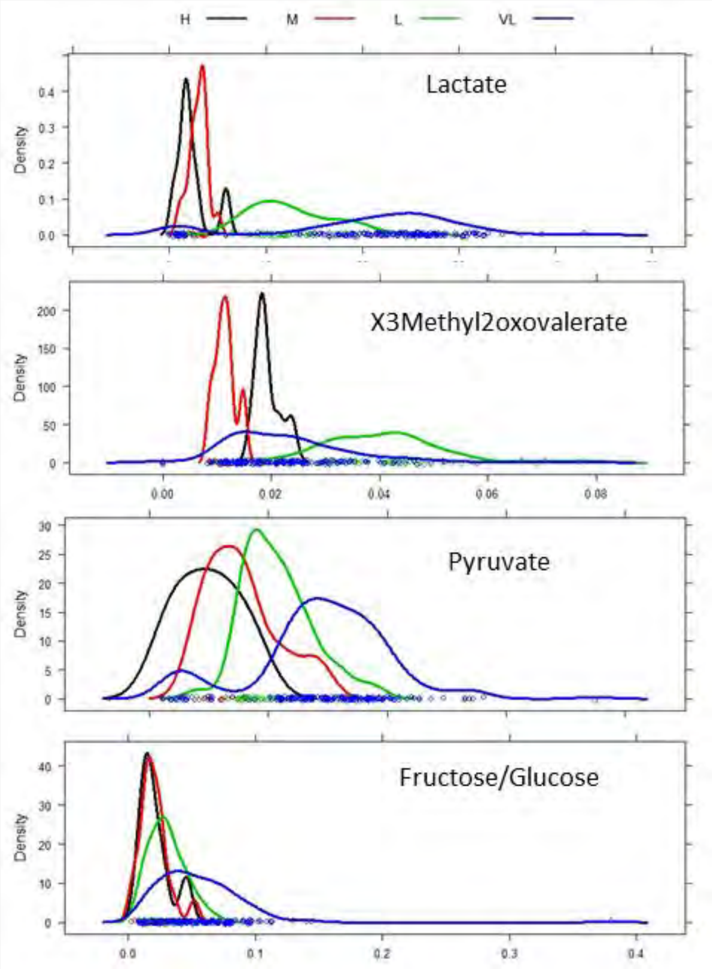


Figure 13 - The distribution of values for 4 metabolites and metabolite ratios identified by the boosting model as biomarkers for discriminating among the 4 categories of dietary intake (H, M, L, and VL denote high, moderate, low, and very low dietary intake, respectfully).

Chapter Summary

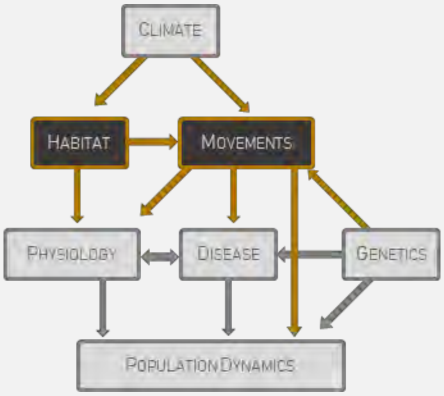
- Linking metabolomics (metabolic intermediates and products of cellular metabolism) to the nutritional and health states of animals may provide insights into the extent nutrition explains variation in demographic rates and expression of respiratory disease.
- Less than 1 mL of serum may provide efficient and quantitative estimates of many 10s to 100s of biological molecules associated with a myriad of physiological processes.
- Work is ongoing to develop a ‘health panel’ that can be readily interpreted by wildlife managers to understand the nutritional status, disease, and physiological stresses on bighorn sheep populations.

Peer-Reviewed Science Products

O’Shea-Stone, G., R. Lambert, B. Tripet, J. Berardinelli, J. Thomson, V. Copié, & R. Garrott. In review. ^{1}H NMR based metabolic profiling distinguishes the differential impact of capture techniques on wild bighorn sheep. Scientific Reports.

CHAPTER 4

MOVEMENTS, MIGRATORY STRATEGIES, & HABITAT MODELS



Objective Provide a descriptive summary of movement patterns and migratory strategies observed in the study populations and interpret the observed movement patterns with respect to the development of diverse migratory behaviors. Develop environment-specific habitat models to support broad restoration efforts across the entire Montana landscape. The fine spatial and temporal data collected from GPS collared ewes from each of the study populations provided an opportunity to gain new and important insights on patterns of movements and habitat selection that is relevant to improving the management and restoration of bighorn sheep.

ANIMAL MOVEMENTS across landscapes and their use of habitats can directly influence vital rates and demographic performance (Manly et al. 2002). Understanding movement patterns and habitat use can, therefore, be valuable for informing management and restoration decisions, particularly given the diversity of management histories and landscapes occupied by bighorn sheep across Montana. The fine spatial and temporal scale location data obtained from the GPS collared ewes across the 8 study populations provided the opportunity to accurately describe, compare, and evaluate variation in movement patterns, migratory behaviors, and habitat selection across populations.

The GPS collars were programmed to record and store location information every 4 hours for a period of approximately 21 months. These collars were further equipped with a mechanism, programmed to release the collar from the animal on a scheduled date, after which the paired VHF collar (see Chapter 1) began transmitting so as to continue survival monitoring for an additional 3-5 years. Using telemetry, field crews navigated to the released GPS collars and retrieved the stored data for analysis. Among all study populations, fix success averaged 95.1% (range 88.7 – 97.6%), and, after censoring for imprecise GPS locations, a total of 643,431 GPS locations were available for spatial analysis, averaging about 71,492 locations per population.

We partitioned our research of the spatial data analyses into 6 categories relevant to specific management and restoration interest. These categories include: 1) a descriptive narrative of seasonal movement patterns within and across study populations, 2) an evaluation of the migratory behaviors within and among study populations that included additional populations located in Idaho, Wyoming, and Colorado for broader insights, 3) the identification and evaluation of potential mineral licks based on low elevation summer migrations of individuals,

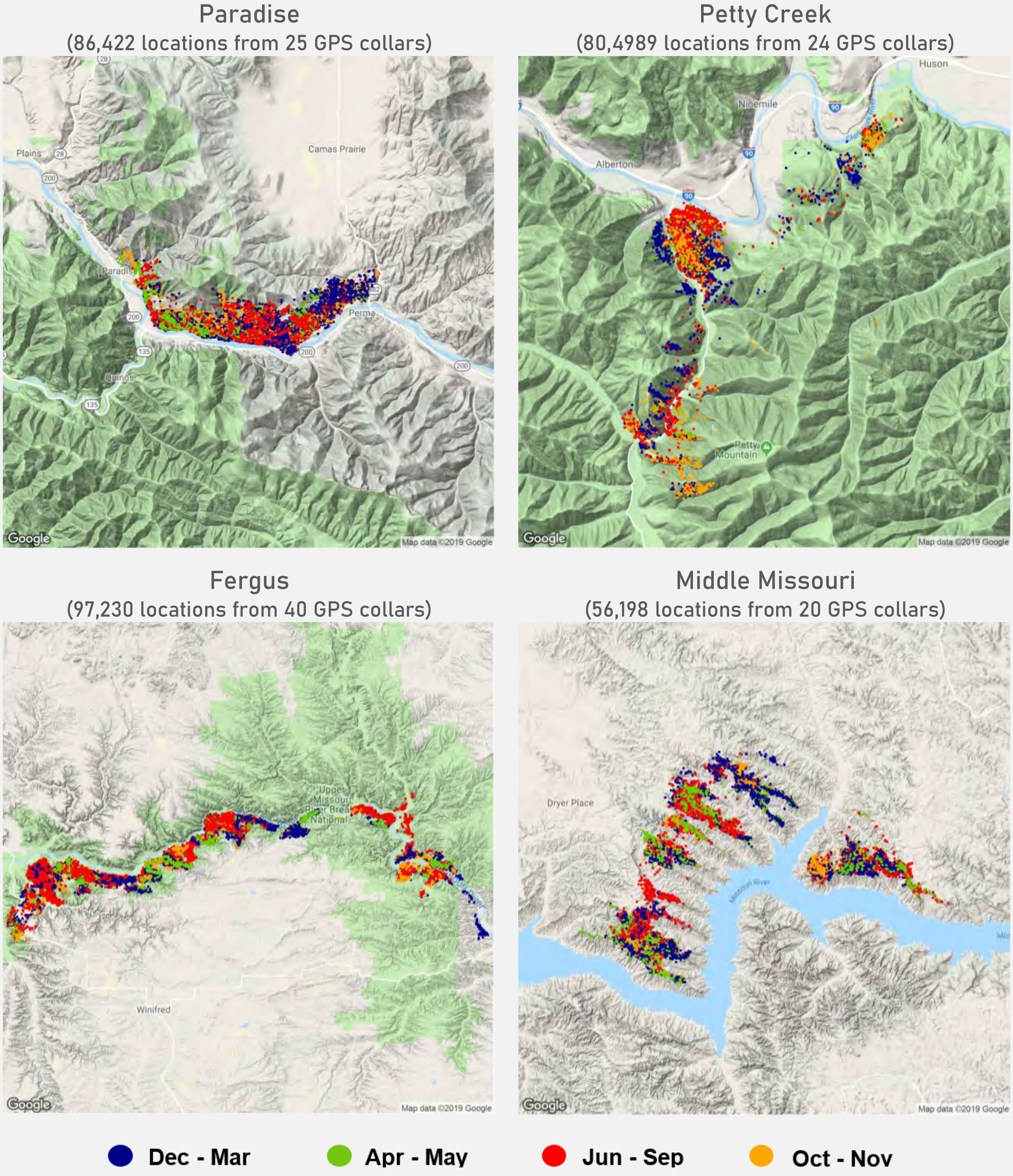
4) habitat modeling specific to the Madison Range and demonstrating intramountain restoration opportunities, 5) migratory behavior-specific habitat modeling for restoration planning in montane environments of western Montana, and 6) habitat modeling for restoration planning in prairie environments of eastern Montana.

SEASONAL MOVEMENTS

Based on the GPS locations, each study population demonstrated general movement tendencies typical of either non-migratory/resident or migratory behaviors (Figure 14, Figure 15, Figure 16). Four populations were non-migratory, remaining resident on their winter ranges throughout the year and included the Paradise, Petty Creek, Middle Missouri, and Fergus populations (Figure 14, Figure 16). Four populations were migratory, including Castle Reef, Stillwater, Hilgard, and Lost Creek (Figure 15, Figure 16). During the summer, these populations demonstrated movements away from lower elevation winter range to higher montane elevations. Movement to high elevation areas during the summer generally varied by individual within the migratory populations, with some individuals occupying lower elevation summer range than others (Figure 16).

MIGRATORY STRATEGIES

Animal migration is one of the most inspiring and important aspects of ecology, yet habitat destruction, barriers along migratory routes, overexploitation, and climate change have resulted in steep declines of migratory behavior across many taxonomic groups (Bolger et al. 2008, Wilcove and Wikelski 2008, Milner-Gulland et al. 2011). While migration continues to decline broadly, GPS technology has enhanced our ability to track animals over small temporal and expansive spatial scales, and in so doing, highlighted the prevalence and diversity of migratory behaviors in native systems that are less impacted by anthropogenic disturbances. Consequently, individual variation in migratory behavior is being increasingly well documented. Ecological theory and empirical results across many migratory taxa have demonstrated population-level demographic benefits resulting from diverse individual migratory behaviors and the congruent diversity in seasonal ranges. For example, the portfolio concept illustrates the demographic benefits of a diverse portfolio of individual migratory


Paradise
(86,422 locations from 25 GPS collars)

Petty Creek
(80,4989 locations from 24 GPS collars)

Fergus
(97,230 locations from 40 GPS collars)

Middle Missouri
(56,198 locations from 20 GPS collars)

Figure 14 - Seasonal locations from GPS collared adult ewes for the 4 non-migratory study populations.

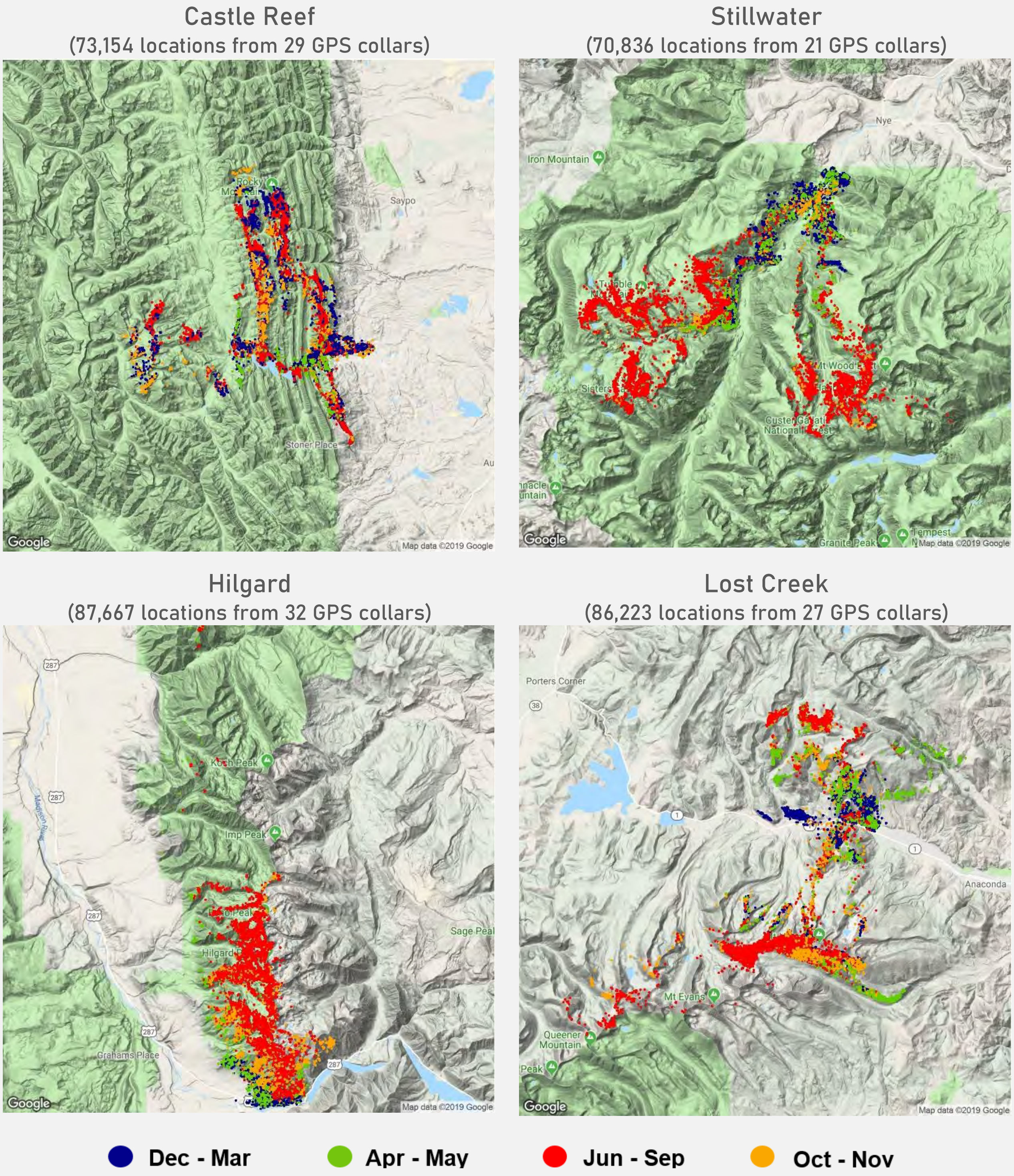


Figure 15 - Seasonal locations from GPS collared adult ewes for the 4 migratory study populations.

behaviors (i.e. life history traits) of anadromous fishes (Schindler et al. 2010). While the dynamics of a single life history trait are inherently volatile, when viewed in aggregate, asynchrony among life history traits results in more stable abundances through time and reduced risk (Schindler et al. 2010, Griffiths et al. 2014). Within migratory ungulates, however, the study of individual variation has largely focused on the ecological (e.g., spatial, temporal, and demographic) differences between resident and migratory components of partially migratory species (i.e., Hebblewhite and Merrill 2009, Middleton et al. 2013, Rolandsen et al. 2017) with little focus on migratory diversity.

We used GPS location data collected from 209 female bighorn sheep to characterize population and individual migration patterns along elevational and geographic continuums for 18 populations of bighorn sheep with different management histories (i.e., restored, augmented, and native) across the western United States. The analysis comprised populations where migratory behavior might be expected, including the 6 montane study populations (excluding Fergus and Middle Missouri) as well as 1 population in Yellowstone

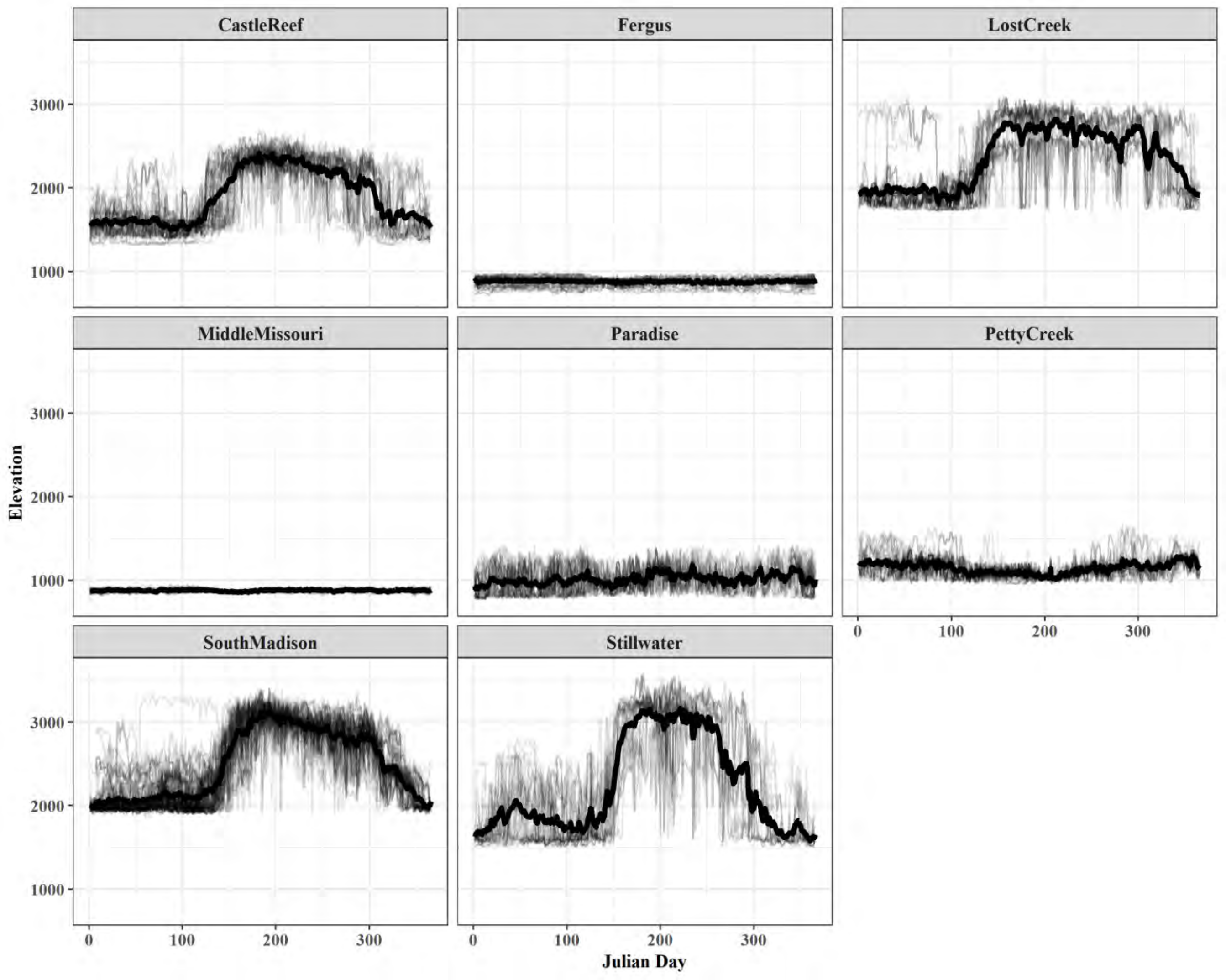


Figure 16 – Elevational profiles of each study population showing individual movements (thin black lines) and the population average (thick black line).

N.P., 7 populations in Wyoming, 2 populations in Idaho, and 2 populations in Colorado (Figure 17; Table 4). We characterized seasonal migrations between summer and winter core ranges, defined using the location data collected from 15-Jan to 28-Feb for winter and 15-Jul to 15-Aug for summer. We characterized geographic distance by measuring the Euclidian distance between centroids (mean coordinates) of the GPS locations collected within the respective core seasonal range date interval. We characterized elevational distance as the seasonal difference between the mean elevations of GPS locations within the respective seasonal periods. Lastly, we described population-level migration using the median elevation and geographic distance and individual variation within a population according to the 10th and 90th percent distribution quantiles among individuals.

Resident individuals, with little to no elevational and geographic distance between core seasonal ranges, occurred in all 3 management histories. Seasonal migrations that spanned elevational gradients (i.e., elevational migrations) were the most common migratory behavior with an average elevational difference of 521 m (± 504 SD), 840 m (± 345 SD), and 484 m (± 413 SD) for restored, augmented, and native populations, respectively. Native populations had a greater range of population-level elevational migrations, which occurred over longer geographic distances in many populations (Figure 18). The average geographic migration distances were 6.5 km (± 5.1 SD), 8.7 km (± 2.5 SD), and 12.4 km (± 8.2 SD) for restored, augmented, and native populations, respectively. While 15 and 11 km marked the near maximum geographic migration distance for restored and augmented populations, native populations tended to move over longer geographic distances, including a maximum median distance of 27 km (Figure 18).

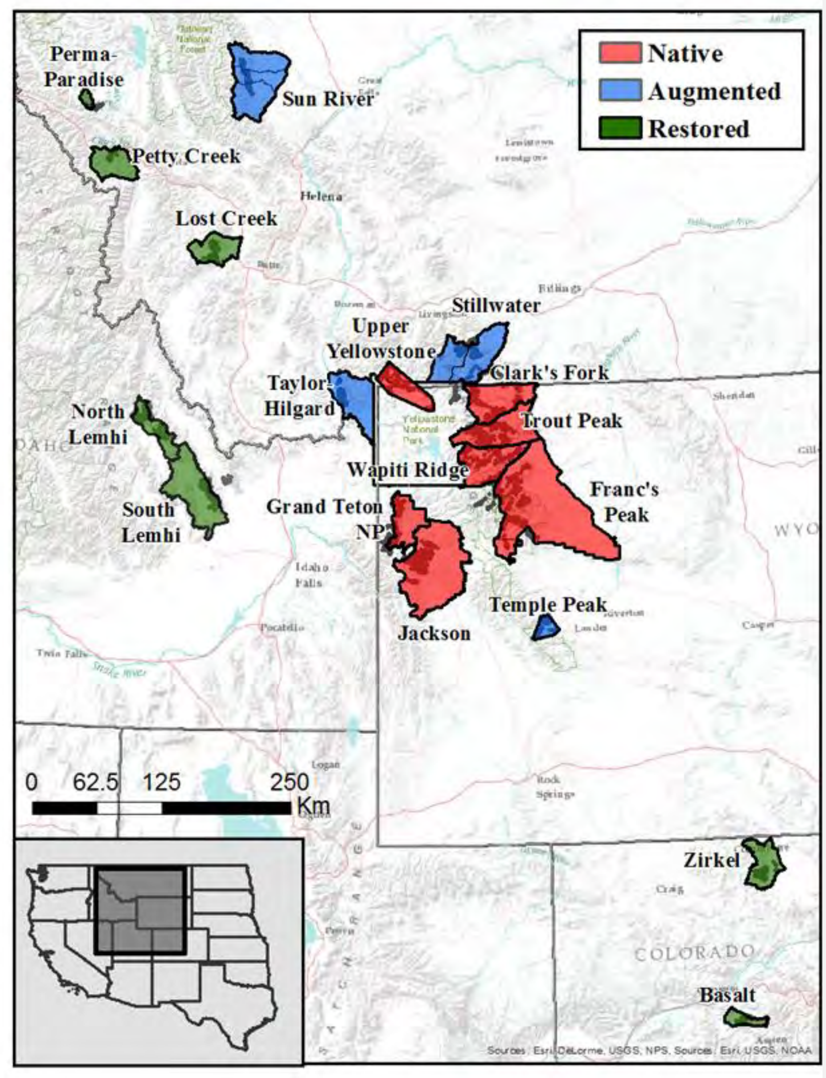


Figure 17 - Native (red; n = 7), augmented (blue; n = 4), and restored (green; n = 7) population units used to characterize female bighorn sheep migration patterns in Montana, Wyoming, Idaho, and Colorado, 2008 - 2017.

There were notable differences in individual variation within a population among the 3 management histories. As predicted, relative to native populations, restored and augmented populations had less variation among individuals with respect to elevational and

Table 4 – Summary information for the study populations in Montana and additional populations in Wyoming, Idaho, and Colorado, 2008 – 2017.

STATE	HERD UNITS					TRANSLOCATION HISTORY			
	NAME	N	MANAGEMENT UNITS[1]	POPULATION ESTIMATE[2]	HERD TYPE	YEAR	NUMBER	SOURCE[3]	MIGRATORY BEHAVIOR OF SOURCE POPULATION
MT	Paradise	14	HD 124	352	Restored	1979	14	WHI	Resident
						2011	22	WHI	Resident
MT	Petty Creek	14	HD 203	160	Restored	1968	16	MT-422	Migratory
						1985	4	NBR	Resident
MT	Lost Creek	10	HD 213	100	Restored	1967	25	MT-422	Migratory
						1985	2	MT-121	Migratory
MT	Hilgard	15	HD 302	280	Augmented	1988	19	MT-121	Migratory
						1989	5	MT-121	Migratory
						1989	19	MT-213	Migratory
						1993	26	WHI	Resident
MT	Castle Reef	12	HD 422, 424	150	Augmented	1960	8	MT-422	Migratory
MT	Stillwater	13	HD 501, 502	75	Augmented	1968	2	MT-422	Migratory
						1970	2	MT-422	Migratory
						1984	3	NBR	Resident
MT	Upper Yellowstone	10	HD 305, northwest YNP	320	Native	–	–	–	–
WY	Clark's Fork	19	HD 1, northeast YNP	600	Native	–	–	–	–
WY	Trout Peak	11	HD 2	700	Native	–	–	–	–
WY	Wapiti Ridge	7	HD 3	850	Native	–	–	–	–
WY	Franc's Peak	17	HD 5, 22	840	Native	–	–	–	–
WY	Grand Teton NP[4]	14	GTNP	100	Native	–	–	–	–
WY	Jackson	16	HD 7	450	Native	–	–	–	–
WY	Temple Peak[4]	8	-	50-75	Augmented	1960	1	WY-Whiskey	Partial
						1964	20	WY-Whiskey	Partial
						1965	20	WY-Whiskey	Partial
						1966	18	WY-Whiskey	Partial
						1971	13	WY-Whiskey	Partial
						1972	39	WY-Whiskey	Partial
						1987	54	WY-Whiskey	Partial
ID	North Lemhi	9	37A, 29	129	Restored	1986	18	OR-Lostine	Migratory
						1988	13	ID-36A	Migratory
						1989	23	ID-36B	Partial
ID	South Lemhi	6	51, 58	40	Restored	1983	19	WY-Whiskey	Partial
						1984	22	WY-Whiskey	Partial
CO	Zirkel	7	S73	120-130	Restored	2004	26	CO-S65	Unk
						2005	14	CO-S65	Unk
CO	Basalt	7	S44	70	Restored	1972	18	CO-S10	Unk

[1] The aggregation of management units within each herd unit is further described in Lowrey et al. 2019.

[2] Estimates were provided by area biologists and determined from local knowledge, minimum counts, and recent trends.

[3] WHI: Wild Horse Island; NBR: National Bison Range.

geographic distance (Figure 18 and Figure 19). The differences were most pronounced for geographic distances, where the majority of native populations had a range of variation between the 90th and 10th percent distribution quantiles that was 2 to 4 times greater than in restored or augmented populations (Figure 19). Moreover, individual migrations in native populations spanned a continuum of elevation and geographic distances. In contrast, rather

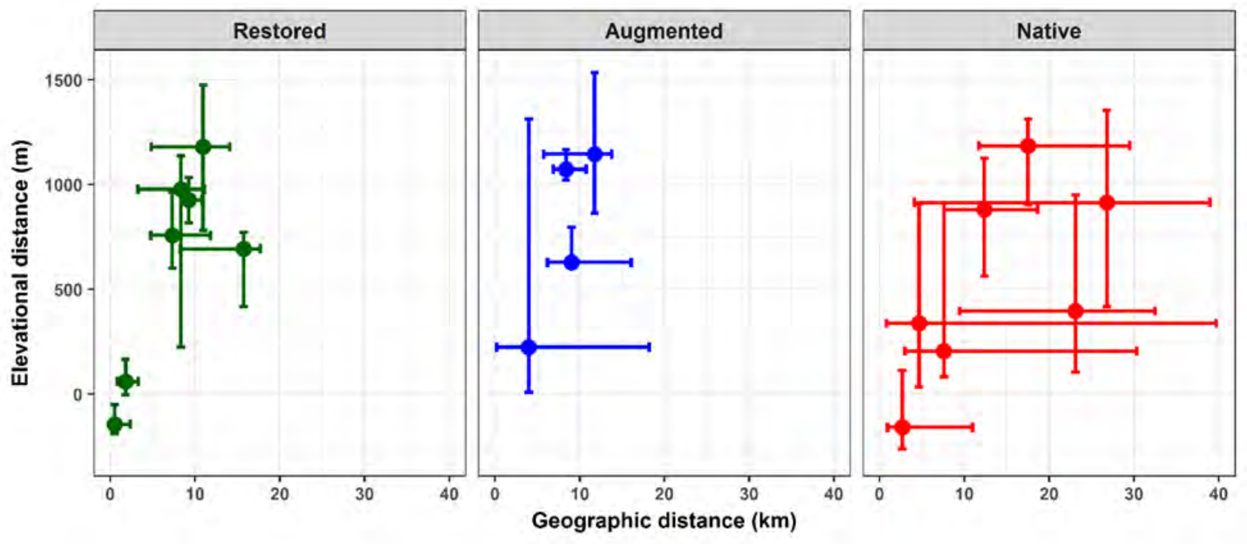


Figure 18 - Migration characterizations with respect to elevational and geographic distance between core seasonal ranges for restored (green), augmented (blue), and native (red) populations of female bighorn sheep in Wyoming, Montana, Idaho, and Colorado, 2008 – 2017. Filled circles represent population-level median values. Individual variability is described with the 10th and 90th percent distribution quantiles. Populations with elevational distance below zero had a winter range that was higher than the summer range. Paradise and Petty Creek are the lower left restored populations, while Grand Teton National Park is the lower left native population.

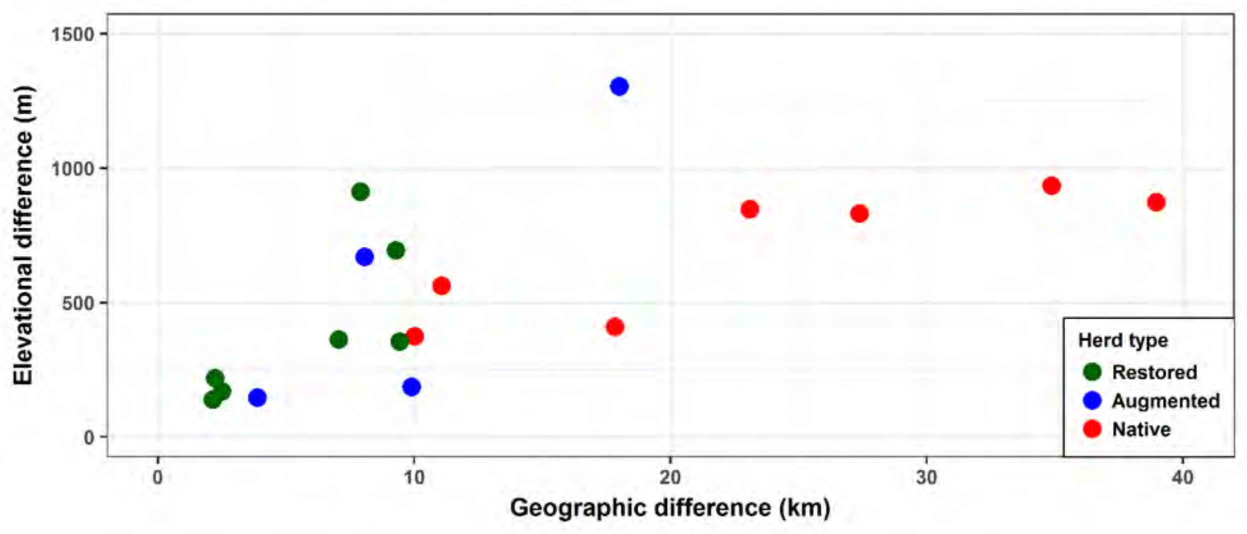


Figure 19 - Range of variation in elevational and geographic distances among individuals within a population, Wyoming, Montana, Idaho, and Colorado, 2008–2017. Each point represents the difference between the 90th and 10th percent quantile for restored (green), augmented (blue), and native (red) populations of female bighorn sheep.

than reflect a continuum of migratory patterns, the limited variation in restored and augmented populations was driven largely by the resident and migrant behaviors characteristic of partially migratory populations (Figure 18).

This work presents a novel and broad-scale characterization of population and individual migration patterns of bighorn sheep from restored, augmented, and native populations using metrics of elevational and geographic distance between seasonal ranges. Although elevational migrations were common among management histories, there was variation in the distances over which elevational migrations occurred. Migrations in native populations occurred over relatively long geographic distances and were characterized by appreciable variation among individuals along both distance continuums and a range of variation that was up to 4 times greater than restored or augmented populations. In contrast, the migrations within restored and augmented populations where shorter, especially with respect to geographic distance, and had notably less variation among individuals within a population. While restoration efforts, largely through translocations, have restored elevational migrations in some areas, our results indicate restoration efforts have not successfully restored long-distance migrations or the migratory diversity observed in native populations.

While nearly a century of bighorn sheep restoration has resulted in modest increases in distribution and abundance, seasonal migrations in restored and augmented populations do not mirror the diversity observed in native populations. Although we do not describe a direct demographic benefit from the longer and more diverse migrations observed in native bighorn sheep populations, the theoretical and empirical evidence supporting migratory diversity in other taxa (Webster et al. 2002, Schindler et al. 2015, Gilroy et al. 2016) suggests future work to link migratory diversity and demography in terrestrial ungulates is warranted. In addition to increasing the abundance and distribution of bighorn sheep on the landscape, we suggest there is value in simultaneously increasing migratory diversity, and in so doing, building resilience to future perturbations and mirroring the migratory portfolios observed in native populations.

SUMMER MIGRATIONS & MINERAL LICKS

Deficiencies in trace minerals are a common cause of impairment to organisms' physiological functions and can negatively affect the demographic vigor of populations. Although we understand a great deal about trace mineral function, requirements, and their effect on production and survival of domestic ungulates, our understanding of trace minerals in wild ungulates is limited. We do know that wild ungulates obtain many essential trace minerals through their diets; however, when ingested plants do not meet the mineral requirements of the animal, ungulates will mitigate deficiencies through geophagia, or the behavior of eating soil (Robbins 1983). This behavior generally occurs at areas called mineral licks where the soil has elevated concentrations of trace minerals compared to the surrounding landscape (Ayotte et al. 2006).

The use of mineral licks by bighorn sheep has been well documented (Geist 1971, Jones and Hanson 1985, Mincher et al. 2007, Rice 2010), but there is little known about the timing and duration of mineral lick use or travel distances for bighorn sheep in the Rocky Mountains. Moreover, only a few studies have investigated mineral concentrations of lick sites used by mountain ungulates (Stockstad 1953, Jones and Hanson 1985, Ayotte et al. 2006, Rice 2010). A qualitative review of elevation profile graphs generated from a large dataset of global positioning system (GPS)-collar locations of seasonally migratory female bighorn sheep in Montana revealed common, short-duration movements during the summer months to low elevations (Figure 20 and Figure 21). We define these movements as summer migrations, and have hypothesized that they are the result of

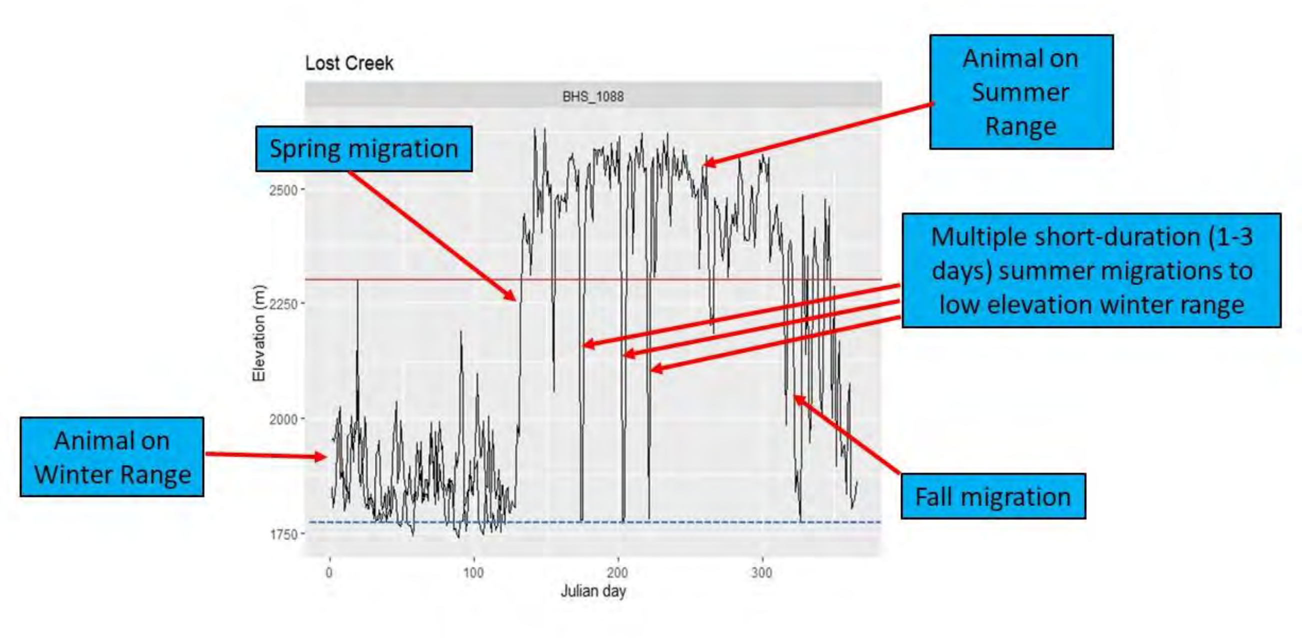


Figure 20 - Example of an elevation profile obtained for an individual bighorn sheep female from the Lost Creek herd based on GPS locations obtained during 2017. The horizontal red line indicates the 10th percentile elevation for the summer period (~Julian days 165-300). The dashed blue line marks the similar lowest elevation for multiple summer migrations. These lowest elevation locations during summer migrations indicate possible visitation to the same site multiple times throughout the summer.

bighorn sheep seeking trace minerals that are limited on summer range.

In this research, we sought to provide insight into these low elevation summer movements by 1) identifying potential mineral lick sites, 2) evaluating low elevation summer migrations of collared bighorn females and how mineral licks influence movement behavior, and 3) to further evaluate the mineral composition of lick sites used by bighorn sheep. Our study area encompassed a broad distribution of the northern Rocky Mountains including western Montana and mountainous regions of the Greater Yellowstone Area within northwestern Wyoming and southeastern Idaho. For our summer migration analysis, we focused on 5 seasonally migratory bighorn sheep herds in Montana: Castle Reef, Lost Creek, South Madison (Hilgard), Stillwater, and Upper Yellowstone (Figure 22).

Identifying Potential Lick Sites Using GPS Movement Data

We located potential mineral licks by identifying clusters of female bighorn sheep locations that occurred on low-elevation winter range during the summer period; the time when a herd was primarily occupying high-elevation summer range. To do this, we first constrained the GPS locations to those below the lowest 10% elevation recorded during a summer period based on the mean daily elevation per individual. We then plotted these low elevation summer locations, placed a 50 m buffer around each, and merged all overlapping buffer points to produce polygons. We focused on polygons with 5 or more locations of 1 or more individuals that made multiple visits to the area throughout the summer period. This reduced the likelihood of including polygons where individuals spent a short time grazing or bedding before moving on and did not return to the area at any other point during the summer period. To further refine the list of potential lick site polygons, we viewed each on satellite imagery to characterize the physical setting, and filtered to those that contained characteristics observed in satellite imagery of 21 known lick sites. These physical characteristics included: bare ground or little to no vegetation due to high animal disturbance, extensive trail networks, and proximity to roads or rock slides (Figure 23).

We analyzed location data from 118 collared adult female bighorn sheep for a total of 180 animal summers (i.e., 1 – 2 summers of location data per individual) across the study herds. Summer location data was available from years within the range of 2012 – 2019. Within each herd, we identified 27 – 46 polygons that had 5 or more locations of 1 or more individuals represented throughout the summer period (Table 5). Of those polygons, at least 5 – 14 per herd, and totally 46 across all herds were considered to be potential mineral lick sites when physical attributes were viewed on satellite imagery (Figure 24). The median number of individuals and locations were generally lower in polygons not considered to be potential lick sites than those that were. Several of the potential mineral licks we described were of likely anthropogenic origin such as mining activity, residential areas, along roads, and livestock salt blocks.

Characterizing Summer Migrations

To characterize summer migrations of the instrumented bighorn sheep in each study herd to the potential and known lick site polygons, we identified patterns of movement that were consistent with the definition of a migration to

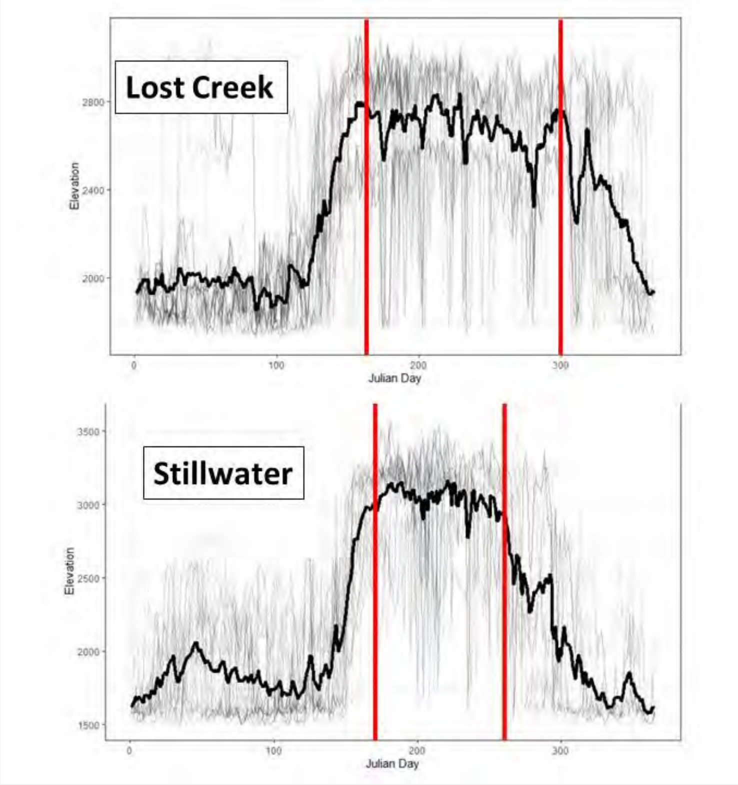


Figure 21 – Example of an elevation profile obtained for an individual bighorn sheep female from the Lost Creek herd based on GPS locations obtained during 2017. The horizontal red line indicates the 10th percentile elevation for the summer period (~Julian days 165-300). The dashed blue line marks the similar lowest elevation for multiple summer migrations. These lowest elevation locations during summer migrations indicate possible visitation to the same site multiple times throughout the summer.

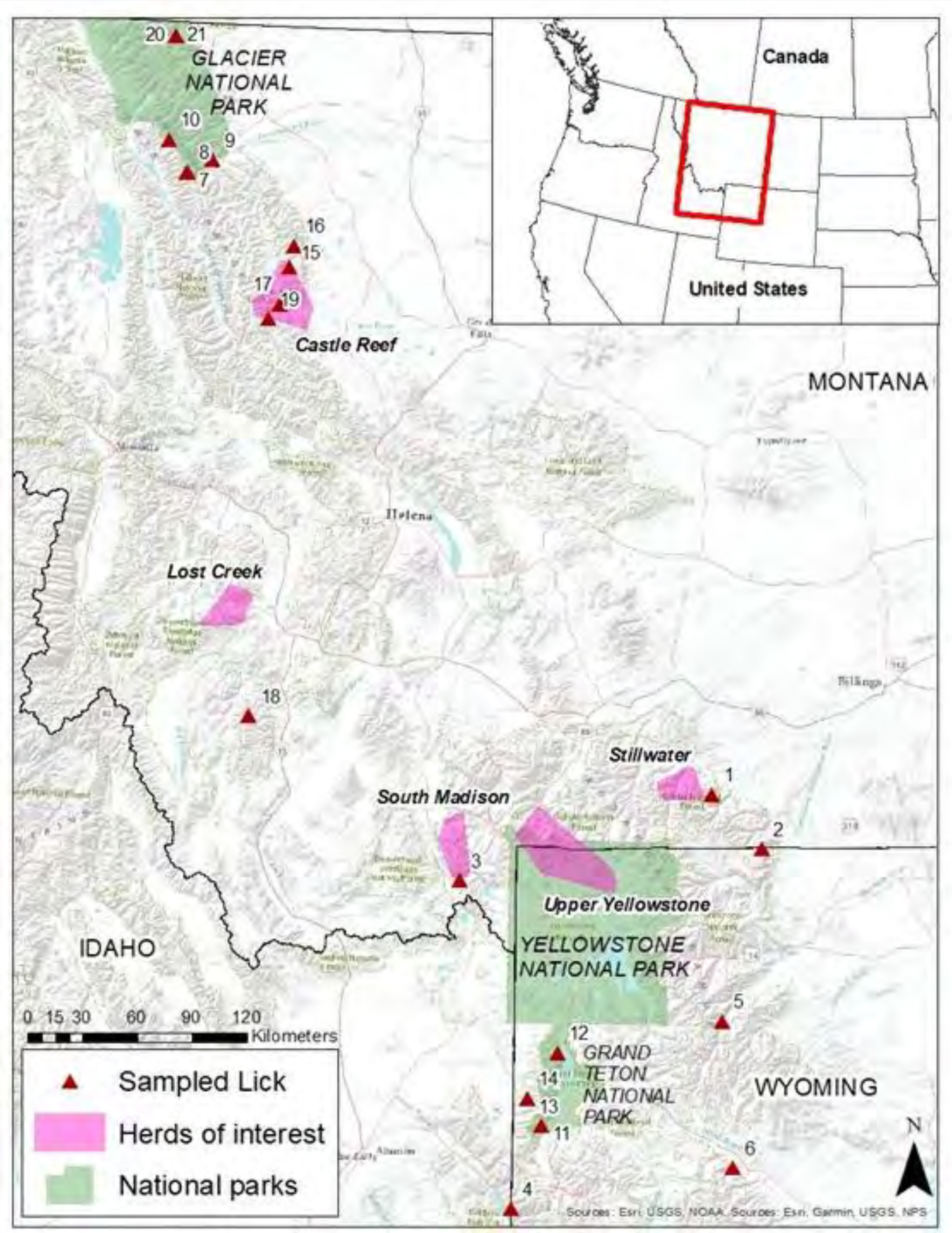


Figure 22 – Our broad study area across the Rocky Mountains of Montana and Wyoming. Location data from a total of 133 collared bighorn sheep females across 5 herds collected from 2012-2018 were used to create minimum convex polygons, depicted in pink. The 21 known lick sites used by bighorn sheep and/or mountain goats that were sampled for soil analysis are shown as red triangles and numbered in the order samples were collected during the fall of 2019 and summer of 2020.

low elevation, i.e., a directed movement from summer range to a low elevation site, and subsequent return to the high elevation summer range. Using all locations of each instrumented individual within the defined summer period, we again calculated the lowest 10% elevation to identify summer migrations to low elevation. For days that the mean elevation was below the 10% elevation, we identified consecutive locations that demonstrated a migration path from an individual's routine high elevation summer range to low elevation locations, and returning again to high elevation summer range.

The total number of summer migrations varied by herd and ranged from 88 to 299. Per summer, individuals had a median of 4 summer migrations in all herds except Lost Creek in which individuals travelled a median of 5 paths per individual summer. Within each herd, we found that the majority of collared females made visits to at least 1 polygon, excluding Upper Yellowstone where only half made these visits (Table 6). The minimum distance travelled to potential licks was generally higher as compared to a typical movements on the high elevation summer ranges. This indicated that bighorn sheep females in our study were expending a higher amount of energy when making visits to polygons suspected of containing lick sites than at other times of the summer. Individuals in each study herd travelled through areas of dense canopy cover, away from ridgelines, and even crossed busy roads to reach potential lick polygons (Figure 25). This is contrary to the habitat features that other studies have shown bighorn sheep to be attracted to (Lowrey 2018, Lula et al. 2020). Many of the collared females likely were accompanied by lambs on these journeys, demonstrating that the resources they are seeking are important enough to justify making movements that increase their and their lambs' risk of mortality.

Evaluating Trace Minerals at Known & Potential Lick Sites Identified from Summer Migrations

We collected 21 composite soil samples during fall 2019 and summer 2020 from mineral lick locations known to be used by mountain ungulates (bighorn sheep and mountain goats) in western Montana and the Greater Yellowstone Area. In addition, we collected soil from a sample of the polygons defined by GPS locations identified as potential lick sites that were accessible and on public land. These included 5 sites on Castle Creek, 3 on Lost Creek, 2 on Stillwater, and 2 on Upper Yellowstone ranges (Figure 22). We chose areas to sample soil within each lick site and potential lick site based on ungulate sign and disturbed areas that indicated high animal use. Depressions, excavations, animal tracks and scat, and trails in bare soil were clues used to identify subsampling sites for soil collection (Figure 26). At 10 of the 12 potential lick sites, we also collected control soil samples in areas with no sign of geophagy that were outside the potential lick site boundary.

At each site, we collected 5-6 soil subsamples distributed across the lick site. We thoroughly mixed the subsamples and then dried and prepared approximately 500 mL of the composited sample as described by Fery and Murphy (2013). Four samples (licks #11-14) were rocks where mountain goats and bighorn sheep were observed by trail cameras ingesting soft limestone from cliff faces. To collect samples from these locations, we used a rock hammer to gather 5 subsamples from the areas of highest animal use. We then used a stainless-steel mortar and pestle to crush the rocks into a powder which we sifted using a 2 mm sieve. All soil samples were analyzed for concentrations of 7 trace minerals by the Brigham Young University Environmental Analytical Lab. We assayed each sample for calcium (Ca), sodium (Na), magnesium (Mg), potassium (K), copper (Cu), selenium (Se), and zinc (Zn) due to their prevalence in animal science literature and past analyses for both domestic and wild ungulates. A pressurized hot water extraction was used to obtain concentrations of soluble selenium (Soil Science Society of America 1996, Webb et al. 2002). The other 6 mineral concentrations were extracted with the Mehlich III reagent (Mehlich 1984). All trace mineral extractions for all sample sites were analyzed using ICP-OES (iCAP 7400, Thermo Scientific, Madison, WI, USA). We compared resulting mineral concentrations of known lick soils sampled during this study with the concentrations of known lick soils from previous studies that were used exclusively by bighorn sheep and/or mountain goats.

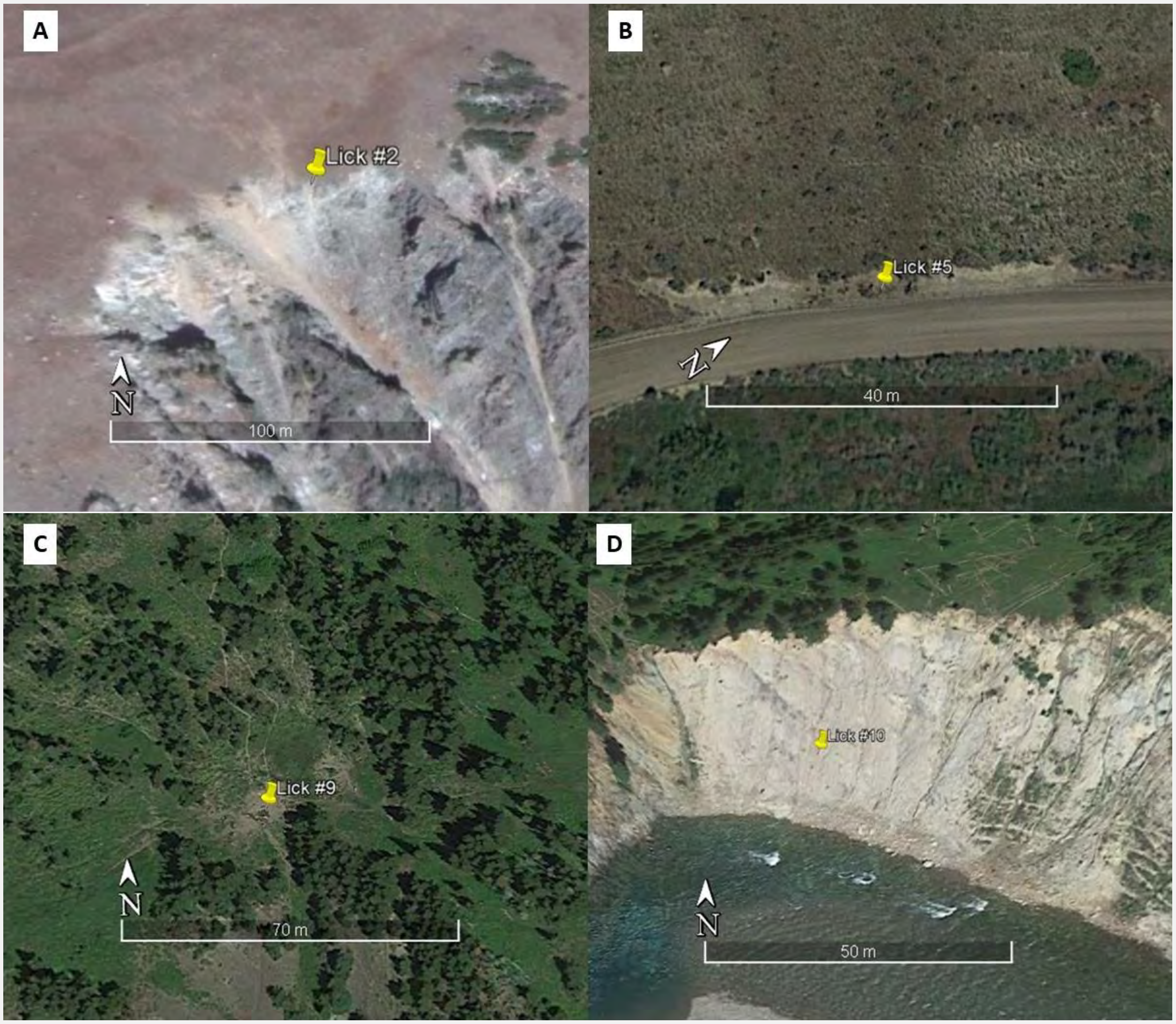


Figure 23 – Satellite imagery of 4 known lick sites sampled for this study in autumn of 2019: Panel A) lick #2 is located at the edge of a high-elevation plateau, note the color variance and disturbance in the soil; panel B) lick #5 is located in a road cut, note the faint trailing above the lick; panel C) lick #9 is a wet lick marked by the bare patch of soil with substantial trailing in the surrounding area; and panel D) lick #10 is a lick located on the steep, eroded slope cut by a creek, note the substantial trailing visible below and to the right of the lick, and in the meadow above the lick.

Our soil samples from known lick sites indicated that calcium, magnesium, and sodium were the most abundant elements assessed with concentrations similar to known lick sites used by mountain goats and/or bighorn sheep reported in the literature (Table 7). Of the 21 lick sites evaluated, 18 had elevated concentrations for a least 1 of the 3 primary trace elements. Four sites had high levels of all 3 elements. Three sites had elevated levels of Ca and Na and 3 sites had elevated levels of Mg and Na. At 3 sites, only Ca was elevated and at 5 other sites, only Na was elevated. The range of concentrations (ppm) for those lick sites with elevated values were 6,348 - 25,930 for Ca (n = 9), 809-10,050 for Mg (n = 7), and 98-19,420 for Na (n = 15). Fourteen of these lick sites were natural in origin, 2 were along road shoulders, 1 was a road cut, and 1 was a traditional livestock salting site.

Table 5 – Polygons created per herd, and general information on visitation and individuals represented within. Values of polygons that weren't considered potential lick sites are noted in parentheses for comparison of individuals and locations represented per polygon.

	Total no. polygons described[a]	No. potential lick polygons[b]	Median no. individuals represented per polygon[c]	Median no. locations represented per polygon[c]
Castle Reef	28	10 (35.7%)	5 (5)	14 (11)
Lost Creek	46	14 (30.4%)	6 (5)	15 (15)
South Madison	33	5 (15.2%)	7 (6)	12 (12)
Stillwater	27	8 (29.6%)	6 (5)	24 (20)
Upper Yellowstone	32	9 (28.1%)	3 (3)	14 (5)

[a] This value is based on polygons created from clusters in which 5 or more locations were represented making separate visits throughout the summer period for each herd.

[b] This value is based on the number of polygons in a herd that, when viewed on satellite imagery, had the physical characteristics that we considered to be similar to known lick sites.

[c] These values are based on polygons we considered to be potential lick sites only.

Table 6 - Summary of various attributes of summer migrations of collared female bighorn sheep visiting potential lick sites.

	Summer migrations with potential lick[a]	Percent of females visiting potential licks[b]	Mode timing of lick visitation[c, d]	Median hours spent within a potential lick[d]	Median minimum distance travelled during migration (km)[d]
Castle Reef	48 (32.2%)	80%	17:00 (1:00 – 21:00)	8 (2 – 70)	14.3 (1.2 – 68.2)
Lost Creek	115 (78.8%)	100%	17:00 (1:00 – 21:00)	10 (2 – 84)	15.1 (0.1 – 32.5)
S. Madison	132 (44.1%)	80%	17:00 (0:00 – 23:00)	15 (5 – 270)	6.4 (0.3 – 16.6)
Stillwater	107 (78.5%)	100%	17:00 (1:00 – 23:00)	8 (2 – 104)	17.2 (0.0 – 31.9)
Upper Yell.	27 (30.7%)	50%	20:00 (0:00 – 23:00)	10 (5 – 50)	2.8 (0.3 – 17.3)

[a] This value is based on the total number of summer migrations recorded for a herd, and the percent is listed in parentheses.

[b] This value is the percent of collared female bighorn sheep that performed at least 1 summer migration containing visitation to a potential lick polygon. This is out of all individuals that summer location data was available for within a herd.

[c] This value is based on the first location of a summer migration that was within a potential lick polygon.

[d] Ranges of values are noted in parentheses.

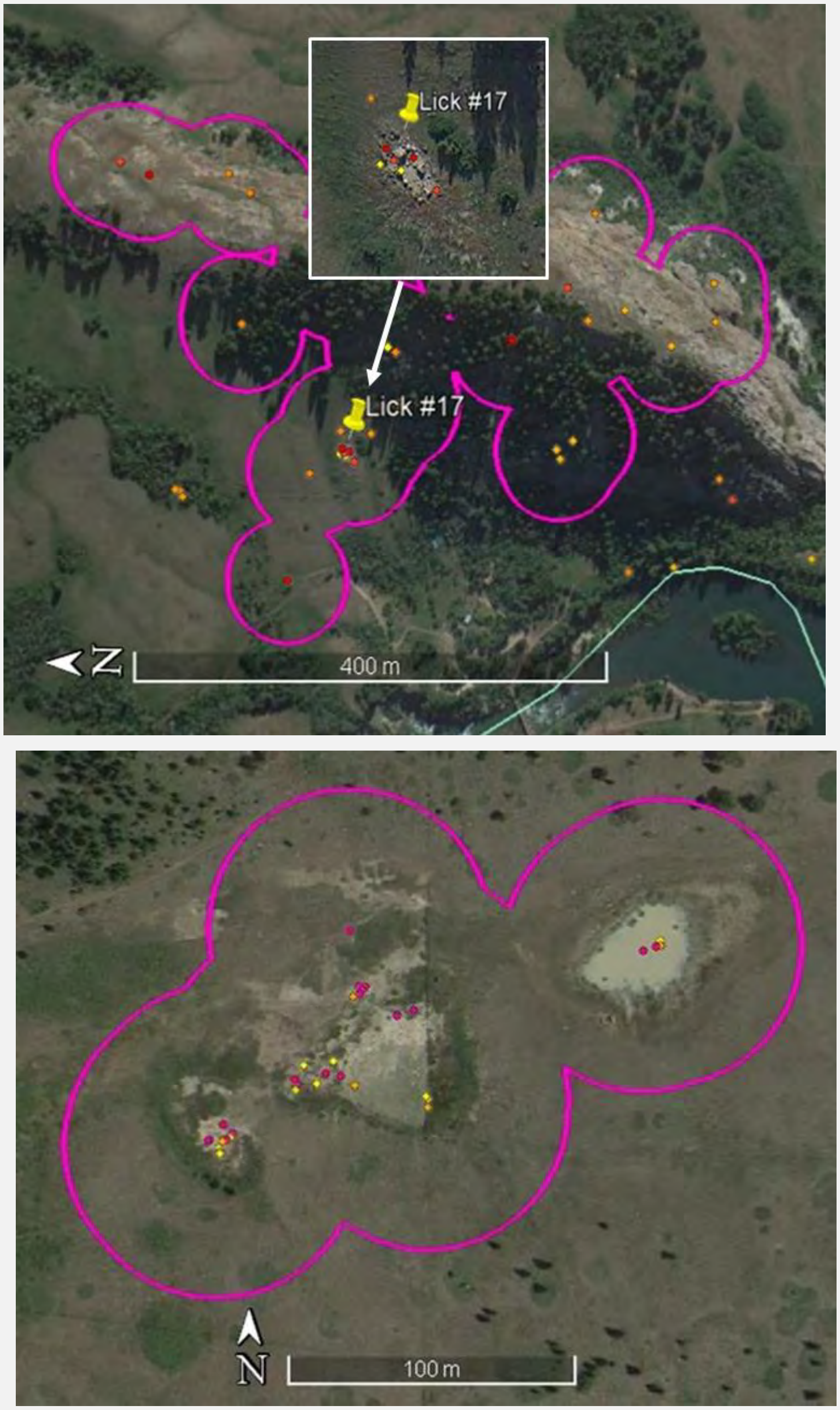


Figure 24 – A Google Earth image of known lick site #17 (upper panel) and a potential lick site identified using GPS location data (lower panel) within the range of the Castle Reef bighorn sheep herd. The colored circles represent the recorded summer locations of 8 different bighorn sheep females of the Castle Reef herd within the lick vicinity of the known lick site and 6 animals in the vicinity of the potential lick site. The violet line illustrates the polygon that was formed from the merging of the 50 m location buffers. The inset is a closer view of the lick site 17 illustrating the area of bare soil at the site and the trail system.

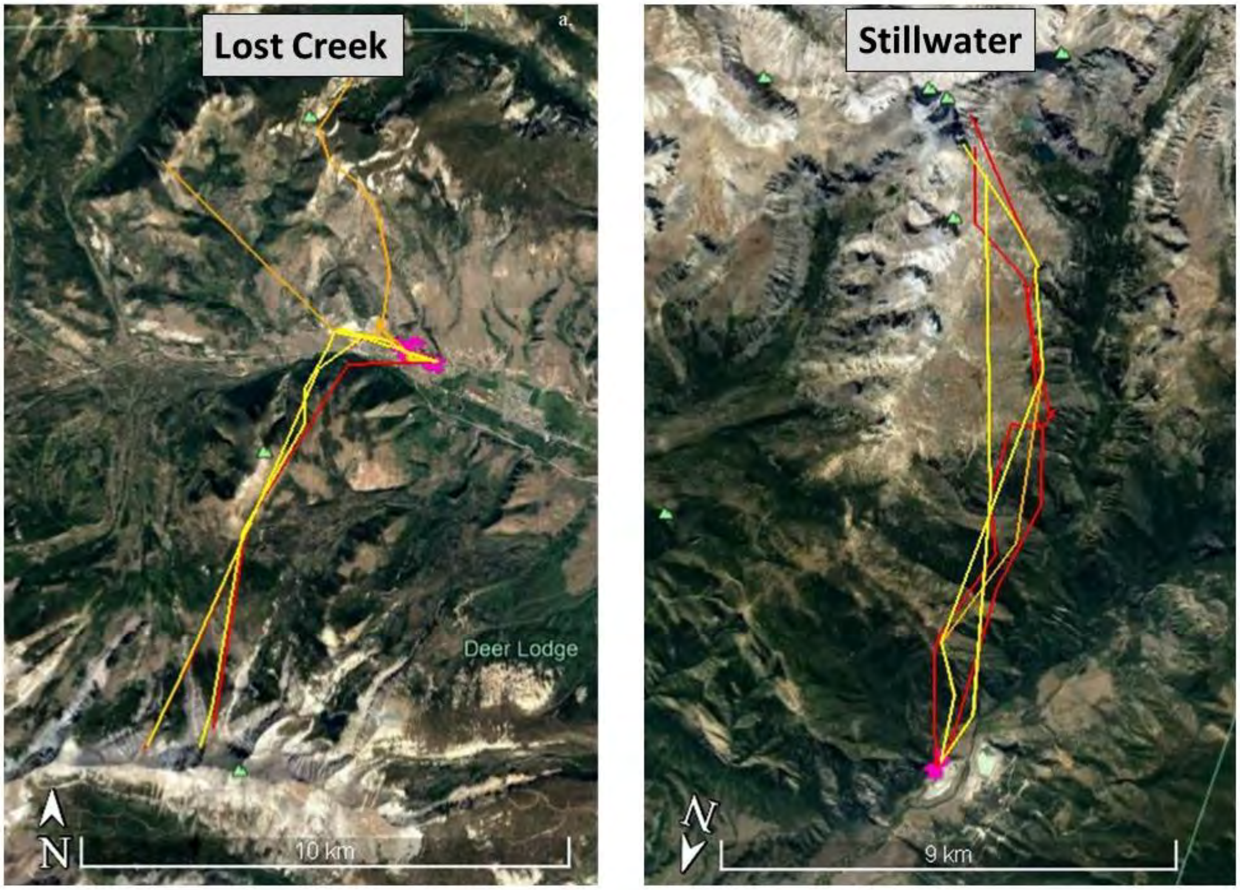


Figure 25 – Examples of the summer migration paths GPS-collared adult female bighorn sheep in the Lost Creek and Stillwater herds. Each panel represents summer migrations by 3 individual bighorn sheep females to potential lick sites (shown as a pink polygon). Note that the paths travel from high-elevation ridges, through heavily wooded areas, with the Lost Creek animals also crossing a busy road.

Figure 26 – An example of a known natural mineral lick (Lick #2, Figure 22) located along the Montana-Wyoming border on the Beartooth Plateau in the northeast corner of the Greater Yellowstone Area. A trail camera documented use of the site by small groups of bighorn sheep and herds of mountain goats frequently exceeding 40 animals in June and July. The inset illustrates the extensive geophagia (note GPS unit for scale in upper right corner of photo).

Figure 27 – An example of an artificial mineral lick site established on high-elevation summer range for the purpose of attracting and capturing bighorn sheep and mountain goats along the northwestern boundary of Yellowstone National Park. The lick was heavily used by both species throughout the summer.

Of the 12 potential lick sites identified through the analysis of GPS movement data where soil samples were collected 11 had elevated concentrations for a least 1 of the 3 primary trace elements. Three sites had high levels of all 3 elements. Two sites had elevated levels of Ca and Na. At 3 sites, only Ca was elevated and at 3 other sites, only Na was elevated. The range of concentrations (ppm) for those potential lick sites with elevated values were 5,181 - 27,260 for Ca (n = 8), 511 - 1,367 for Mg (n = 3), and 83 - 4,668 for Na (n = 7). These values were similar to those reported in the literature and evaluated in our study for soil samples collected at known lick sites used by bighorn sheep and/or mountain goats. Control soil samples were collected at 8 of the potential lick sites and generally contained substantially lower concentrations of the elevated elements than the sample collected within the suspected lick site. The exceptions were 1 potential Lost Creek lick site and 1 potential Castle Reef lick site where Ca was the only elevated element but concentrations were similar to the control samples. Both of these sites were located in areas with limestone outcrops. Of the 11 potential lick sites with elevated concentrations of at least 1 primary trace element (suggesting the sites serve as licks), 7 were natural, 3 were associated with mining, and 1 was along the shoulder of a road. In addition, a potential lick site identified on winter range of the South Madison herd was associated with a private residence where a livestock salt block was known to exist. Numerous potential lick sites identified using the animals' movement data were located on small subdivisions, ranches, and other private lands where we suspect salt blocks may have been deployed.

Collectively, the data from our study and those similar studies reported in the literature indicate that for many bighorn sheep populations, ewes are likely seeking calcium, magnesium, and sodium to a higher degree than

Table 7 – Soil mineral concentrations (ppm) for known lick sites used by bighorn sheep and mountain goats collected in this study and concentrations reported in the literature for other known lick sites in North America (Stockstad 1953, Jones and Hanson 1985, Ayotte et al. 2006, and Rice 2010).

		Mineral concentrations (ppm)						
		Ca	Mg	Na	K	Cu	Se	Zn
This study								
	n[a]	21	21	21	21	21	12[b]	21
	Median	5828	428	241	207	1.56	0.09	10.10
	Standard Deviation	6650	2311	4184	262	2.98	0.18	5.53
	Minimum	932	172	33	94	0.37	0.01	0.33
	Maximum	25930	10050	19420	1212	14.30	0.64	20.40
Literature								
	n	30	30	30	30	3	0	3
	Median	4433	559	100	162	3.50		2
	Standard Deviation	4773	1170	344	548	0.96		1
	Minimum	625	36	35	34	2.00		2
	Maximum	14000	3200	1092	2000	3.80		4

[a] *Number of sampled licks reported for each trace mineral.*

[b] *Nine samples below detectable concentration*

other minerals, and these 3 elements are likely limited on many summer ranges. Calcium is important in ruminant physiology for processes such as blood clotting, muscle contraction, normal teeth and bone development, adequate heart rhythm, and enzyme activation as well as aspects of pregnancy and lactation (Subcommittee on Beef and Cattle Nutrition 2000). Magnesium is highly involved in metabolism, and the immune function of lambs (Underwood and Suttle 1999, Ataollahi et al. 2018). As previous studies have noted, potassium intake will increase in the spring when forage becomes succulent again (Watts and Schemnitz 1985, Ayotte et al. 2006). An increase in potassium causes a subsequent decline in an animal's ability to retain magnesium and sodium, which may cause animals to seek mineral lick soils to balance these deficiencies. Sodium is the mineral most commonly thought of when mineral supplementation is mentioned, and one of the most important when considering its role in the body. It is responsible in the processes of the transmission of nerve impulses, membrane potential maintenance, and water metabolism control. When deficient, livestock can experience a reduction in the ability to absorb magnesium and a decrease in milk yield (Underwood and Suttle 1999, Subcommittee on Beef and Cattle Nutrition 2000).

Conclusions & Management Suggestions

Our soil sample and movement analyses indicate that bighorn sheep females are visiting mineral licks during the majority of summer migration events, making mineral licks an important habitat feature for managers to be aware of. This is especially the case when bighorn sheep are crossing busy roads and through dangerous forested terrain. Bighorn sheep managers should consider mapping and inventorying mineral licks, both natural and anthropogenic, used by bighorn sheep under their jurisdiction. Sharing this lick site information with multiple land management agencies would increase the aid in conserving and maintaining movement corridors to and from seasonal ranges. When reintroducing bighorn sheep to new areas, the consideration of establishing specially formulated artificial licks (i.e. salt blocks) where the bighorn sheep summer, can help ensure they have access to trace minerals. This would also eliminate the need for the naïve herd to risk making long forays in an unknown landscape and leaving the desired reintroduction area when searching for sources of trace minerals during periods of deficiencies. Establishing artificial licks on summer range of established bighorn sheep herds, particularly those with poor lamb:ewe ratios, could deter these females from travelling long distances with their lambs through risky landscape features. Such artificial mineral licks have been successfully deployed on summer ranges during this study. The mineral blocks were contained in small tubs staked to the ground and covered with a metal mesh to prohibit soil contamination and were authorized by federal land managers and deployed in Yellowstone National Park and National Forest wilderness for summer animal capture operations (Figure 27). Authorization was contingent on removing the mineral licks well before the initiation of fall hunting seasons.

The attraction of bighorn sheep to sources of trace minerals during the summer lamb-rearing season also provides the opportunity to experiment with delivery of medications that may mitigate the impacts of respiratory disease in lambs. Most bighorn herds host a suite of bacterial pathogens associated with pneumonia (see Chapter 2). Once exposed adult ewes can become asymptomatic carriers and transmit the pathogens to naive lambs, with lamb-lamb contact exacerbating transmission when animals are in nursery groups during the spring and summer. Mortality of 2 - 4 month old lambs can be substantial and chronic in some herds, depressing lamb recruitment and demographic vigor of populations for years, and in some herds for decades. Pneumonia in domestic lambs and calves is also common and several effective medications to combat bacterial pneumonia are routinely used in the livestock industry. The 2 most common medications are sodium sulfamethazine (Sulmet) and

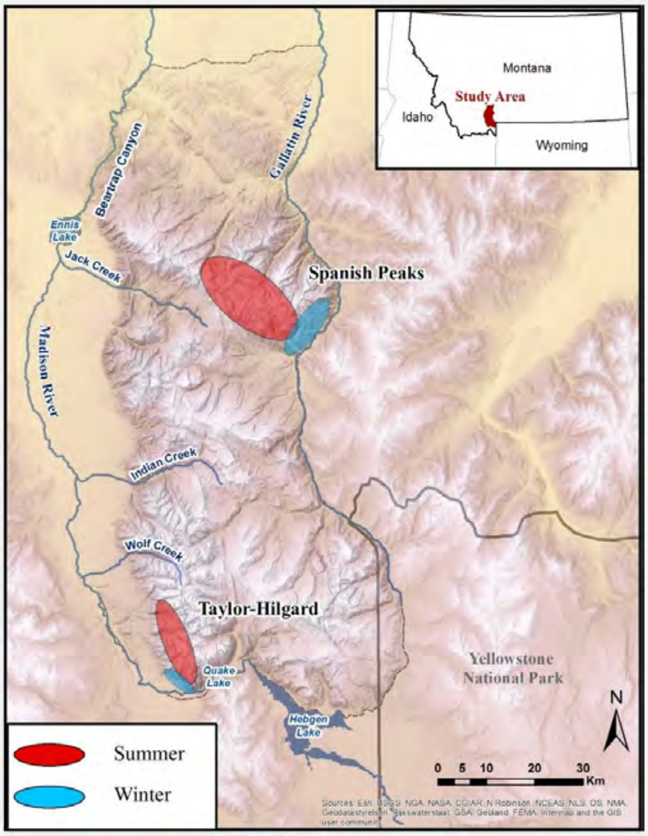


Figure 28 - Madison Range with estimated seasonal home ranges (summer = red, winter = blue) for the Spanish Peaks and Hilgard study populations

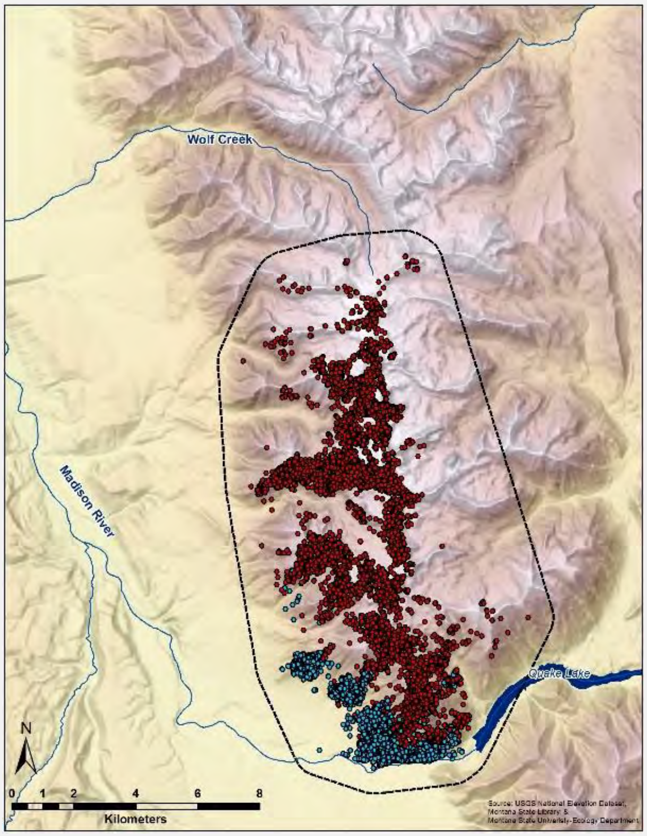


Figure 29 - The annual range (black dotted line) encompassing summer (red) and winter (blue) GPS locations for bighorn sheep in the Hilgard study population.

chlortetracycline HCL (Pennchlor 64). Both are soluble powders that are dissolved in water, often with an additional flavoring agent, and readily consumed by animals. Wildlife managers currently have no tools to try to mitigate chronic lamb pneumonia and the strong attraction of ewes to trace mineral sites during the summer lamb-rearing season presents an opportunity to deliver these medications to evaluate if protocols can be developed to moderate the impacts of bacterial pathogens on lamb survival and improve herd performance.

MADISON RANGE HABITAT MODEL FOR INTRAMOUNTAIN RESTORATION

As habitat specialists, bighorn sheep rely on rugged terrain offering good visibility as security from predators and are dependent upon the seasonal availability of forage (Geist 1971). Due to this specialization, bighorn sheep habitat is often naturally fragmented within a geographic area (e.g. mountain range) resulting in localized populations with discrete seasonal ranges (Demarchi et al. 2000, Singer et al. 2000a). Anthropogenic induced fragmentation of habitat may constrain populations of bighorn sheep into increasingly small and isolated patches of habitat (Shackleton et al. 1999) discouraging natural exploration of surrounding habitat (Smith et al. 1999) and potentially leading to seasonal deficiencies in forage (Festa-Bianchet 1988b, Enk et al. 2001).

The Madison Mountain Range (Figure 28) located along the western edge of the Greater Yellowstone Ecosystem (GYE), hosts the Hilgard and Spanish Peaks study populations. However, historical accounts suggest that bighorn sheep occurred throughout the range (Montana Fish Wildlife and Parks 2010). Both populations are considered isolated from each other and have seen little expansion into surrounding habitat during their eight-decade management history (Montana Fish Wildlife & Parks 2013). In particular, the Hilgard population has demonstrated little range expansion and re-colonization of historic wintering habitat despite steady population growth above management objective (n = 120). As a case study, we sought to determine if habitat was the primary factor limiting the distributions of bighorn sheep within the Madison Range and, if not, evaluate the potential for restoration.

Using GPS data collected from the Hilgard population and a suite of habitat covariates expected to influence habitat selection (Lowrey et al. 2019), we built summer and winter resource selection function (RSF) habitat models. RSFs produce spatially explicit predictive models by quantifying the relationship between how animals use important resources relative to the availability of those resources within a defined extent, thus linking a species to a set of habitat characteristics (Boyce and McDonald 1999, Manly et al. 2002). For our analysis, we defined the extent of availability as the Hilgard annual range (Figure 29).

We adopted a tiered approach in developing our models (Franklin et al. 2000) and used corrected Akaike's information criterion (AIC_c) to select our most supported summer and winter models. Within the tiered approach, we evaluated multiple functional forms (i.e., linear, quadratic, and pseudothreshold) and spatial grains (Meyer and Thuiller 2006, Laforge et al. 2015) for appropriate covariates and compared similar landscape covariates bringing forward the most explanatory covariates in our top summer and winter models (Lula et al. 2020).

Our results indicated that bighorn sheep within the Hilgard population generally selected for resources at larger spatial grains (500 m and 1000 m) indicating that they perceived these resources at a broader geographic extent. During the summer, bighorn sheep selected for

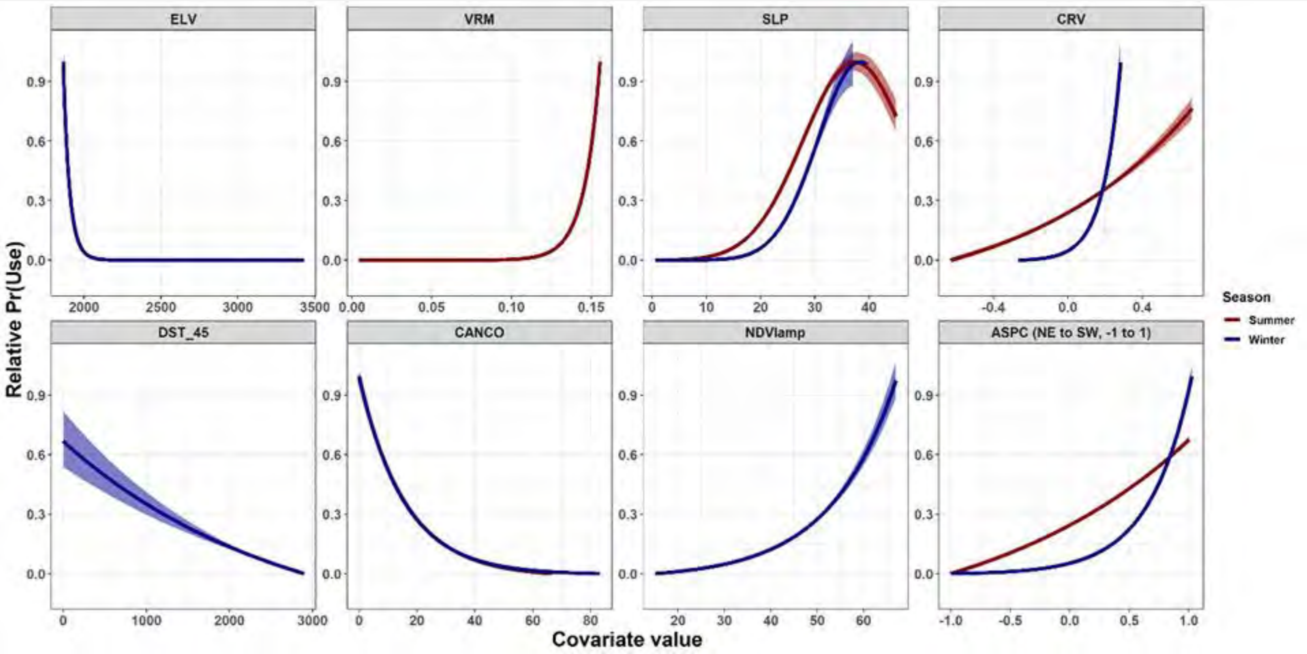


Figure 30 - Predictions of the relative probability of use for the top covariates in the summer (red) and winter (blue) RSF models for bighorn sheep in the Hilgard study population. Predictions were generated across the observed covariate range with all other covariates held at their mean value.

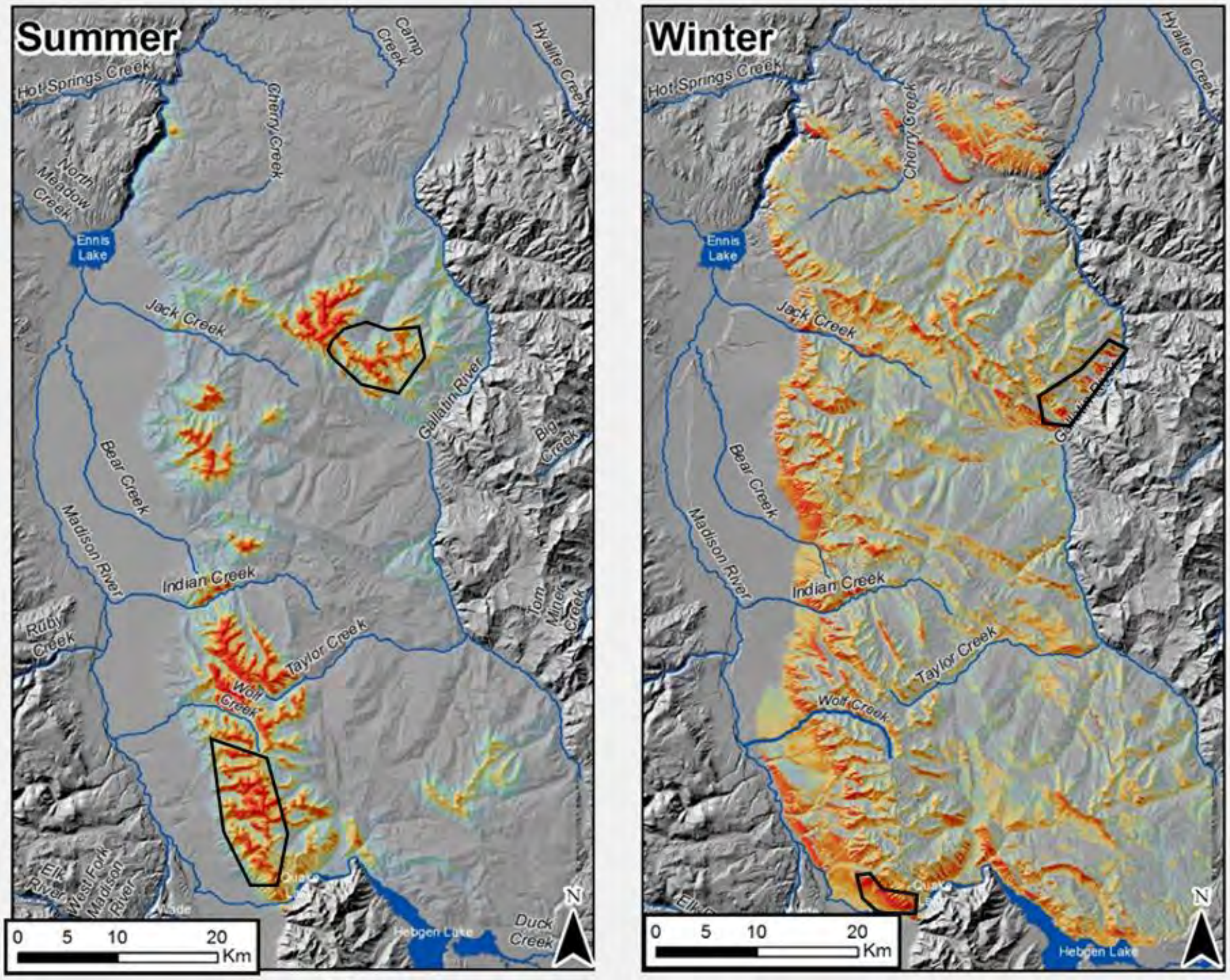


Figure 31 - Seasonal RSF model results extrapolated to the Madison Range where RSF scores were classified into 10 equal-area bins based on the seasonal predictions within the Hilgard study population annual range. Cool (blue) and warm (red) colors represent low and high RSF values, respectively. Black polygons represent known distributions of extant bighorn sheep populations.

rugged terrain (VRM), steep slopes, convex curvatures (i.e., ridgelines), decreased canopy cover, and southwestern aspects (Figure 30). Winter habitat was characterized by selection for low elevations, steep slopes, convex curvatures, southwestern aspects, high summer NDVI amplitude, and smaller distances from slopes ≥ 45° (Figure 30).

Predicted winter habitat largely occurred within the Madison Valley, along the low-elevation, southwest facing aspects associated with reduced snow cover (Figure 31). Consistent with the migratory behavior observed in both Madison Range bighorn sheep populations, predicted summer habitat occurred within more mountainous regions of the Range, essentially as 3 contiguous patches along high elevation ridgelines (Figure 31). We validated our results using *k*-fold validation (Boyce et al. 2002) and with additional GPS data from bighorns collared during the 2016 Hilgard capture, the 2016-2018 MFWP Wolf Creek translocations, and the 2018 MFWP Spanish Peaks capture. Our model validations were successful, predicting bighorn sheep locations within and outside of the

Hilgard annual range as well as in the Spanish Peaks (Figure 31).

Our model results indicate that habitat availability is not constraining bighorn sheep distributions within the Madison Range. The distributions of predicted habitat and the migratory behaviors of the Hilgard and Spanish Peaks populations suggest that the Range may have historically supported a much broader distribution of bighorn sheep consisting of localized wintering populations that then migrated to shared high-elevation summer ranges.

To explore the potential for restoration within the Madison Range, we linked our winter RSF model to 2 measures of abundance within the Hilgard population (Boyce and McDonald 1999, Boyce and Waller 2003). We used the Hilgard population management objective (n_{min} = 120) and the 5 year maximum observed population count (2013 – 2018, n_{max} = 255) to estimate densities of bighorn sheep on Hilgard winter range. We then applied those densities to our extrapolated results and estimated a range of abundance values, assuming all potential habitat were occupied and resources used similarly to the Hilgard population. Our results indicate that winter habitat within the Madison Range may be capable of supporting between 780 and 1,730 bighorn sheep, which is between 2 to 4 times the number currently estimated within the range.

Given our results, we conclude that habitat is not the primary constraint on bighorn sheep distributions within the Madison Range and that the available habitat may be capable of supporting a significantly higher abundance of bighorn sheep. We hypothesize that the Madison Range historically supported a naturally fragmented distribution of bighorn sheep, similar to that found in other native GYE populations, consisting of localized wintering populations that utilize shared summer ranges.

Bighorn sheep exhibit especially strong fidelity to established seasonal ranges (Bleich et al. 1996) and recent work has demonstrated that knowledge of the broader landscape is culturally transmitted between generations (Jesmer et al. 2018). We therefore speculate that cultural transmission may have been critical in maintaining localized wintering populations and that historic extirpation resulted in an overall reduction of the broader geographic landscape known to the remaining populations. Once extirpated, wintering populations are unlikely to be naturally reestablished by neighboring populations given the high fidelity that female bighorn sheep exhibit to their natal home range (Bleich et al. 1996). We speculate that this behavioral tendency, in combination with subsequent disease related die-offs and factors such as increased predator densities, may have effectively suppressed the remaining populations of bighorn sheep within the Madison Range (i.e., Hilgard and Spanish Peaks) from expanding into adjacent habitats by lengthening the number of generations needed to explore, learn, and eventually colonize areas of unoccupied habitat (Jesmer et al. 2018).

Our model results provide managers with a useful tool for identifying future translocation sites that maximize the probability of population establishment and a blueprint with which to help monitor the success of restoration efforts. Given our predicted seasonal distributions of habitat, the strong behavioral fidelity that bighorn sheep exhibit towards seasonal ranges (Geist 1971, Festa-Bianchet 1986) and the slow generational process by which populations of animals accumulate geographic knowledge (Sasaki and Biro 2017, Jesmer et al. 2018), it may be useful to consider a progressive series of short-range translocations into adjacent winter habitat using animals from either the Hilgard or Spanish Peaks populations as a source. By moving animals within the same geographic region, short-range translocations may reduce the risk of novel pathogen introduction (Butler et al. 2017) and perhaps maintain migratory behavior. Furthermore, by moving animals with an established knowledge of the broader landscape, rather than introducing naïve animals to a novel landscape, short-range translocations may promote exploration and decrease the number of generations needed to naturally recolonize unoccupied habitat.

Although our results can most directly be applied to management within the Madison Range, the underlying implications of our research may be worth considering in the context of broader restoration as well. Our habitat predictions within a single mountain range supporting two well-established native populations of bighorn sheep indicated that the potential for further restoration was greater than previously realized. As managers face increasingly complex biological and social constraints to restoring and maintaining bighorn sheep populations, the implication that other mountain ranges may contain unrealized potential could provide new opportunities for creating and enhancing persistent populations of bighorn sheep. We address this idea in a forthcoming manuscript that considers potential opportunities in the mountain ranges of western Montana and in the following section for the prairie environments of eastern Montana where distributions of bighorn sheep occurred historically.

BEHAVIOR-SPECIFIC HABITAT MODELS AS A TOOL TO INFORM UNGULATE RESTORATION

Across North America, many ungulate species that experienced historic population declines and range contractions are now broadly distributed across their native ranges after the implementation of successful restoration programs (Picton and Lonner 2008, IUCN/SSC 2013). Translocation, the intentional movement of animals from one area to another, has a long history in wildlife and fisheries management and is the foundation of native fauna restoration (Griffith et al. 1989). Recently, technological advances have provided managers tools to inform translocation programs and increase the probability of translocation success. For example, wildlife researchers and management practitioners routinely collect location data from devices on marked individuals to construct habitat models (Boyce and McDonald 1999, Johnson et al. 2006). Such models can identify covariates that influence habitat selection and be predicted spatially to generate habitat maps across large landscapes. Within the context of translocation, habitat selection models can help to identify unoccupied habitat or possible translocation sites (Niemuth 2003, Cianfrani et al. 2010) and are an effective tool to increase the probability of translocation success (Griffith et al. 1989, Zeigenfuss et al. 2000).

The broad deployment of animal tracking devices also has highlighted the diversity of migratory behaviors both among populations as well among individuals within a single population. For example, partial migration in which a population subset is migratory while the remaining individuals are resident on a shared winter range, is common among migratory wildlife species (Chapman et al. 2011). Resident and migrant population segments can have different selection patterns seasonally (Hebblewhite and Merrill 2009, Barker 2018), yet the multiple behaviors are often pooled when creating habitat models. The process of pooling multiple migratory behaviors results in an 'average' habitat model that might not describe the more nuanced habitat characteristics of any single population segment (Lowrey et al. 2019, Spitz et al. 2020). Moreover, when selection patterns are strongly divergent among migratory behaviors, pooling location data across multiple migratory behaviors can produce a mismatch in which the models used to inform translocations do not reflect the migratory behavior of translocated individuals. For example, habitat models constructed from location data collected from migrants may misinform translocations where residents are used as the source population.

We used a large spatial dataset collected from 8 bighorn sheep populations (totaling 194 females) across western Montana (Figure 32) to build behavior-specific habitat models with the purpose of characterizing differences in seasonal selection patterns between resident and migrant population segments, and generating broad spatial predictions of bighorn sheep habitat to inform future translocations. Migratory behaviors of the study populations included elevational migrations in 6 populations (Castle Reef, Lost Creek, South Madison, Spanish Peaks, Stillwater, and the Upper Yellowstone) and resident behavior in 2 populations (Paradise and Petty Creek, see Lowrey et al. 2020 for additional details). In general, migrant populations had low-elevation winter ranges and high-elevation summer ranges while residents remained at low elevations year-round.

We constructed separate winter (24-Nov to 6-May) and summer (7-June to 7-Oct) resource selection models for the migrant and resident populations using non-collinear combinations of covariates that have been shown to influence bighorn sheep resource selection across western United States and Canada (Lowrey et al. in review). We included quadratic forms of elevation and slope, allowing selection for these resources to peak at intermediate values, and a pseudothreshold (natural log) form for slope variance, allowing selection for rugged terrain to asymptote at a threshold value. We evaluated aspect, canopy cover, distance to steep terrain,

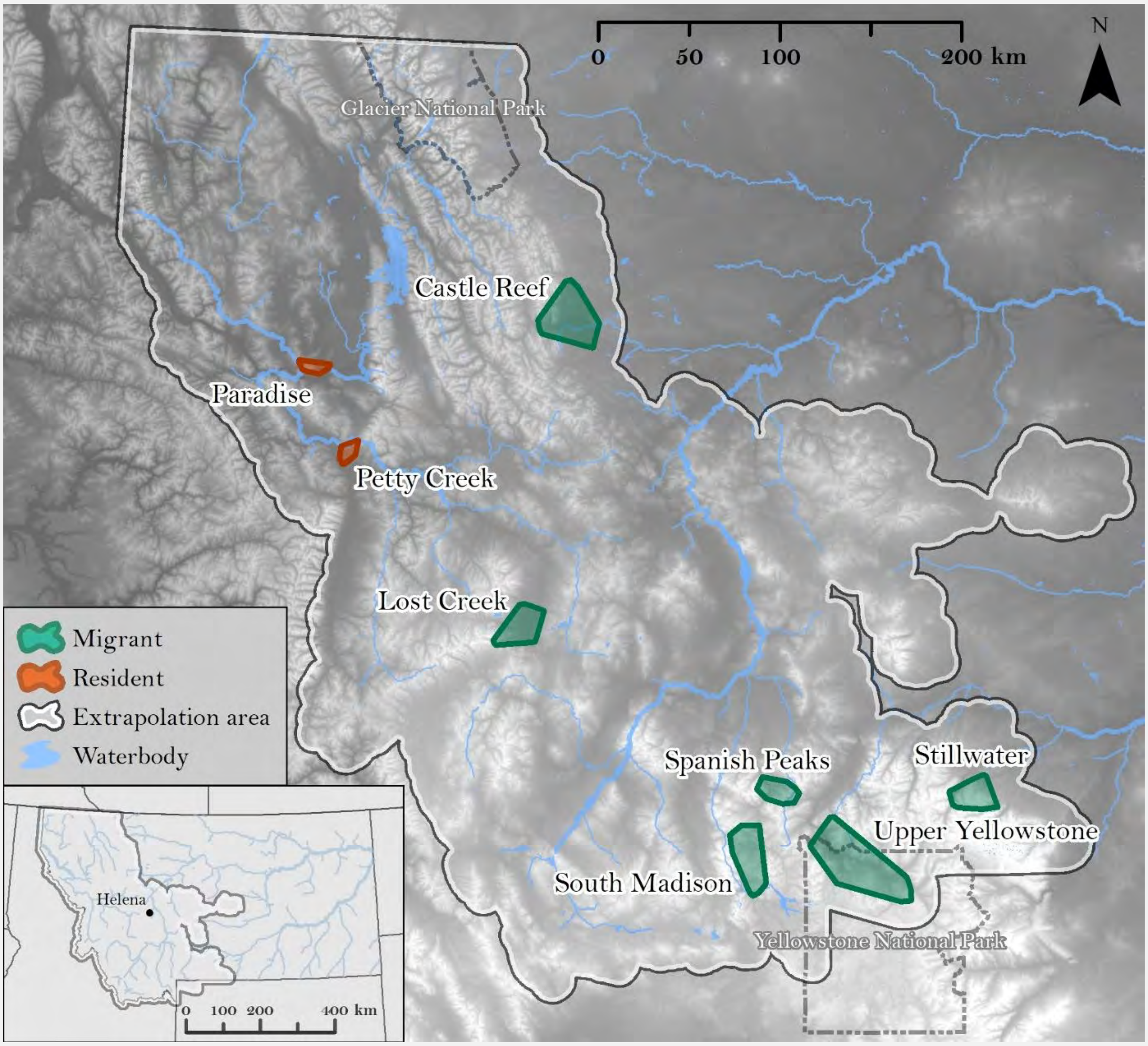


Figure 32 - The 8 study populations used to model migratory and resident female bighorn sheep resource selection in the montane regions of western Montana, USA, 2012–2018. Migrant populations are shown in green while the residents are shown in orange. The study areas were defined using an annual minimum convex polygon which we buffered by 2.5 km. Elevation is represented by the dark grey (low) to light grey (high) gradient.

elevation, slope, and slope variance in all models and included NDVIAmp in summer and SWE in winter. For all models we employed a used-available design (i.e., Design II; Manly et al. 2002) where values associated with individual GPS locations represented the "used" set and "availability" was sampled from population-level annual ranges.

Because of the relatively small sample of resident populations (n = 2) and their limited distribution, we were unable to spatially predict the resident seasonal models due to the narrow distribution of covariate values relative to the broad prediction area across western Montana. However, we observed a strong correlation between the winter migrant model and resident annual use for both the Petty Creek and Paradise populations, indicating that the winter migrant model also represented resident annual use (Lowrey et al. In review).

We demonstrated 2 examples of how the model predictions could be used to inform bighorn sheep translocations with the purposes of: 1) establishing a new population within unoccupied historic range, and 2) expanding the distribution of existing populations through within-mountain range translocations. For the first example, we used the model predictions of relative habitat quality in the Tendoy Mountains to: 1) identify areas with a relatively high probability of translocation success based on landscape characteristics, and 2) identify the most appropriate migratory behavior(s) to target as a source population that best matches the landscape attributes of the Tendoy Mountains. For the second example, we explored the potential to expand current distributions of bighorn sheep through short-distance translocations from a single population to unoccupied, adjacent areas in the same mountain range. These 'intra-mountain' translocations have reduced risk of introducing novel pathogens or pathogen strains and have shown positive results in California and Montana bighorn sheep restoration programs, but require habitat near or adjacent to extant populations (Epps et al. 2010, Montana Fish Wildlife & Parks 2013). To do this, we characterized bighorn sheep habitat, as indexed by the RSF bin values, adjacent to extant populations in western Montana by summing the frequency of each RSF bin value within a 15 km buffer surrounding (but not including) each seasonal polygon. This provided a characterization of the habitat surrounding each of the existing populations and allowed us to: 1) evaluate the potential for broad implementation of intra-mountain translocations across western Montana, and 2) identify and discuss existing populations that provided examples of possible restoration scenarios with abundant habitat adjacent to winter and summer ranges, abundant habitat adjacent to only the winter range, or little habitat adjacent to both seasonal ranges.

The final winter model for both migrants and residents contained aspect, canopy cover, distance to steep terrain, slope, slope variance, and SWE. The final summer model for both migrants and residents contained aspect, canopy cover, distance to steep terrain, elevation, slope, slope variance, and NDVIAmp. Although there were differences in strength of selection, specifically in summer, selection patterns were generally similar among migrants and residents within each season (Figure 33). The notable exception was elevation in summer, where residents selected for low elevations while migrants selected for relatively high elevations, as expected. This was the strongest difference between the 2 migratory behaviors and the only covariate for which migrants and residents had an opposite direction of selection.

Throughout the prediction area, bighorn sheep habitat was centered around mountainous areas in all seasons (Figure 34). Habitat for migrants in summer was broadly distributed across rugged and steep areas at high elevations. Winter migrant and resident habitat was broadly dispersed throughout the prediction area at mid to low elevations proximal to steep and rugged slopes.

Our predictions within the Tendoy Mountain restoration area indicated widespread seasonal habitat with relatively large percentages of preferred habitat areas associated with high RSF bin values for both seasons (Figure 35). We identified winter ranges within the northern, central, and southern parts of the restoration area which could be used as release sites to place animals into the highest quality habitat. Given the broad expanses of seasonal habitat for migrants, sourcing individuals from migrant populations would be appropriate for future translocations. Additionally, because of the strong correlation between migrant selection patterns in winter and resident annual use, resident individuals could also be considered in future translocation efforts.

Our characterization of bighorn sheep habitat adjacent to extant populations in western Montana generally indicated a positive relationship between the RSF bin values and their relative abundance surrounding the extant populations (Figure 36). Across the extant populations, many showed potential for intra-mountain translocations to expand the current distribution, while for others this restoration strategy would not be recommended because our models predicted little habitat adjacent the current populations. For example, the summer and winter range of the Stillwater population and winter range of the Beartooth WMA population were embedded within a broad distribution of bighorn sheep habitat. For other

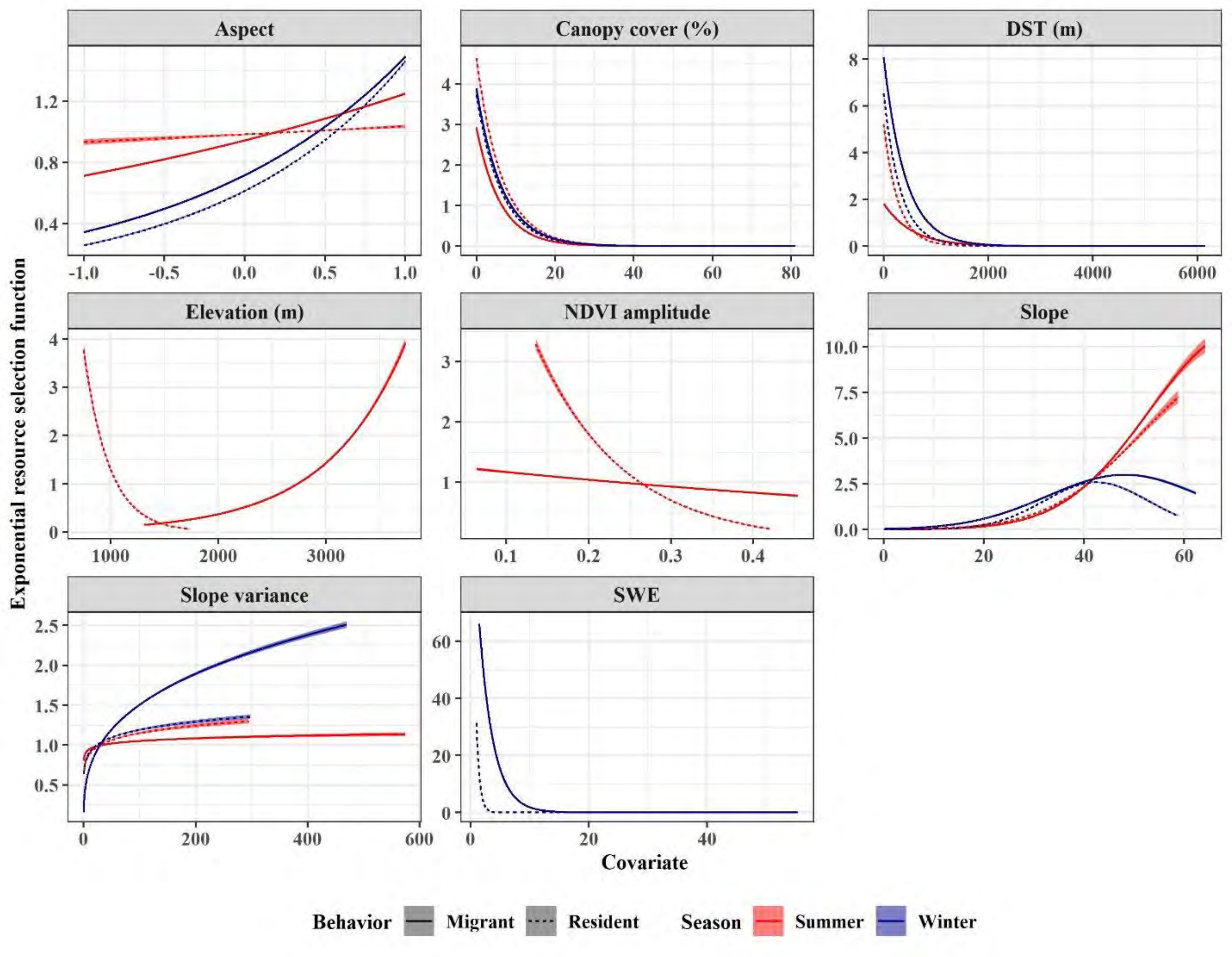


Figure 33 - Prediction plots showing the relationship with the exponential resource selection function (±95% CI) for the seasonal behavior-specific habitat models (summer = red, winter = blue, migrant = solid line, resident = dashed line) developed for female bighorn sheep, western Montana, USA, 2012–2018. DST = distance to steep terrain, SWE = snow water equivalent.

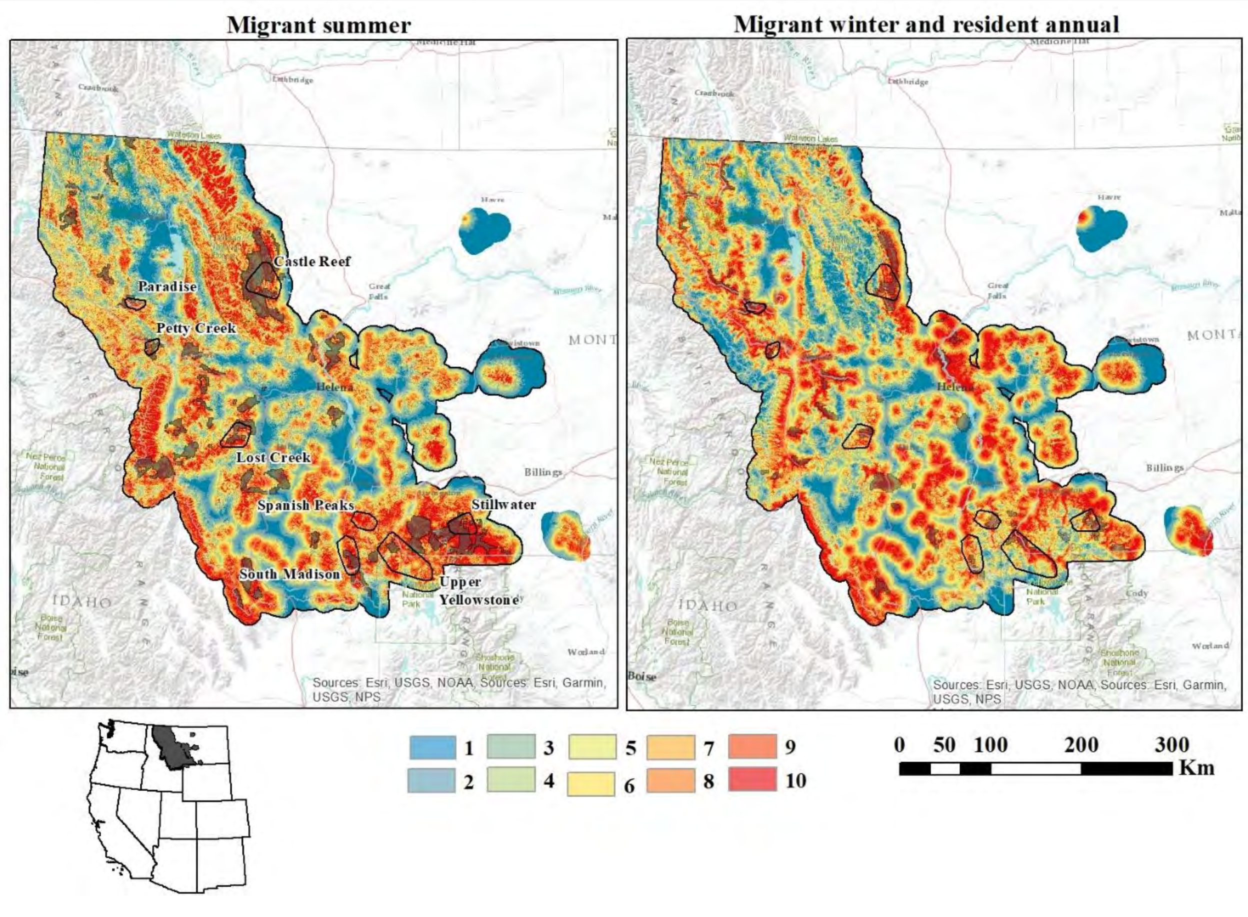


Figure 34 - Seasonal resource selection model predictions for migratory bighorn sheep, which in winter also characterizes resident annual use, western Montana, 2012–2018. Resource selection bins are numbered 1–10 (blue–red). The population annual minimum convex polygons (black) are shown as are the generalized expert opinion bighorn sheep seasonal ranges (dark grey).

populations, for example Ural Tweed in northwest Montana, there was relatively little habitat adjacent to the existing distribution of animals.

By combining the behavior-specific approach with a broad GPS data set, our models provided several advantages over the existing habitat models available in Montana. Our models: 1) provide separate summer and winter habitat predictions, 2) were constructed with a large sample of GPS locations from collared individuals as opposed to management data collected from annual surveys, and 3) were validated using multiple methods and data sources that span western Montana. When paired with other habitat models for bighorn sheep throughout the state, there is now a state-wide habitat map to help inform restoration in both mountain and prairie (DeVoe et al. 2020) regions of Montana. Additionally, the behavior-specific approach allows managers to match the migratory behavior of source populations with landscape characteristics of the area being restored.

By characterizing unoccupied areas that are adjacent to the known distribution of bighorn sheep across western Montana, our work expanded on Lula et al. (2020) in evaluating the potential of habitat to limit bighorn sheep distributions more broadly across the state. We

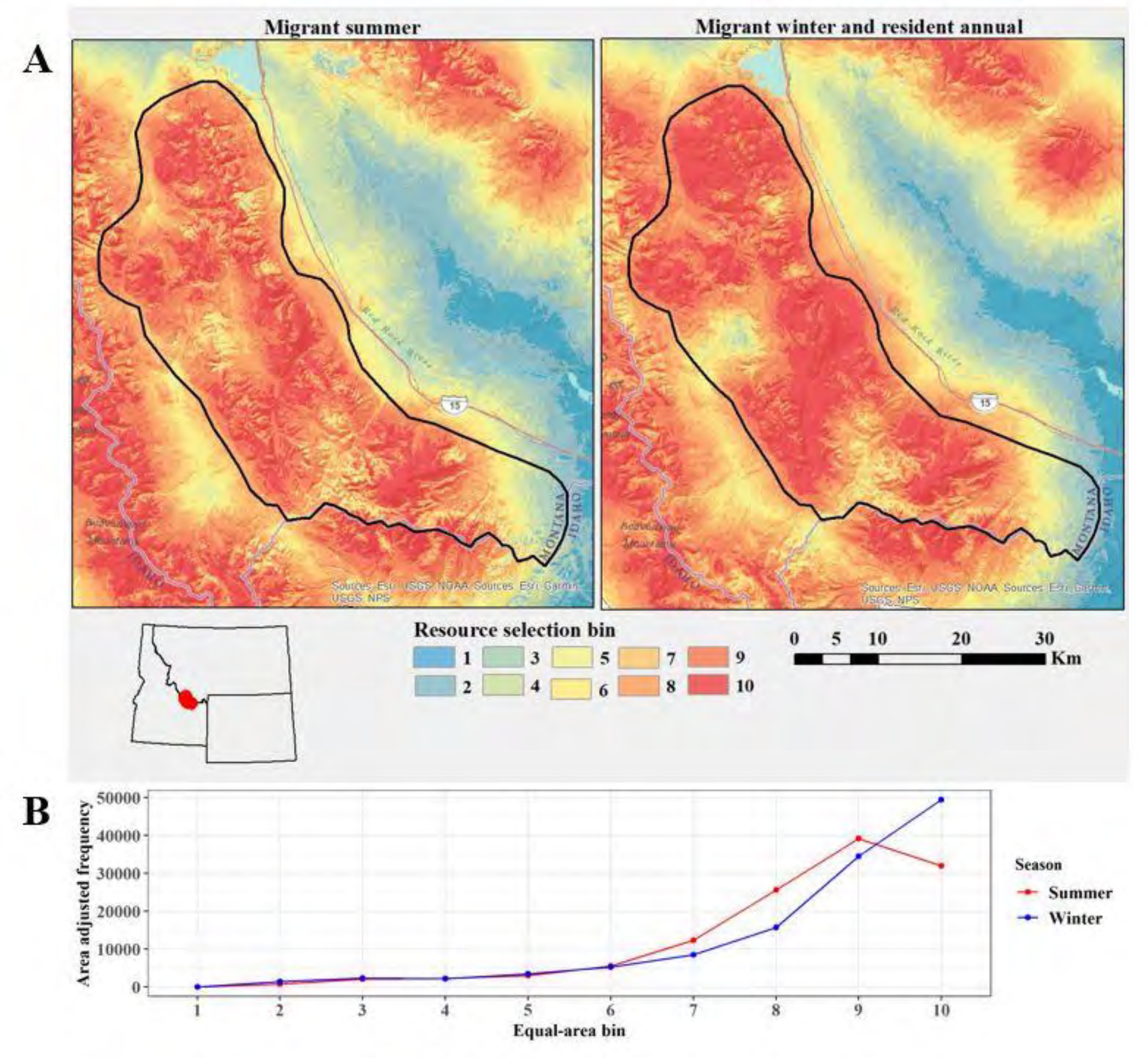


Figure 35 – A. Summer and winter migrant model predictions in the Tendoy Mountains, southwest Montana, USA 2012–2018. Because of the strong correlation between winter selection of migrants and resident annual use, we interpreted the winter model for both migrants in winter and residents annually. B. The frequency of occurrence of RSF bin values from the seasonal migrant models predicted across the Tendoy Mountains.

observed a larger proportion of preferred habitat areas surrounding many existing populations and suggest there is broad restoration potential through intra-mountain translocations. We encourage local working groups to further evaluate the restoration potential of these areas beyond the 15 km buffer distance included in our analysis.

Throughout our study system, residency occurred exclusively on low elevations. However, in other systems residency can also occur at high-elevations where bighorn sheep remain at elevations ≥ 3,000 m annually (Courtemanch et al. 2017, Spitz et al. 2020). Models of high-elevation winter or resident ranges may help to restore bighorn sheep into these historic ranges where there is reduced

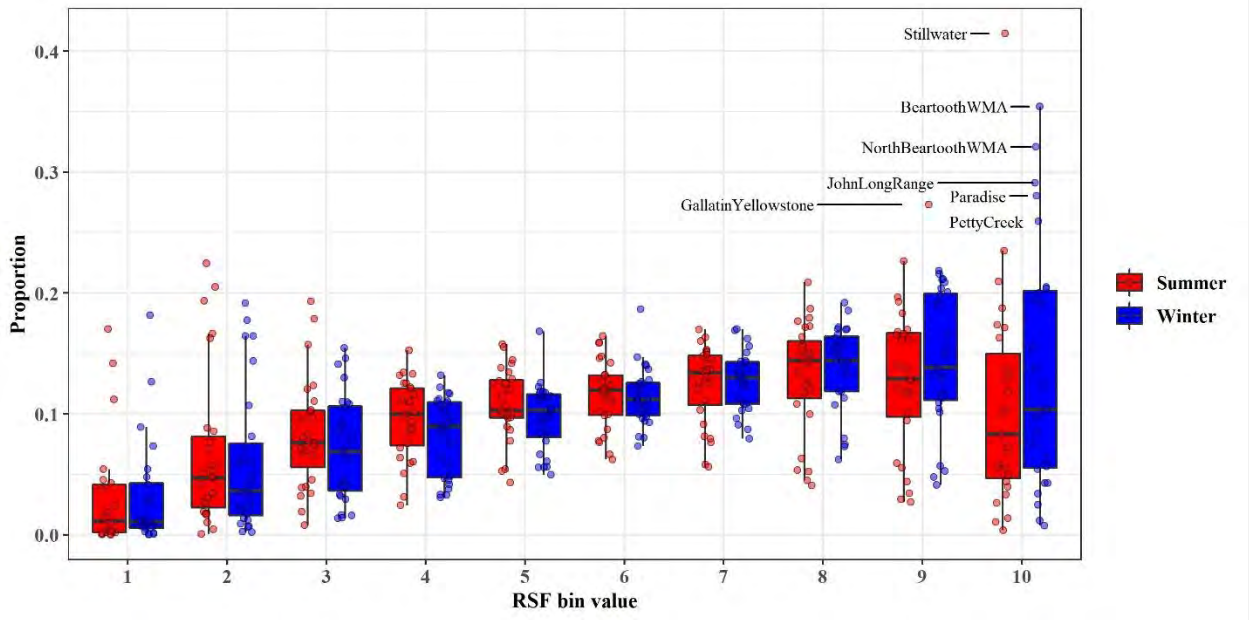


Figure 36 - Boxplots (and raw data points) summarizing the seasonal distribution of the proportion of each RSF bin within the 15km buffer surrounding the general bighorn sheep distribution polygons. The points with a proportion > 0.25 are labeled with the population name for reference.

risk of overlap with domestic livestock and associated deleterious pathogens (Wyoming Game and Fish Department 1980, Lowrey et al. 2020).

While the difference in selection with respect to elevation for residents and migrants was expected, the behavior-specific approach provided a framework for explicitly modeling differences in selection between the two migratory behaviors. In our study, incorporating the behavior-specific relationships resulted in models with additional biological relevance and utility in informing future translocation. Just as generating sex-, age-, or season-specific models is common practice in wildlife research, our study extends these principles to generating models for population components with unique migratory behaviors where selection patterns vary among individuals. While evaluating the need for multiple habitat models for different migratory behaviors may not always result in behavior-specific models, such evaluations are an important aspect of the modeling approach with broad implications for ecology, conservation, and management. As the need for translocations increases across taxa, novel tools and practices will help to ensure that the efficacy of translocations meet the growing demand for their implementation.

PRAIRIE HABITAT MODEL FOR RESTORATION PLANNING

Bighorn sheep occupy a diversity of rugged landscapes that include montane, desert, and prairie environments (Buechner 1960, Geist 1971). Extending throughout the Northern Great Plains ecoregion of Montana, Wyoming, North Dakota, South Dakota, and Nebraska (Omernik and Griffith 2014), the prairie regions account for a substantial portion of historic bighorn sheep range, which, in the prairie regions, is distributed primarily along river corridors with rough and deeply eroded badlands topography (Buechner 1960, Toweill and Geist 1999). Bighorn sheep, namely the Audubon's bighorn sheep ecotype (*Ovis canadensis auduboni*, Cowan 1940), were entirely extirpated from the prairie regions, with the last known individual harvested in the 1920's in South Dakota (Toweill and Geist 1999).

In Montana, approximately 35% of the recognized historic range of bighorn sheep extended throughout the prairie region of the central and eastern part of the state (Couey 1950, Buechner 1960). Currently, only 4 of the 49 populations of bighorn sheep in Montana exist in relatively small, isolated regions of the prairie primarily along the Missouri and Yellowstone Rivers – the primary river corridors that historically supported large, well-interconnected populations (Couey 1950, Buechner 1960). The current populations residing along the Missouri River are some of Montana's most abundant and stable and are sought after by hunters owing to exceptional horn-growth in the rams (Montana Fish, Wildlife and Parks 2010). Despite this, little research to inform restoration efforts has been directed at bighorn sheep in the prairie region of Montana and, therefore, little is known about their patterns of resource selection or the availability of habitat across their historic range. Given the current and historic distributions of bighorn sheep, we propose that the historic range in eastern Montana is capable of supporting additional prairie populations of bighorn sheep.

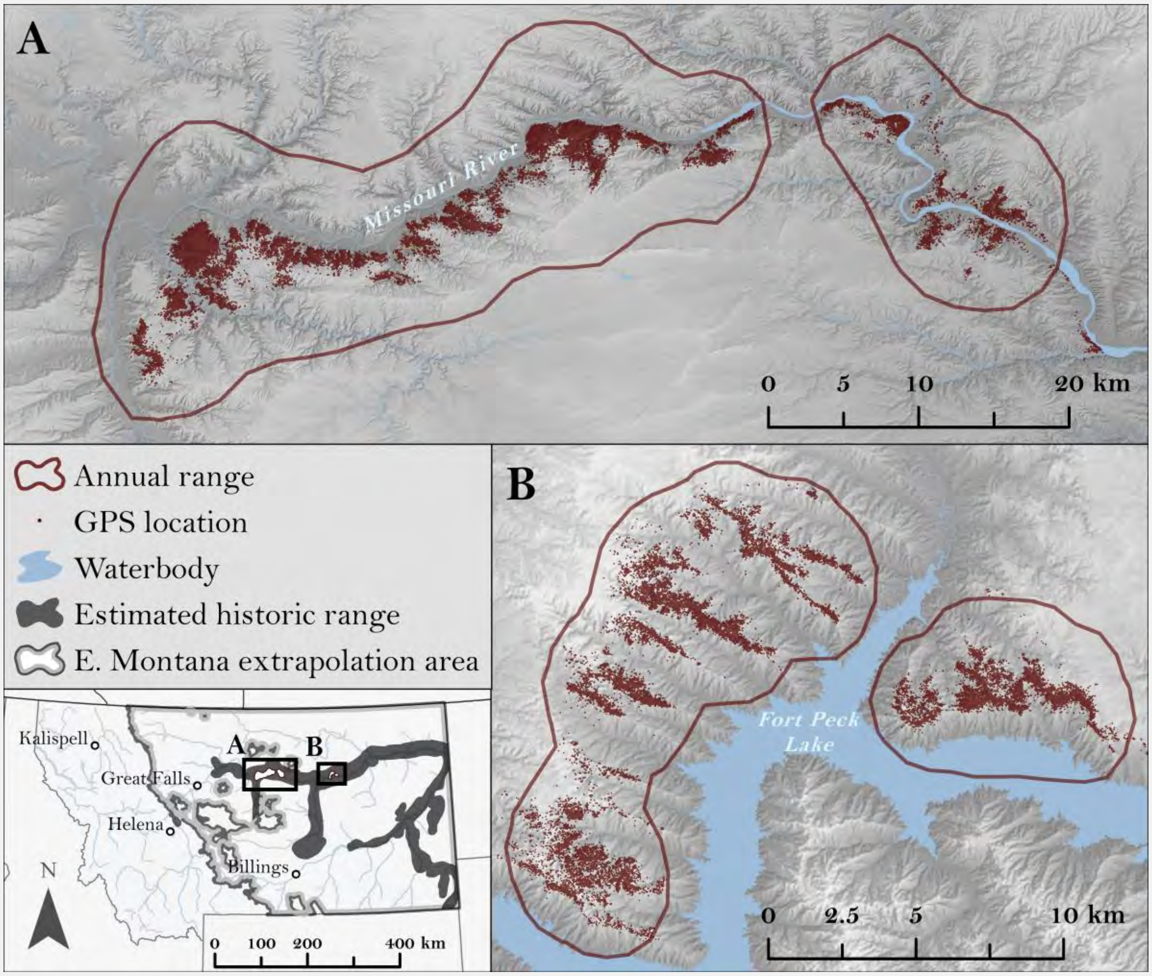


Figure 37 - GPS locations of 43 prairie bighorn sheep instrumented in the Fergus (panel A) and Middle Missouri Breaks (panel B) study areas in eastern Montana, 2014–2018. Inset map shows the extrapolation area across the prairie region and encompassing the estimated historic range of bighorn sheep (Couey 1950) and study areas. The study areas represent annual ranges (red lines) that were defined based on a 95% kernel density estimate of all locations for each population. Elevation is represented by the dark grey (low) to light grey (high) gradient. Note differences in panel scales.

Montana's management plan for restoring bighorn sheep prioritizes the establishment of new populations in areas of identified habitat that are primarily public lands accessible by the hunting public and that were historically occupied by bighorn sheep (Montana Fish, Wildlife and Parks 2010). Further, given the potential of domestic sheep to transmit pathogens that can cause respiratory disease and increased mortality risk in bighorn sheep (Besser et al. 2013, 2014), state and federal policy restricts translocations to areas that have some level of separation (e.g., physical barriers or distances ≥23 km) of domestics and bighorn sheep (Montana Fish, Wildlife & Parks 2010, Wild Sheep Working Group 2012, U.S. Forest Service 2015). Under these policies, restoration of bighorn sheep to Montana's prairie region is challenging owing in particular to the heterogeneity of public and private lands and the distribution of domestic sheep on private lands that occur in the region. Even so, substantial restoration opportunities may exist across these heterogeneous ownerships as well as outside the recognized historic range. There is therefore a need to identify habitat across the prairie region in both privately and publicly owned lands located both within and outside of the estimated historic range to provide the state's wildlife agency with information for developing restoration actions for establishing populations of bighorn sheep to public-land dominated regions of the prairie and for supporting a broader restoration program. While separation of domestic and bighorn sheep is an important consideration, data on the distribution of domestic sheep does not exist at a broad scale. Managers, however, can combine information on bighorn sheep habitat with local knowledge of domestic sheep distributions to make translocation decisions.

We sought to provide wildlife managers with tools for understanding patterns of habitat selection and supporting restoration efforts in prairie landscapes of Montana. Using GPS data collected from the Fergus and Middle Missouri study populations (Figure 37) and a suite of habitat covariates expected to influence habitat selection (DeVoe et al. 2020), we built an annual RSF habitat model. To build this model, we used a tiered approach based on AIC_c and selected the most supported model. Within the tiered approach, we evaluated multiple functional forms (i.e., linear, quadratic, and pseudothreshold) of the covariates and compared similar landscape covariates bringing forward the most explanatory covariates in our top model. We used the model to predict habitat across the prairie region of eastern Montana and identify potential translocation sites both within and outside of the estimate historic range. We summarized our predictions of habitat across land ownership for currently unoccupied areas of the estimated historic range to characterize restoration potential under the constraints of public land restoration within the historic range.

Our results indicated that prairie bighorn sheep generally selected for areas with steeper and more rugged terrain (VRM) and lower canopy cover and $NDVI_{Amp}$ values (Figure 39). We validated the model using *k*-fold validation (Boyce et al. 2002) which resulted in good predictive performance. Predicted habitat was generally patchily distributed along river corridors and drainages with heterogeneous terrain across the prairie region and accounted for 19% (7,211 km^2) of the estimated historic range currently unoccupied by bighorn sheep (38,046 km2; Figure 38). Our extrapolation results indicate that the prairie region is not constrained by lack of available habitat and is capable of supporting a broad distribution of bighorn sheep. Habitat primarily occurred on private, Bureau of Land Management, and U.S. Fish and Wildlife Service lands (Table 8).

To further explore the potential for restoration within currently unoccupied regions of the estimated historic range in the prairie region, we coupled our habitat model to count data from annual surveys of the hunting districts (HD 482 for Fergus and HD 622 for Middle Missouri) of the 2 study populations (Boyce and McDonald 1999, Boyce and Waller 2003). We used the maximum population count surveyed during 2014 – 2018 (n_{min} = 226 from HD 482 and n_{max} = 318 from HD 622) to estimate densities of

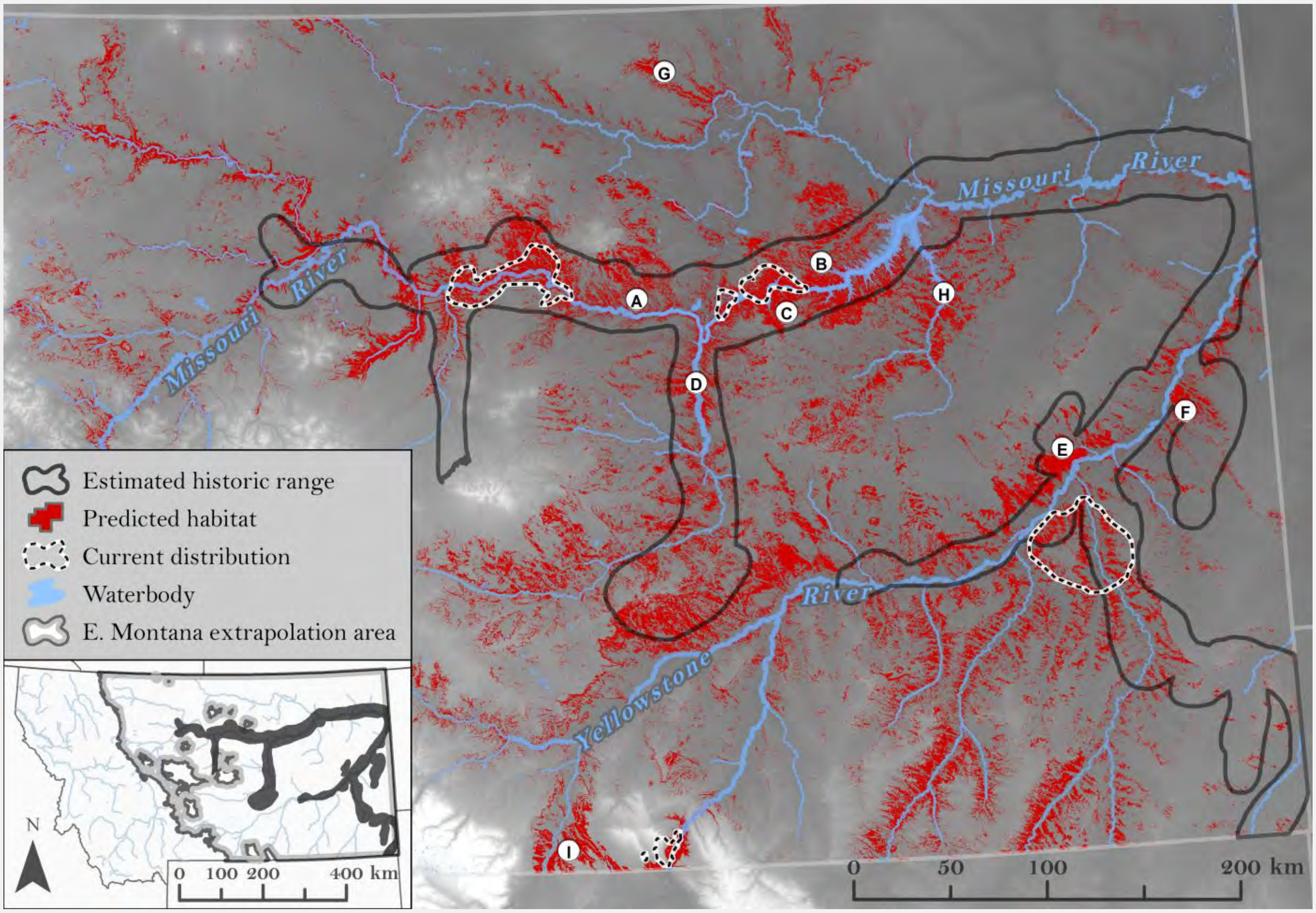


Figure 38 - Extrapolation of the prairie habitat model overlaid on elevation (low = dark grey, high = white) and the current distribution of bighorn sheep in the prairie region. Potential translocation sites are identified by alphabetical characters and described in Table 9.

bighorn sheep in the respective hunting districts. We then applied those densities to our extrapolated results and estimated a range of abundance values, assuming all potential habitat were occupied and resources used similarly to the 2 study populations. Our results indicate that the currently unoccupied areas of the estimated historic range may be capable of supporting between 1,327 and 3,457 bighorn sheep, which is about 2 – 3 times the number currently estimated in Montana's prairie region.

To provide guidance for organizations interested in the recovery of bighorn sheep, we used the predicted habitat to identify 9 potential translocation sites based generally on the availability of contiguous habitat juxtaposed on lands largely under public ownership (Figure 38 and Figure 40; Table 9). Within the estimated historic range, these sites included regions: 1) between HD 680 and 622 and north of the Missouri River, 2) east of HD 622 and the Middle Missouri Breaks study area, 3) south of HD 622 and the Middle Missouri Breaks study area located across Fort Peck Lake of the Missouri River, 4) along the lower reaches of the Musselshell River on both the west and east sides, 5) northwest of the Yellowstone River across from the confluence of the Powder River, and 6) southeast of the Yellowstone River encompassing Makoshika State Park. Outside of the estimated historic range, these included regions adjacent to: 1) the upper reaches of Cottonwood Creek that flows into the Milk River, 2) Big Dry Creek and the Dry Arm of Fort Peck Lake of the Missouri River, and 3) the Clarks

Fork of the Yellowstone River located north of the Montana state border and west of HD 503.

Given our results, we conclude that there is extensive habitat beyond the current distribution of bighorn sheep in the prairie region and considerable potential for additional restoration opportunities both within and outside of the recognized historic range of bighorn sheep. The current populations restored to the prairie have proven to be demographically robust and provide substantial consumptive and non-consumptive opportunities to the public and local residents, suggesting that continued restoration efforts in the prairie could achieve similar results. Although some patches of predicted habitat may be too small to support persistent populations, and would reduce the estimated number of bighorn sheep that is supportable by the predicted habitat, these patches may be valuable for promoting the exploration of unoccupied habitat and providing important linkages between larger patches of predicted habitat (Bleich et al. 1996).

The broad and mosaicked distribution of private lands (Figure 40) that contain habitat limits the restoration potential under current policies requiring any new populations to be established in areas of primarily public land that are accessible by the hunting public and within bighorn sheep historic range (Montana Fish, Wildlife and Parks 2010). Unless policies are implemented that allow establishment of populations on large tracts of private land that may not be adjacent to public land or permit public hunter access, broad efforts to restore bighorn sheep to their former range would require strong collaboration between private and public jurisdictions to lessen the restrictions of public hunter access on private lands. In addition, liberalizing restoration constraints to areas outside the historic range, which is not entirely known, could considerably enhance restoration successes in Montana.

Restoration efforts may also be constrained by the distribution of domestic sheep given state and federal policies for maintaining separation between the two species to reduce the likelihood of transmitting disease-causing pathogens to bighorn sheep (Montana Fish, Wildlife and Parks 2010, Wild Sheep Working Group 2012, U.S. Forest Service 2015). Identifying translocation sites that reduce the risk of commingling between these species is critical. Future restoration planning and implementation will need to occur at the local level with state, federal, and private partners who have knowledge of domestic sheep distributions in order to further define potential restoration sites. Given domestic sheep operations and policies to maintain separation of domestic and bighorn sheep populations, our estimates of predicted habitat and abundance are likely overestimates of the practical reality of restoration potential. To realize the full biological potential of bighorn sheep restoration in the prairie region, innovative partnerships between natural resource agencies and private landowners are needed to resolve the obstacles related to commingling of bighorns sheep with domestic livestock. This may include incorporating into translocation decisions an assessment of the level of risk that the public and resource agencies are willing to accept in regards to pathogen transmission.

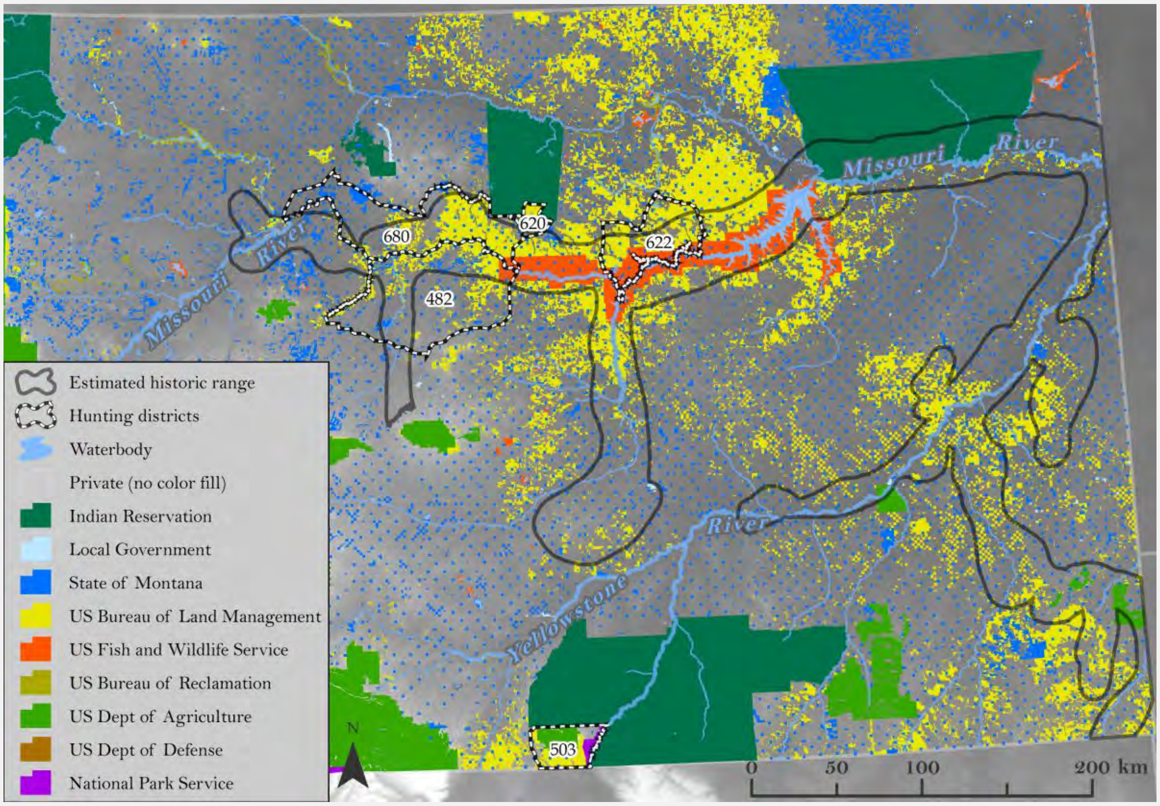


Figure 40 - General land ownership, Montana hunting districts (http://gis-mtfwp.opendata.arcgis.com), and estimated historic range of bighorn sheep overlaid on elevation (low = dark grey, high = white) in the prairie region of eastern Montana.

Table 8 - Summary of predicted bighorn sheep habitat in currently unoccupied areas within the estimated historic range for each land ownership in the prairie region. The total area of the currently unoccupied historic range was 38,045 km^2 and of habitat in the currently unoccupied historic range area was 7,211 km^2.

Land ownership	Proportion (area km^2)	Proportion of habitat (area km^2)
Private	0.6208 (23,634.55)	0.4412 (3,185.93)
Bureau of Land Management	0.1743 (6,635.86)	0.3303 (2,385.05)
U.S. Fish and Wildlife Service	0.0644 (2,450.70)	0.1507 (1,088.06)
State of Montana	0.0591 (2,251.55)	0.0684 (493.68)
U.S. Forest Service	0.0084 (318.21)	0.0036 (25.64)
Fort Peck Indian Reservation	0.0691 (2631.88)	0.0030 (21.41)
Water	0.0009 (33.26)	0.0012 (8.73)
U.S. Dept. of Agriculture	0.0015 (56.35)	0.0007 (5.13)
City Government	0.0005 (19.95)	0.0005 (3.97)
County Government	0.0008 (29.10)	0.0002 (1.46)
U.S. Dept. of Defense	0.0002 (6.17)	0.0002 (1.21)
Bureau of Reclamation	0.0001 (4.54)	0.0001 (0.57)
Fort Belknap Indian Reservation	<0.0001 (0.01)	<0.0001 (0.01)

Table 9 – Potential translocation sites within and outside the estimated historic range in the prairie region. Region IDs correspond to Figure 38. Sites were identified as areas of relatively contiguous predicted habitat generally the same size as or larger than the annual ranges of the study populations and positioned on lands largely publicly owned.

REGION ID	NAME	LOCATION DESCRIPTION
A	Upper Missouri Breaks-West	Between HD 680 and 622 and north of the Missouri River, including the Duval Creek, Siparyann Creek, Rock Creek, Sevenmile Creek, CK Creek, and Beauchamp Creek drainages
B	Lower Missouri Breaks	East of HD 622 and the Middle Missouri Breaks study area, including the Sutherland, Willow, and Beaver Creek drainages
C	Missouri Breaks-Prairie	South of HD 622 and the Middle Missouri Breaks study area located across Fort Peck Lake of the Missouri River, including the Devils, Seven Blackfoot, and Billy Creek drainages
D	Lower Musselshell River	Lower reaches of the Musselshell River on both the west and east sides, including the Blood Creek, Sacagawea River, Lodgepole Creek, and Nancy Russell Creek drainages
E	Crooked Creek	Crooked and Lost Boy Creek drainages, northwest of the Yellowstone River across from the confluence of the Powder River
F	Makoshika	Southeast of the Yellowstone River encompassing Makoshika State Park
G	Upper Cottonwood Creek	Upper reaches of Cottonwood Creek that flows into the Milk River
H	Big Dry Creek/Arm	Big Dry Creek and the Dry Arm of Fort Peck Lake of the Missouri River
I	Upper Clarks Fork of the Yellowstone	North of the Montana state border adjacent to the Clarks Fork of the Yellowstone River, primarily east of State Route 72, west of HD 503, and including the Silvertip, Hunt, and Cottonwood Creek drainages

Chapter Summary

- Seasonal locations from GPS collared adult female bighorn sheep demonstrated that 4 study populations (Paradise, Petty Creek, Fergus, and Middle Missouri) were primarily non-migratory and 4 were primarily migratory (Castle Reef, Stillwater, Hilgard, and Lost Creek); however, some individual variability existed within populations.
- Relative to native populations, restored and augmented populations had less variation among individuals with respect to elevation and geographic migration distances, with the majority of native populations having a range of variation in geographic distances of 2 – 4 times greater than restored or augmented populations.
- Limited migratory diversity in restored and augmented populations may be an additional limiting factor to demographic performance and range expansion. Preserving native migrations and matching migratory patterns of source populations with local landscape attributes for restoring and augmenting populations may improve success of broad restoration efforts and increase the resiliency of populations to perturbations.
- Natural forage on high-elevation summer ranges are typically deficient in essential trace minerals and bighorn sheep routinely alleviate deficiencies by conducting repeated short-duration migrations to natural and anthropogenic 'lick' sites. Licks are an important habitat attribute for bighorn sheep populations that are likely required to maintain animal health and population demographic vigor. Summer migrations by ewes with lambs to low-elevation licks likely increase risk from predation and other sources of mortality.
- Habitat modeling in the Madison Range suggests that significant potential for intramountain restoration exists that could double to quadruple the number of bighorn sheep currently residing in the Range. The habitat model provides managers with a useful tool for identifying future translocation sites that maximize the probability of population establishment and for monitoring the success of restoration efforts.
- Migratory behavior-specific habitat modeling in the montane environments of western Montana indicate that broad restoration potential exists which could be achieved through translocations that match the migratory strategy of source populations with landscape characteristics of the area being restored. Restoration in many areas could be achieved through translocations to establish new populations within unoccupied historic range or through intra-mountain translocations from adjacent existing populations.
- Habitat modeling in the prairie environments of eastern Montana indicate that considerable restoration opportunities exist both within and outside of the recognized historic range of bighorn sheep that could double to triple the number of bighorn sheep that currently occupy Montana's prairie region. We identify 9 potential translocation sites in the prairie region to guide potential restoration efforts.

Peer-reviewed Science Products

Lowrey, B., K. Proffitt, D. McWhirter, P. White, A. Courtemanch, S. Dewey, H. Miyasaki, K. Monteith, J. Mao, J. Grigg, C. Butler, E. Lula, & R. Garrott. 2019. Characterizing population and individual migration patterns among native and restored bighorn sheep (*Ovis canadensis*). Ecology and Evolution. https://doi.org/10.1002/ece3.5435.

Lowrey, B., D. McWhirter, K. Proffitt, K. Monteith, A. Courtemanch, P.J. White, T.J. Paterson, S. Dewey, & R. Garrott. 2020. Individual variation creates diverse migratory portfolios in native populations of a mountain ungulate. Ecological Applications. https://doi.org/10.1002/eap.2106.

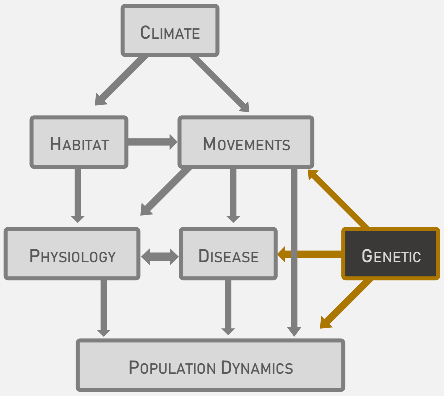
Lula, E., B. Lowrey, K. Proffitt, A. Litt, J. Cunningham, C. Butler, & R. Garrott. 2020. Is habitat constraining bighorn restoration? a case study. Journal of Wildlife Management. https://doi.org/10.1002/jwmg.21823.

DeVoe, J., B. Lowrey, K. Proffitt, & R. Garrott. 2020. Restoration potential of bighorn sheep in a prairie region. Journal of Wildlife Management. http://doi.wiley.com/10.1002/jwmg.21922.

Lowrey, B. DeVoe, J., K. Proffitt, & R. Garrott. In review. Behavior-specific habitat models as a tool to inform ungulate restoration. Ecosphere.

CHAPTER 5

GENOMICS



Objective Describe the collection and genotyping of samples for a diversity of bighorn sheep populations to perform genomics studies addressing management questions. Provide the optimal per population sample size for genetic assessment of bighorn sheep populations. Describe the evaluation of genetic uniqueness and differentiation among sampled populations, genetic results of past translocations, and the possibility for a founder effect in reintroduced populations. Quantify inbreeding at the population (herd) level and determine if inbreeding levels are associated with population attributes and management history. Compare inbreeding levels with disease history and population demographic performance. Describe the relationship of population genetics to ecological and evolutionary processes influencing restoration and conservation.

GENETIC INVESTIGATIONS were added to the Montana Bighorn Sheep Study project in 2016 as an integral component of a comprehensive research program to address potential limiting factors in bighorn sheep restoration, conservation, and management. For example, genetic consequences of inbreeding in small populations can impact recruitment, and local adaptations and can influence translocation success. Comparing genetics of different bighorn sheep populations could potentially provide information to describe genetic connectivity and diversity of examined populations, as well as discover links between population demography and genetics. Genetics research may also serve to inform evaluation of genetic diversity in current or previously small populations, aid in selection of potential source populations for augmentation or reestablishment projects, determine what populations have low genetic diversity and might benefit from augmentation, discover what populations are genetically unique, and examine potential links between genetics and population history of respiratory diseases.

The Ovine array is a new genetic analysis technique originally developed for domestic sheep that provides considerable promise for advancing bighorn sheep genetics research. The Ovine array contains approximately 700,000 single nucleotide polymorphisms (SNPs), with approximately 24,000 markers that are informative for Rocky Mountain bighorn sheep (Miller et al. 2015). This technique represents a significant advancement in genetic analysis of bighorn sheep, as most previous studies have used microsatellites and less than 200 genetic markers. In addition, the Ovine array provides the potential to map informative SNPs to genomic areas of known function. The Ovine array provides the capability to conduct whole genome genotyping of bighorn sheep and can serve to increase understanding of population genetics.

GENERATING HIGH-QUALITY GENOTYPE DATA

We have built an archive of over 500 high-quality bighorn sheep genotypes from different populations across Montana, Wyoming,

Table 10 – High quality genotypes derived from genetic samples (gene cards, ear biopsy punches, tissue, nasal swabs, and/or DNA extractions) for different animals from Montana, Wyoming, Colorado, California, and Canada.

POPULATION	MANAGEMENT AGENCY	SAMPLES CURRENTLY ASSAYED
Castle Reef*	MFWP	25[c]
Fergus*	MFWP	30[c]
Petty Creek*	MFWP	25[c]
Lost Creek*	MFWP	25[c]
Middle Missouri Breaks*	MFWP	25[c]
Paradise*	MFWP	25[c]
Stillwater*	MFWP	24[a,c]
Hilgard* (Taylor-Hilgard)	MFWP	30[c]
Galton	MFWP	5[c]
Highlands	MFWP	17[c]
Spanish Peaks	MFWP	20[c]
Tendoys	MFWP	25[a,c]
Wild Horse Island	MFWP	25[a,c]
Glacier National Park & Waterton Lakes National Park	NPS & Parks Canada	95[b]
Beartooth-Absaroka metapopulation	WY Fish & Game & NPS	90[a]
Dinosaur National Monument	NPS	20[d]
Sierra Nevada	CA Dept. of Fish & Wildlife	5[c]
TOTAL		511

** Populations in the Montana statewide research project*

[a] Analysis of these samples was funded by the Wild Sheep Foundation, Holly Ernest at the University of Wyoming, and Gray Thornton from the Wild Sheep Foundation.

[b] Analysis of these samples was funded by the Glacier National Park Conservancy, the National Geographic Society, Glacier National Park, and the National Science Foundation Graduate Internship Program.

[c] Analysis of these samples was funded by Montana Fish, Wildlife and Parks.

Colorado, and California in the U.S. and Alberta and British Columbia in Canada available for genomic analysis (Table 10). Genotyped samples were available due to past capture efforts coordinated by Montana Fish, Wildlife and Parks, Wyoming Game and Fish Department, the Greater Yellowstone Area Mountain Ungulate Project, Yellowstone National Park, Glacier National Park, USGS, and California Department of Fish and Wildlife. We collected multiple types of genetic samples, including gene cards, biopsy ear punches, and whole blood. Collection using gene cards involves placing 2-4 drops of whole blood directly from the syringe onto each of the 4 circles of filter paper on an FTA Classic gene card. Montana Fish Wildlife and Parks has been

collecting DNA using gene cards since 2004. To obtain DNA of greater quality than gene cards can provide, we also collected biopsy ear punches, whole blood, and tissue from hunter-harvested animals. Biopsy punches were obtained from ear cartilage during ear tagging and stored frozen in diluted ethanol.

EXTRACTION OF GENETIC SAMPLES & ASSESSMENT OF DNA QUALITY

During extraction of bighorn sheep genetic samples at MSU, we gained information regarding the quality of DNA that can be extracted from different types of bighorn sheep genetic samples in our lab. While gene cards provide a relatively low-cost method to store genetic samples at room temperature over long periods of time, we found that there are some limitations to their use for genomic analysis. Older gene cards that have not been stored with desiccant in foil pouches over long periods of time provided extractions with lower overall quality and occasionally required multiple extraction attempts to achieve suitable quality for SNP genotyping. More recently collected gene cards that were stored in foil pouches provided higher quality DNA extractions than the older cards. However, these samples were not sufficiently high quality to consider sequencing uses with currently available technology. In addition, despite thorough assessment of DNA quality and quantity in our lab prior to genotyping, a small number of the gene card extractions provided low quality SNP genotyping results.

Thus, we also collected ear punch and whole blood samples for genomic analysis. Ear punches were collected using a single use biopsy punch tool to capture ear cartilage prior to ear-tagging and stored frozen in 90% ethanol. Ear punch extractions generally provided greater quality and concentrations of extracted DNA than gene card extractions. We also collected whole blood samples from a limited number of captured animals that can provide extractions suitable for sequencing when extracted within days of capture. In addition, we extracted DNA from tissue sampled from hunter-harvested animals that provided high quality extractions.

EVALUATING SAMPLE SIZE TO ESTIMATE GENETIC MANAGEMENT METRICS

In April 2018, we published an empirical simulation study in the peer-reviewed journal Molecular Ecology Resources. This study quantified genetic attributes of bighorn sheep populations with a range of different population attributes to investigate genomic kinship within and between populations and estimate an optimal sample size per population for evaluating genetic diversity and distance (Flesch et al. 2018). The literature provides little insight into this issue, and, while we had a target of 15 animals per population in the pilot study, a formal evaluation of sample size requirements aided in generating the highest quality data for the resources invested. Sample size may impact genetic inference, as genetic uniqueness, genetic distance, and inbreeding could be assessed differently, depending on the sampling scheme and the total number of bighorn sheep evaluated (Weir and Cockerham 1984, Schwartz and McKelvey 2009). Thus, we determined the optimal number of animals to sample from each population for genetic analyses. Information regarding optimal sample size served to maximize genetic insight for management and limit costs associated with genetic sample collection, processing, and analysis.

We analyzed genetic material from 30 individuals from each of 4 different populations that we predicted would differ in genetic characteristics due to population dissimilarities that included origin (native/reintroduced), population size, bottleneck history, degree of connectivity, and augmentation history. The 4 populations provided samples across a spectrum of these population attributes and included Fergus, Hilgard, and Glacier National Park in Montana and the Beartooth Absaroka in Wyoming. We took 10,000 random sub samples of 5, 10, 15, 20, and 25 individual bighorn sheep per population unit to evaluate the effect of sample size on estimated variance and relative bias. We evaluated mean kinship (Manichaikul et al. 2010) within each population to determine how related individuals were on average in the same area (an inbreeding metric). This effort addressed our first objective of our original genetics study proposal, which was to determine optimal sample size for genetic assessment of bighorn sheep populations.

Our simulation results indicated that a sample size of 20-25 animals per population is adequate for assessing intra- and inter-population mean kinship. Within populations, the Beartooth Absaroka and Glacier National Park had similar mean kinship values normally distributed around 0. These native metapopulations had lower intrapopulation relatedness than the Fergus and Hilgard populations, which had more complex population histories. A comparison of a native metapopulation (Glacier) and a reintroduced population (Fergus) using the mean kinship metric is in Figure 41. This figure also demonstrates that estimates regarding within population relatedness differences between the 2 different populations do not clearly differentiate until a sample size of 25. To address our hypothesis, we also examined the variance and mean squared error of the mean kinship estimate for each population. Mean squared error was dominated by variance, rather than bias relative to the 30 sample estimate, and mean squared error decreased with increasing sample size for all populations. In regard to kinship between populations, differences in kinship among population comparisons were also more clearly differentiated at a sample size of 25 (Figure 42). Thus, we decided to use 20-25 samples per population to evaluate population genomics of additional populations that were assessed through the statewide study (Table 10).

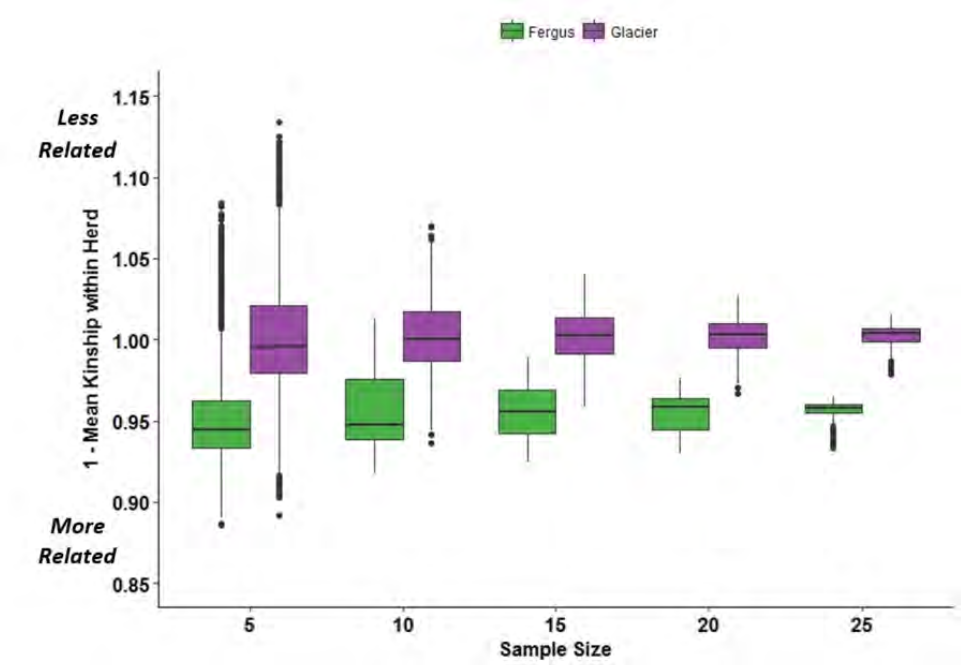


Figure 41 - Boxplots of intrapopulation kinship estimates based on 10,000 replicate simulations using empirical SNP genotypes from populations of bighorn sheep, including 1 minus mean kinship by increasing sample size. Centerlines represent the median, box limits represent the 25th and 75th percentiles, whiskers indicate 1.5 multiplied by the interquartile range from the 25th and 75th percentiles, and points represent outliers. Different populations are indicated by color, including Fergus (green) and Glacier (purple).

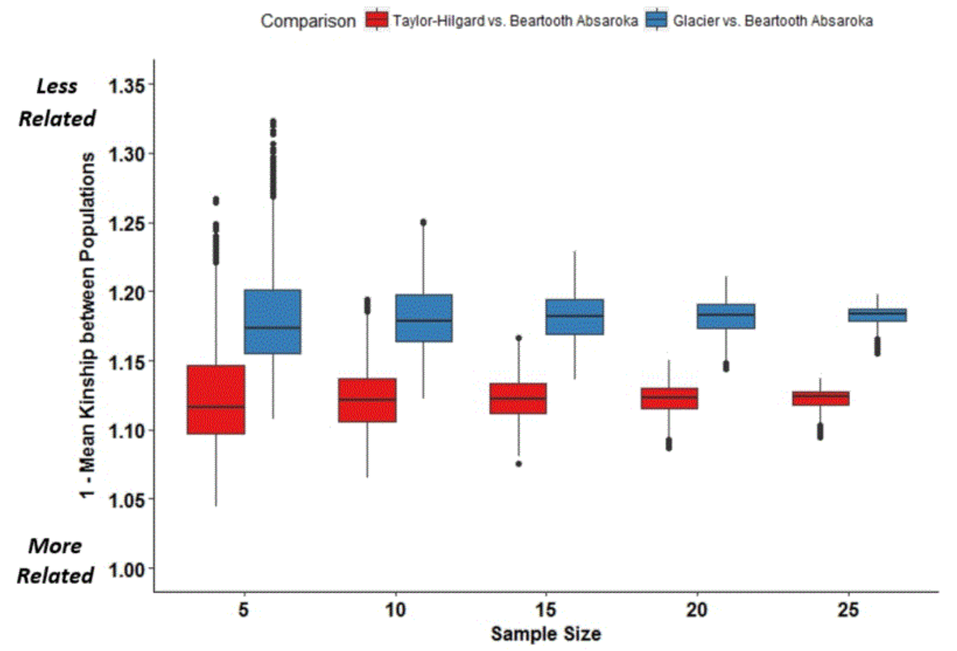


Figure 42 - Boxplots of interpopulation relatedness estimates based on 10,000 replicate simulations using empirical SNP genotypes from populations of bighorn sheep, including 1 minus mean kinship by increasing sample size per individual population included. Centerlines represent the median, box limits represent the 25th and 75th percentiles, whiskers indicate 1.5 multiplied by the interquartile range from the 25th and 75th percentiles, and points represent outliers. Different population comparisons are indicated by color.

COMPARISON OF GENETICS AMONG POPULATIONS

Bighorn sheep restoration often involves translocation efforts to reintroduce and augment populations. We examined the genomic consequences of bighorn sheep translocations and population isolation, to enhance understanding of evolutionary processes that affect population genetics and inform future restoration strategies and genetic management of bighorn sheep. We evaluated the genomics of Rocky Mountain bighorn sheep populations with different origins and translocation histories. The origin of bighorn sheep populations included native (indigenous) populations and reintroduced populations with different founder sizes and number of generations since establishment. Some reintroduced and native populations received augmentations (translocations into an existing population), whereas others remained isolated. Using this diverse set of populations, we evaluated 1) genetic differences among native and reintroduced populations, 2) unassisted past and present gene flow between populations (i.e. gene flow indicating natural movements of individuals), 3) relative contributions of past augmentations (i.e. reproductive success of translocated individuals), and 4) population genetic differences in reintroduced populations from their initial source (i.e. evolution of reintroduced populations). We synthesized this information to inform risk and benefit assessments of future augmentations and reintroductions.

Study Populations

We evaluated 16 Rocky Mountain bighorn sheep populations found in Montana, Wyoming, Colorado, Utah, and the Canadian provinces of Alberta and British Columbia (Figure 43). We sought to sample at least 20-25 individuals per population, based on sample size simulations that determined sampling less than this number would introduce an unacceptable level of uncertainty to estimates of genomic kinship between populations (Flesch et al. 2018). We evaluated 7 native populations, including 1 population of 80-90 animals with no augmentations (Galton), 3 small to moderately-sized populations (80-200 animals) with augmentation attempts (Spanish Peaks, Hilgard, and Stillwater), and 3 large, continuous populations (380-3800 animals), including Beartooth-Absaroka, Castle Reef, and Glacier. Castle Reef is a geographic portion of a large, spatially-structured population (a collection of subpopulations that occupy distinct geographic areas but are linked by animal movement) and is expected to have connectivity across 4 administrative units (Figure 43). Beartooth-Absaroka and Glacier provided baseline genetic examples of large, spatially-structured populations prior to widespread fragmentation of the species' range and without an extensive history of augmentations. Due to large population size and range, we had a greater sample size of 90–95 individuals to represent each of these 2 populations. The Beartooth-Absaroka population spanned multiple management units along the eastern portion of the Greater Yellowstone Area, including Yellowstone National Park and Wyoming hunt units 1, 2, 3, 5, and 22 (Figure 43). Wyoming units 5 and 22 each received an augmentation from a nearby native population, Whiskey Mountain. The Glacier population spanned the U.S.-Canada border, including Glacier and Waterton Lakes National Parks. A large lake and adjacent forest forms at least a partial geographic barrier to most bighorn sheep movement within Glacier, so in some analyses we assessed the Glacier population in units north and south of this drainage (Figure 43; Tosa et al. *in prep*).

We evaluated 9 reintroduced populations. For 8 of those populations, 1 or all of the initial founding sources were included in this study (Figure 43). We considered translocations within 3 years of the first reintroduction event to an unoccupied area as part of the potential founding source. Founder size in reintroduced populations ranged from 8 to 53 bighorn sheep. Based on a generation length of 6 years, estimated using the mean age of reproductively active females, there were 5 to 11 generations since establishment of the reintroduced study

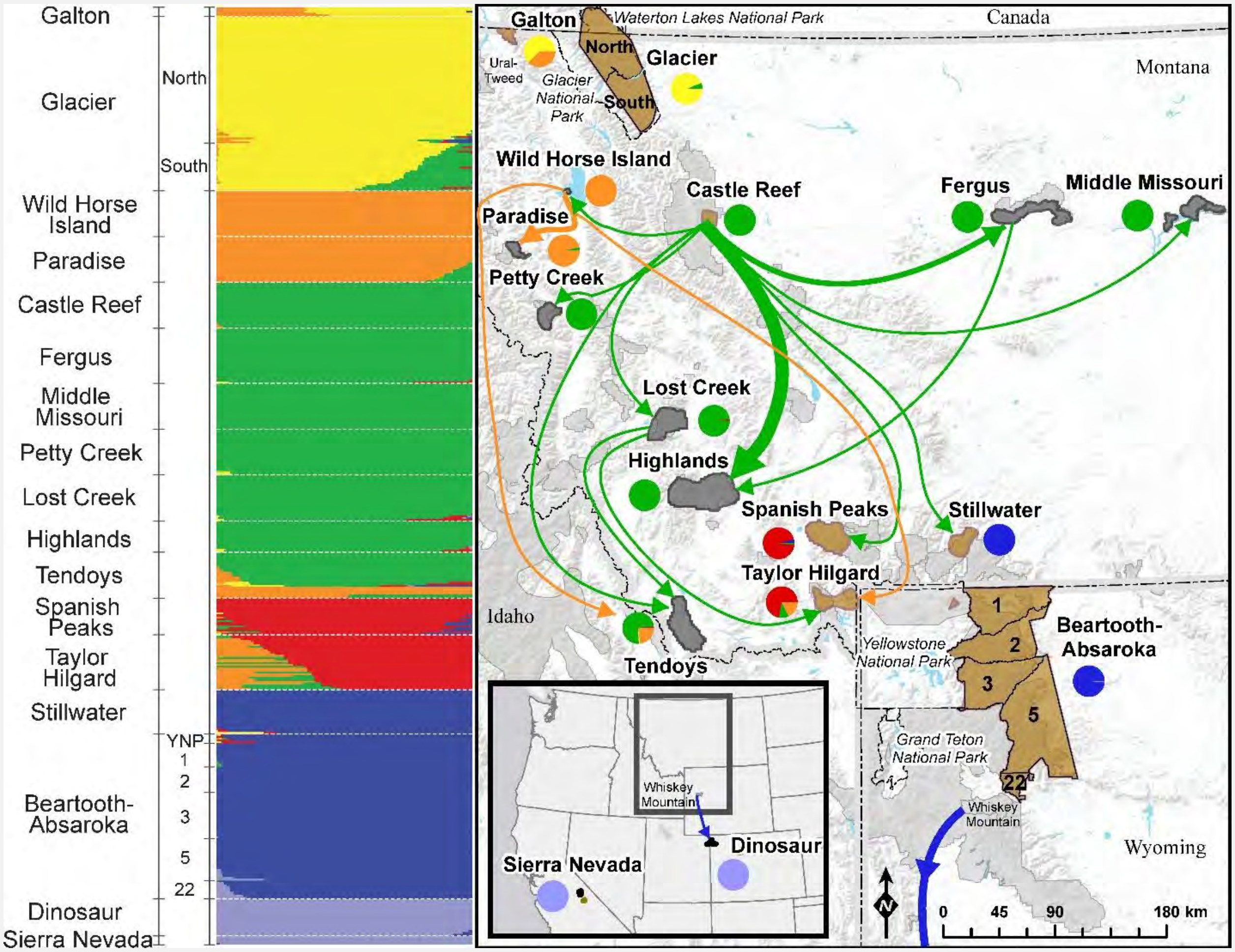


Figure 43 - Map of fastStructure (K=6) results for Rocky Mountain (O.c. canadensis) and Sierra Nevada (O.c. sierrae) bighorn sheep populations genotyped using the HD Ovine array. Approximate distributions of native populations are brown polygons; reintroduced populations are black polygons. A pie chart of population-level fastStructure group assignments is next to each population. All known translocation events between Rocky Mountain bighorn sheep populations in this study are shown by arrows. Arrows point generally to the recipient population and do not represent exact release location. Arrow thickness is proportional to number of translocations; arrow color corresponds to the predominant fastStructure group assignment of the source population. Approximate bighorn sheep ranges, including populations not in this study, are shown in gray polygons for Idaho, Wyoming, and Montana (Montana Fish, Wildlife and Parks 2008, Wyoming Game and Fish Department 2012, Thomas 2019). Hunt unit boundaries for Beartooth-Absaroka are labelled and truncated to bighorn sheep range. State boundaries are designated by dashed lines outlined in gray; national park boundaries in the study area are designated by dashed lines.

populations (Hogg et al. 2006, Johnson et al. 2011). Montana Department of Fish, Wildlife and Parks (2010) defined 5 ecological regions that contain bighorn sheep populations within the state, including prairie/mountain foothills, prairie/breaks, northwest montane, mountain foothills, and southern mountains.

To include a reference outgroup in our analyses, we evaluated 5 Sierra Nevada bighorn sheep (*Ovis canadensis sierrae*), which are considered a different subspecies than Rocky Mountain bighorn sheep (Wehausen et al. 2005, Buchalski et al. 2016). Three Sierra Nevada samples were obtained from the native Sawmill population, and 2 samples came from the reintroduced Wheeler population, which was founded by Sawmill individuals. We gathered records for all known translocations received by study populations, including translocations that originated from areas not included in the study. Where both the source and recipient populations were in this study, approximately 0 to 8 generations occurred since augmentation.

Methods for Population Structure, Ancestry, & Kinship Analyses

We used multiple analyses with different methods and assumptions to address our research objectives. To estimate global ancestry and assess the number of genetically unique populations (K clusters) in our dataset, we used a variational Bayesian framework implemented in fastStructure software (Pritchard et al. 2000, Raj et al. 2014, Pina-Martins et al. 2017). To identify clusters of samples without relying on assumptions used in fastStructure models, we completed a nested multidimensional scaling (MDS) analysis using KING v2.1.4 (Manichaikul et al. 2010). To model genetic drift among populations defined by geography while accounting for unassisted dispersal and translocations, we estimated a maximum likelihood bifurcating tree of populations using Treemix v1.13 (Pickrell and Pritchard 2012). We conducted a 3-population test for admixture to examine gene flow between pre-defined populations (Reich et al. 2009, Pickrell and Pritchard 2012). Finally, we estimated mean kinship between populations using KING v2.1.4 to evaluate genetic differences in reintroduced populations from their founding source and to inform future augmentation decisions by identifying potential source and recipient populations with minimal mean kinship (Manichaikul et al. 2010).

Population Structure & Past Gene Flow

To evaluate genetic differences and gene flow among populations, we estimated the number of genetic populations and ancestry proportions for each individual and population using a fastStructure analysis. Our fastStructure analysis of 17 bighorn sheep populations suggested there were 6 genetic clusters (K). Three clusters consisted of pairs of native populations that were geographically proximate, including Glacier with Galton, Spanish Peaks with Hilgard, and Stillwater with Beartooth-Absaroka. Two other genetic groups encompassed source and receiving populations of reintroductions. Wild Horse Island and Paradise formed a distinct cluster because Wild Horse Island was the sole source for the Paradise reintroduction and later augmentation. The Castle Reef cluster encompassed the greatest number of populations, as Castle Reef bighorn sheep or their descendants founded the other populations in the genetic group, including Fergus, Middle Missouri, Petty Creek, Lost Creek, Highlands, and Tendoys. The 6th cluster included geographically distant Dinosaur and outgroup Sierra Nevada. In the K=7 analysis, Petty Creek formed its own cluster, and in the K=9 analysis, Lost Creek formed its own cluster, which may be due to past augmentations from sources not included in this study.

We detected past augmentations and natural gene flow between genetic groups, where multiple clusters were identified within a population. The following translocation events were detected, indicating that translocated individuals survived and reproduced successfully at the release site: Wild Horse Island to Hilgard, Lost Creek to Hilgard, Wild Horse Island to Tendoys, and Castle Reef to Tendoys (Figure 43). The results of an augmentation from Castle Reef to Spanish Peaks were unclear, as the Castle Reef cluster was detected in fewer than 5 individuals in Spanish Peaks. Augmentations of 2 males to Spanish Peaks and Stillwater from Castle Reef were not detected in multiple analyses, suggesting that after these 2 augmentation events, the translocated individuals did not survive or reproduce. During breeding season, adult male bighorn sheep can wander long distances between mountain ranges and potentially depart the augmentation destination, which may result in no genetic contribution to the intended recipient population. In addition, because bighorn sheep are a polygynous species, a small number of dominant rams may competitively exclude translocated males due to female mate preference for residents or poor condition after transport/release, suggesting that translocating a greater proportion of females may be more effective for augmentation. We also detected augmentations from populations that were not directly sampled but were also augmentation sources to populations in our study. The Whiskey Mountain population (Figure 43; Wyoming hunt unit 10) provided 2 augmentations that likely made a genetic contribution to Wyoming hunt units 5 and 22 in the Beartooth-Absaroka (Wild Sheep Working Group 2015, Love Stowell et al. 2020). Whiskey Mountain contributed 3 augmentations to the Dinosaur population, which was detected by a shared cluster between Dinosaur and augmented hunt units 5 and 22. Finally, our results suggested historical gene flow between Glacier and geographically proximate Castle Reef, as Castle Reef shared a cluster with the southern unit of Glacier.

Genetic Distinctiveness & Recent Gene Flow

We identified 4 clusters of populations in the MDS analysis and evaluated the 4 MDS clusters separately (Figure 44). The cluster shown in Figure 44A encompassed all populations found outside of Montana, including the Sierra Nevada, Dinosaur, and the Beartooth-Absaroka, as well as Stillwater. Most Stillwater individuals showed some differentiation from the Beartooth-Absaroka, even though the 2 populations were grouped into a single fastStructure cluster. However, 4 Stillwater individuals were similar to Beartooth-Absaroka genotypes. Outgroup Sierra Nevada and geographically distant Dinosaur were distinct from the other populations.

Figure 44B, which generally depicted the extensive history of translocations from Castle Reef, included 9 out of 17 populations, similar to the fastStructure results. Wild Horse Island, Lost Creek, and Petty Creek were all founded by Castle Reef but were more dissimilar to their founding source than Fergus, Middle Missouri, and Highlands, indicating different influences on the evolution of these populations. Paradise was similar but somewhat distinct in comparison to its founding and only augmentation source, Wild Horse Island. Tendoys results were reflective of the population's translocation history from 5 different sources. Most individuals in the Tendoys were more similar to Castle Reef, which provided an augmentation, than Lost Creek, which founded the population. Four Tendoy individuals grouped with Wild Horse Island, the source of 1 augmentation. Three Tendoy individuals plotted partway between Castle Reef/Lost Creek and Wild Horse Island, likely representing hybrids from these 2 lineages. One Tendoy individual had an outlier genotype in the overall analysis (Figure 44C), which may represent an individual descended from neighboring populations in Idaho or 2 augmentations from areas not in the study.

Figure 44D included Hilgard and Spanish Peaks, which appeared distinct in the subset analysis, despite being grouped into the same fastStructure cluster. When compared to all other populations, Spanish Peaks was more similar to the nearby native populations of Stillwater and Beartooth-Absaroka, whereas Hilgard was more similar to its augmentation sources, Lost Creek and Wild Horse Island (Figure 44C). Figure 44E included Glacier and Galton, where Glacier genotypes generally separated within the population based on location, north and south of a large lake drainage, and Galton was distinct from Glacier.

Population Tree & Genetic Contributions from Augmentations

We used Treemix to evaluate a bifurcating tree of populations defined by geography that accounted for gene flow. The population tree generated by Treemix was consistent with fastStructure, as the nodes on the tree generally grouped together populations found in the same fastStructure clusters (Figure 45). Sierra Nevada was defined as the outgroup. Geographically distant Dinosaur was the least related to all other evaluated Rocky Mountain bighorn sheep populations. Stillwater and Beartooth-Absaroka were grouped together, followed by Spanish Peaks and the Hilgard. Glacier formed its own branch, as Galton was not evaluated due to low sample size. The remaining populations grouped together were influenced by a history of translocations from Castle Reef and its descendant populations. The population founded by Castle Reef that showed the highest divergence was Wild Horse Island, and the founding of Paradise by Wild Horse Island was accurately represented in the population tree. Reintroduction of the Tendoy population by founders from Lost Creek was accurately depicted in the population tree, despite later augmentations directly from Castle Reef. Petty Creek and Lost Creek showed greater drift parameters from Castle Reef than Fergus and Middle Missouri, consistent with MDS and fastStructure results, although this may be because these populations received augmentations from other populations not included in this study, rather than just genetic drift.

The Treemix model identified 4 augmentation events between specific populations (Figure 45, orange lines). All 4 identified gene flow events represented known augmentations where shared ancestry between populations was identified by fastStructure. The direction of the plotted augmentation event was the least stable feature of the Treemix analysis, and we reversed the direction of augmentation events identified 3rd and 4th, as the direction was known. Translocation weight estimated the proportion of alleles contributed by the source population, assuming admixture occurred in 1 generation (Pickrell and Pritchard 2012). The 3-population test further supported genetic contributions of Wild Horse Island, Castle Reef, and Lost Creek to the Tendoys from translocations. In addition, the 3-population test suggested natural gene flow between Stillwater and the Beartooth-Absaroka, consistent with Figure 44A.

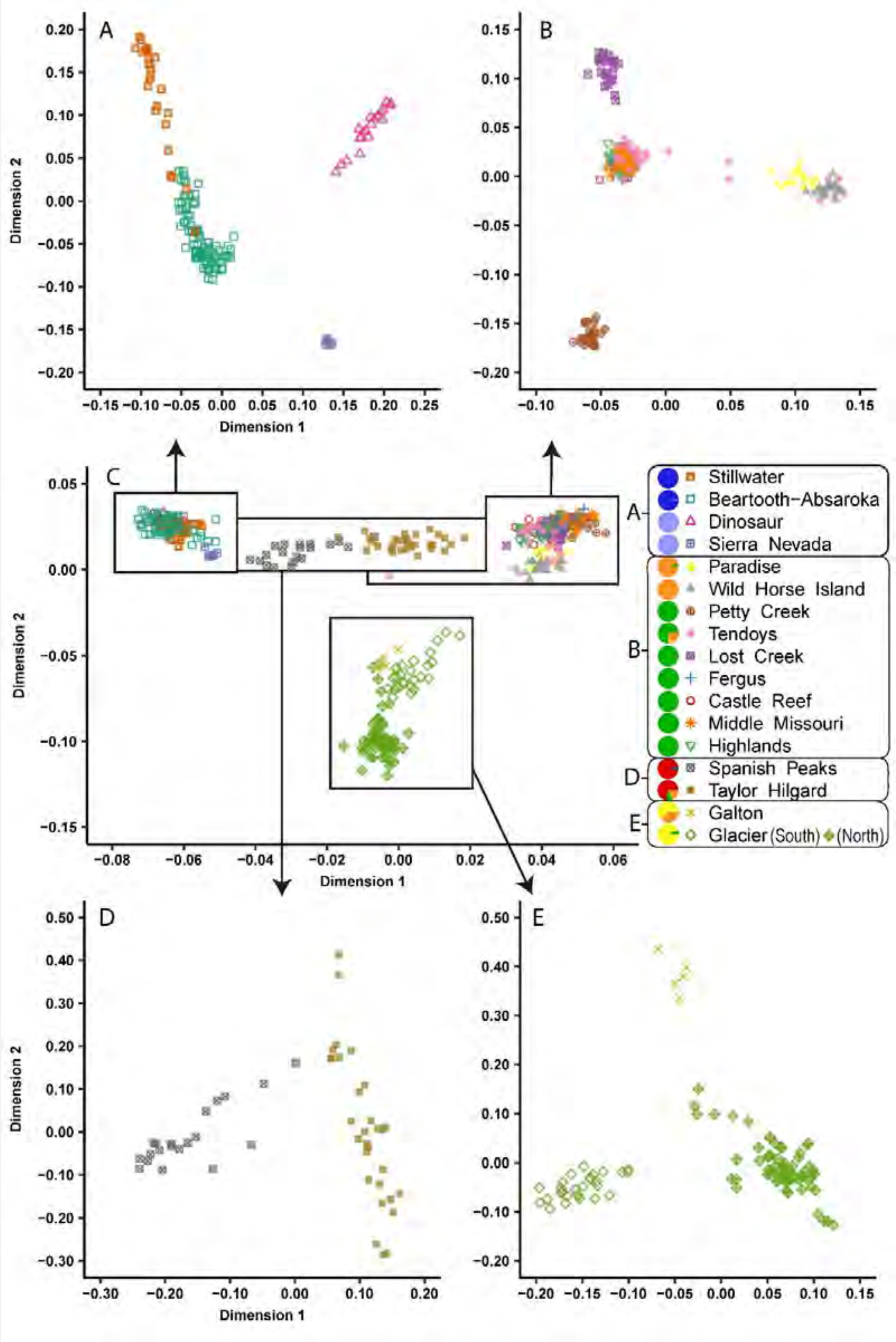


Figure 44 - Multi-dimensional scaling (MDS) results for individual HD Ovine array genotypes from 17 bighorn sheep populations, including an analysis of all populations (C) and subset analyses (A, B, D, and E) based on clusters of populations in panel C. The legend defines symbols for each population and pie charts of population-level fastStructure group assignments (K=6).

Comparing Translocation History & Genomic Analyses

To identify which reintroduction and augmentation efforts made a genetic contribution to the recipient population, we synthesized genomic evidence from fastStructure, MDS, and Treemix for 24 different translocation events where both populations were included in the study. For all 8 reintroduced populations with founding source data, genetic contribution of the original founding group(s) to the contemporary population was suggested by at least 2 analyses. Fifteen out of 24 translocations were augmentations, including 11 unique pairs of source and recipient populations. Four out of 11 augmentation pairs could not be assessed for genetic contribution, as the source population was the same as or genetically similar to the founding source. Of the remaining 7 source and recipient augmentation pairs, we detected 5 augmentation pairs in multiple analyses, including Wild Horse Island to Hilgard, Lost Creek to Hilgard, Wild Horse Island to Tendoys, Castle Reef to Tendoys, and Beartooth-Absaroka (due to augmentation from Whiskey Mountain) to Dinosaur. Augmentations to Spanish Peaks and Stillwater from Castle Reef were not detected in multiple analyses, suggesting that after these 2 translocation events, translocated individuals did not survive or reproduce.

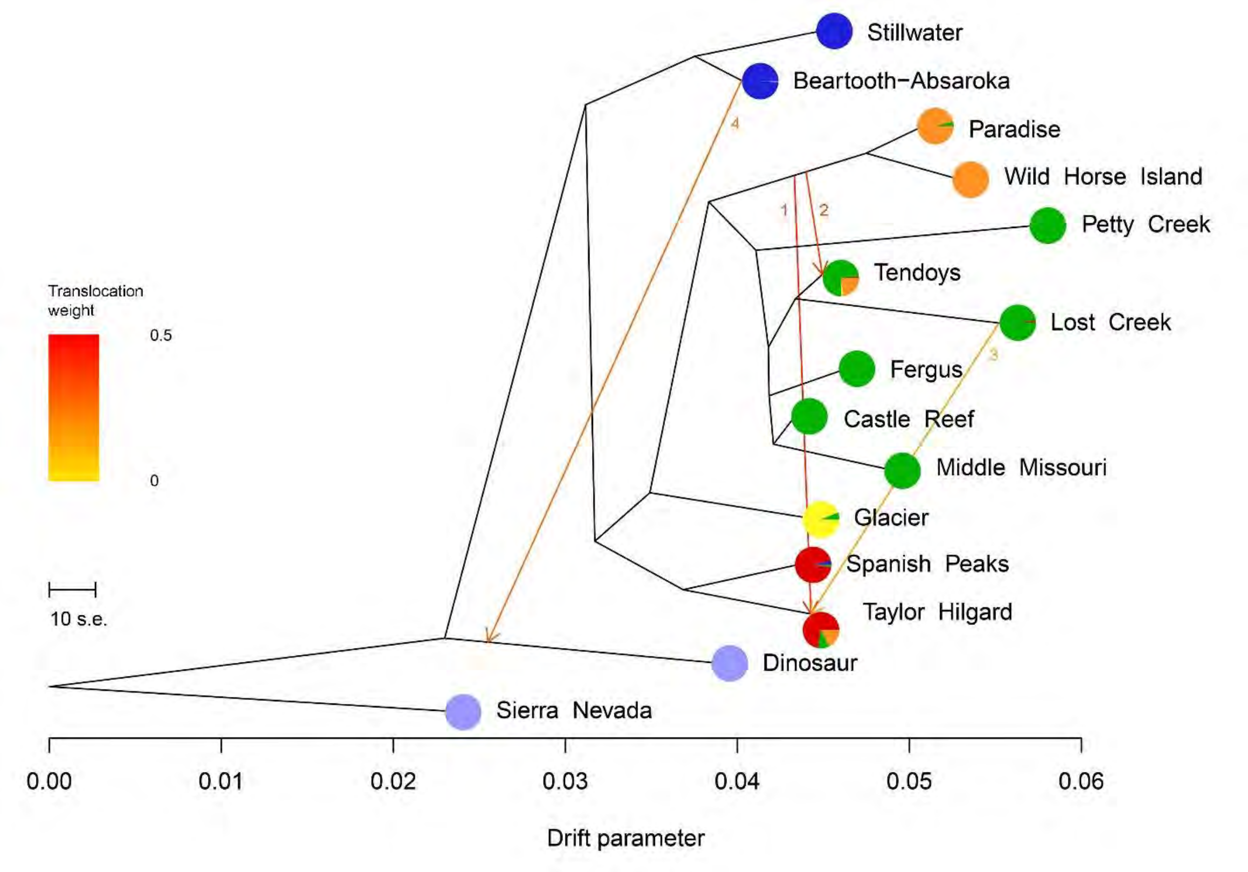


Figure 45 - Treemix population tree with 4 detected translocations (orange lines) plotted for 14 Rocky Mountain bighorn sheep populations with 20 or more genotypes. Sierra Nevada was defined as the outgroup. Horizontal axis scale bar defines 10 times the mean standard error of the sample covariance matrix; horizontal branch length is proportional to genetic drift amount. Pie charts of population-level fastStructure group assignments (K=6) are shown for each population.

Comparing Mean Kinship Between Populations

We estimated mean kinship between bighorn sheep populations to inform future augmentation efforts by identifying potential source and recipient populations with minimal mean kinship (Figure 46). Mean kinship values ranged from -0.868 (most unrelated) to -0.001 (most related). All mean kinship values between populations were negative, indicating that allelic correlations were less than expected under an assumption of Hardy-Weinberg equilibrium (Frankham et al. 2017). A lack of Hardy-Weinberg equilibrium between populations was consistent with the geographic isolation of most populations and a limited number of recent translocations (Figure 43). The Sierra Nevada outgroup and geographically distant Dinosaur had the lowest mean kinship with all other populations. The highest mean kinship was found between Middle Missouri and Castle Reef (-0.001, standard deviation of 0.032), because Middle Missouri was founded by 1 translocation of 28 Castle Reef bighorn sheep in 1980 and had no other augmentations.

We estimated mean kinship between reintroduced populations and their founding source to evaluate 6 attributes that could affect population evolution since reintroduction. These attributes included founder population size, number of generations since population establishment, number of augmentations from the founding source, number of source populations, number of augmentations from other sources, and level of connectivity with neighboring populations. As the number of augmentations from other areas and the number of source populations increased, mean kinship with the founding source generally decreased. All 6 examined population attributes likely influenced evolution of reintroduced populations to differing extents, which complicated our interpretation of which attributes were dominant. However, application of this approach with a greater sample size could serve as a method to evaluate which population attributes influenced reintroduced population evolution and genetic divergence from the founding source.

Conclusions

We examined the genomic consequences of bighorn sheep restoration to enhance understanding of evolutionary processes, such as drift from isolation and unassisted gene flow, that affect population genetics and to inform genetic management of fragmented populations and future restoration strategies. Building on other studies that draw conclusions from only a few populations or limited genetic markers, our study design maximized insight from an observational study by employing standardized sampling of 14 bighorn sheep populations with differing management histories distributed across the northern Rocky Mountain region, a standardized set of SNP markers, and a suite of contemporary analytical tools. Our results provide insight on genomic distinctiveness of native and reintroduced populations, the relative success of reintroduction/augmentation efforts and their associated attributes, and guidance for selection of source populations and translocation strategies to aid in restoration of bighorn sheep.

Genetic structure and connectivity across native populations prior to fragmentation due to human activities can serve as a baseline goal for bighorn sheep restoration in other areas. Glacier and Beartooth-Absaroka served as examples of large, continuous native populations, and our results suggested that gene flow within these populations was influenced by geographic distance and a natural barrier. Genetic differences between the north and south portions of Glacier suggested partial fragmentation due to a large, central lake that may serve as a barrier to extensive gene flow (Figure 43, Figure 44). In addition, we observed isolation-by-distance within Stillwater and Beartooth-Absaroka, as results for Stillwater, Yellowstone, northern hunt units 1-3, and southern hunt units 5 and 22 suggested small

	Beartooth−Absaroka	Castle Reef	Dinosaur	Fergus	Galton	Glacier	Highlands	Lost Creek	Middle Missouri	Paradise	Petty Creek	Sierra Nevada	Spanish Peaks	Stillwater	Taylor Hilgard	Tendoys
Beartooth−Absaroka 90																
Castle Reef 25	−0.168 (0.038)															
Dinosaur 20	−0.210 (0.088)	−0.278 (0.078)														
Fergus 30	−0.202 (0.116)	−0.019 (0.096)	−0.320 (0.138)													
Galton 5	−0.224 (0.054)	−0.191 (0.039)	−0.343 (0.085)	−0.228 (0.116)												
Glacier 95	−0.208 (0.110)	−0.169 (0.120)	−0.341 (0.147)	−0.206 (0.163)	−0.163 (0.121)											
Highlands 17	−0.194 (0.125)	−0.034 (0.133)	−0.325 (0.158)	−0.052 (0.155)	−0.235 (0.137)	−0.205 (0.168)										
Lost Creek 25	−0.278 (0.072)	−0.087 (0.061)	−0.383 (0.090)	−0.130 (0.111)	−0.285 (0.072)	−0.264 (0.142)	−0.135 (0.153)									
Middle Missouri 25	−0.201 (0.052)	−0.001 (0.032)	−0.307 (0.078)	−0.035 (0.098)	−0.212 (0.047)	−0.198 (0.129)	−0.058 (0.140)	−0.101 (0.069)								
Paradise 25	−0.252 (0.043)	−0.096 (0.035)	−0.347 (0.069)	−0.128 (0.102)	−0.185 (0.037)	−0.229 (0.133)	−0.151 (0.141)	−0.171 (0.069)	−0.096 (0.037)							
Petty Creek 25	−0.343 (0.058)	−0.129 (0.045)	−0.437 (0.068)	−0.156 (0.100)	−0.345 (0.060)	−0.328 (0.145)	−0.183 (0.156)	−0.201 (0.083)	−0.126 (0.047)	−0.209 (0.047)						
Sierra Nevada 5	−0.731 (0.142)	−0.798 (0.145)	−0.715 (0.135)	−0.827 (0.171)	−0.841 (0.158)	−0.868 (0.227)	−0.846 (0.250)	−0.855 (0.172)	−0.794 (0.147)	−0.844 (0.154)	−0.863 (0.147)					
Spanish Peaks 20	−0.104 (0.055)	−0.133 (0.045)	−0.257 (0.091)	−0.168 (0.114)	−0.212 (0.059)	−0.188 (0.113)	−0.166 (0.131)	−0.228 (0.079)	−0.163 (0.059)	−0.211 (0.053)	−0.300 (0.065)	−0.773 (0.151)				
Stillwater 24	−0.100 (0.120)	−0.206 (0.122)	−0.277 (0.150)	−0.244 (0.167)	−0.264 (0.127)	−0.252 (0.159)	−0.235 (0.169)	−0.310 (0.141)	−0.236 (0.128)	−0.279 (0.129)	−0.375 (0.143)	−0.828 (0.227)	−0.147 (0.121)			
Taylor Hilgard 30	−0.147 (0.134)	−0.114 (0.148)	−0.288 (0.174)	−0.148 (0.180)	−0.212 (0.150)	−0.199 (0.173)	−0.149 (0.181)	−0.185 (0.168)	−0.142 (0.158)	−0.162 (0.162)	−0.283 (0.178)	−0.817 (0.265)	−0.097 (0.147)	−0.190 (0.175)		
Tendoys 25	−0.167 (0.074)	−0.024 (0.069)	−0.295 (0.101)	−0.051 (0.119)	−0.185 (0.063)	−0.174 (0.127)	−0.066 (0.142)	−0.117 (0.084)	−0.041 (0.071)	−0.087 (0.073)	−0.165 (0.076)	−0.805 (0.163)	−0.137 (0.079)	−0.209 (0.133)	−0.111 (0.149)	
Wild Horse Island 25	−0.272 (0.068)	−0.130 (0.072)	−0.378 (0.095)	−0.162 (0.124)	−0.202 (0.078)	−0.248 (0.142)	−0.180 (0.153)	−0.207 (0.098)	−0.132 (0.078)	−0.030 (0.081)	−0.240 (0.088)	−0.876 (0.183)	−0.236 (0.079)	−0.302 (0.140)	−0.177 (0.164)	−0.098 (0.108)

Figure 46 - Mean kinship between Rocky Mountain and Sierra Nevada bighorn sheep populations evaluated using the HD Ovine array. Standard deviation from the mean is in parentheses. Smaller values indicate lower mean kinship. Sample size for each population is next to each population name on the y-axis; populations with fewer than 20 genotypes are labeled in red.

genetic differences within a larger population (Figure 43, Figure 44; Love Stowell et al. 2020). Between populations, distances over 100 km and non-contiguous mountain ranges generally reduced detection of past and present unassisted gene flow. For example, native Galton and Castle Reef were genetically different and about 170 km apart in linear distance (Figure 43). Populations outside of Montana and Wyoming, including Sierra-Nevada and Dinosaur, were distinct from one another and all other populations (Figure 44). In contrast, we did not detect an influence of geographic distance on genetic similarities between restored populations, due to the strong genetic influence of translocations. This is consistent with patterns observed in restored white-tailed deer (*Odocoileus virginianus*) populations, where little genetic differentiation existed due to translocations to repopulate previously occupied areas (Budd et al. 2018).

Our results identified native populations that had historical gene flow but were recently fragmented. To identify populations that were recently fragmented, we evaluated MDS and 3-population test results. The fastStructure model can provide useful information regarding global ancestry (estimated ancestry proportions from each population for each individual), but it has lower accuracy with uneven sampling and assumes random mating, which is frequently inaccurate for wild populations (Alexander et al. 2009, Puechmaille 2016, Frankham et al. 2017). Thus, while STRUCTURE models can evaluate admixture, they lack a temporal assessment of fragmentation (Frankham et al. 2017). In contrast, MDS can identify separation among populations without an assumption of random mating, and the 3-population statistic is a test to detect admixture, meaning that genetically differentiated populations interbred (Reich et al. 2009, Frankham et al. 2017). Our MDS and 3-population test results suggested that most populations were genetically isolated from one another, except for Stillwater and Beartooth-Absaroka. This is likely because Stillwater and Beartooth-Absaroka are in geographic proximity and part of a continuous, spatially-structured population. However, examined populations could have gene flow with nearby populations not evaluated in this study, and additional genetic sampling of neighboring populations may be useful to more thoroughly evaluate genetic isolation in specific populations.

Gaps between formerly connected populations are areas where managers could prioritize reestablishing connectivity. Filling gaps in distribution can be an important part of species restoration, in addition to establishing new populations (Watson and Watson 2015, Stewart et al. 2017). Spatially-structured populations can also help prevent extirpation of an entire population after an epizootic by localizing outbreaks in smaller groups and lowering probability of disease spread (Altizer et al. 2003, Lopez et al. 2005). We detected 2 pairs of native populations that had past connectivity but were recently fragmented, including Spanish Peaks/Hilgard and Castle Reef/south Glacier. These populations were similar in fastStructure and Treemix analyses, but the 3-population test and MDS suggested a lack of contemporary gene flow. The linear distances between recently fragmented populations were comparable to those for populations with recent gene flow. Stillwater and Beartooth-Absaroka are about 25 km apart in linear distance, whereas about 35 km separate south Glacier and the nearest bighorn sheep range connected to Castle Reef and 28 km separate Spanish Peaks and Hilgard.

To evaluate evolution of newly founded populations and inform reintroduction planning, we compared reintroduced populations with their founding source. Other studies have suggested that bighorn sheep reintroduction efforts may be more successful when founders are sourced from either matching environmental conditions, due to greater recruitment when ecotypes are matched, or native populations, due to typically higher levels of genetic diversity (Fitzsimmons et al. 1997, Singer et al. 2000c, Bleich et al. 2018). We evaluated 8 populations that originated from reintroductions where a founding source was in our study. Castle Reef was the source of 6 out of 8 reintroduced populations, and the remaining 2 were started by reintroduced populations initially founded by Castle Reef. The overall success of reintroductions from Castle Reef suggested that genetic diversity sourced from the native population may have been more important than matching environmental conditions. Sourced from mountain foothills, Castle Reef animals successfully established populations not only in similar environments as that of the source location (Lost Creek and Highlands), but also in semiarid prairie/river breaks environments (Fergus and Middle Missouri) and an island with weather influenced by the Pacific Ocean maritime effect (Wild Horse Island; Montana Department of Fish, Wildlife and Parks 2010). However, local adaptation in bighorn sheep populations is still possible, given that native populations found in different ecological regions were genetically differentiated, but genetic distinctiveness could also be explained by genetic drift (Figure 43). If ecologically matched native populations have low genetic diversity or are not available for a reintroduction, multiple sources could be used, as maximizing genetic diversity will increase adaptive potential in a new environment (Broadhurst et al. 2008, Olson et al. 2013, White et al. 2018).

After selecting source population(s), it is typically recommended to use a large number of founders to replicate the genetic profile of the founding source, but the number recommended to capture genetic diversity can range from 20-50 and depend on the species (Taylor and Jamieson 2007, Weeks et al. 2011, Jamieson and Lacy 2012). It is useful to evaluate how much reintroduced populations genetically diverged from their founding source, to assess if the released number of founders successfully represented the source's genetic diversity and to determine if genetic drift occurred, which is the main process by which small populations lose genetic variation, as in reintroduced populations of Alpine ibex (*Capra ibex ibex*; C. Lacy 1987, Templeton 2006, Biebach and Keller 2009). Thus, greater genetic divergence from the source population may indicate a loss of genetic variation in the reintroduced population due to chance. We expected that reintroduced populations established with a greater number of founders would have greater mean kinship with the founding source, as a large number of animals would be more genetically representative of the source. However, when we compared founder sizes ranging from 8 to 53 bighorn sheep to kinship with the founding source, we did not find a clear relationship. Thus, kinship in many of the reintroduced populations may have been driven by other genetic influences, as 6 out of 8 reintroduced populations received translocations from at least 2 sources. Wild Horse Island, Lost Creek, and Petty Creek had greater divergence from their founding source than the other 5 reintroduced populations (Figure 43, Figure 44, and Figure 45). All 3 populations were founded by 25 or fewer animals from the same source, received an augmentation from at least 1 other source, and had 8-11 generations for genetic drift or selection. For example, Wild Horse Island showed the greatest divergence from its founding source, Castle Reef (Figure 43, Figure 45). Wild Horse Island was founded by 8 animals and experienced 11 generations of isolation, except for 2 rams translocated from Ural-Tweed in 1987. Thus, a founder effect, genetic drift or selection over time, and genetic input from a different source population likely influenced population genetics of Wild Horse Island. In contrast, Middle Missouri had the highest mean kinship with the same founding source, Castle Reef, after 1 reintroduction event of 28 animals, 6 generations of isolation, and no augmentations. While multiple influences on the evolution of reintroduced populations complicated identifying which affected divergence the most, our results suggested that augmentations from other source populations that were genetically distinct from the founder source likely was the dominant factor in determining the levels of divergence between source and recipient populations in our study. Future genomic studies could target reintroduced populations with different founder sizes and no augmentations to gain clearer insights to inform the number of bighorn sheep recommended for reintroductions.

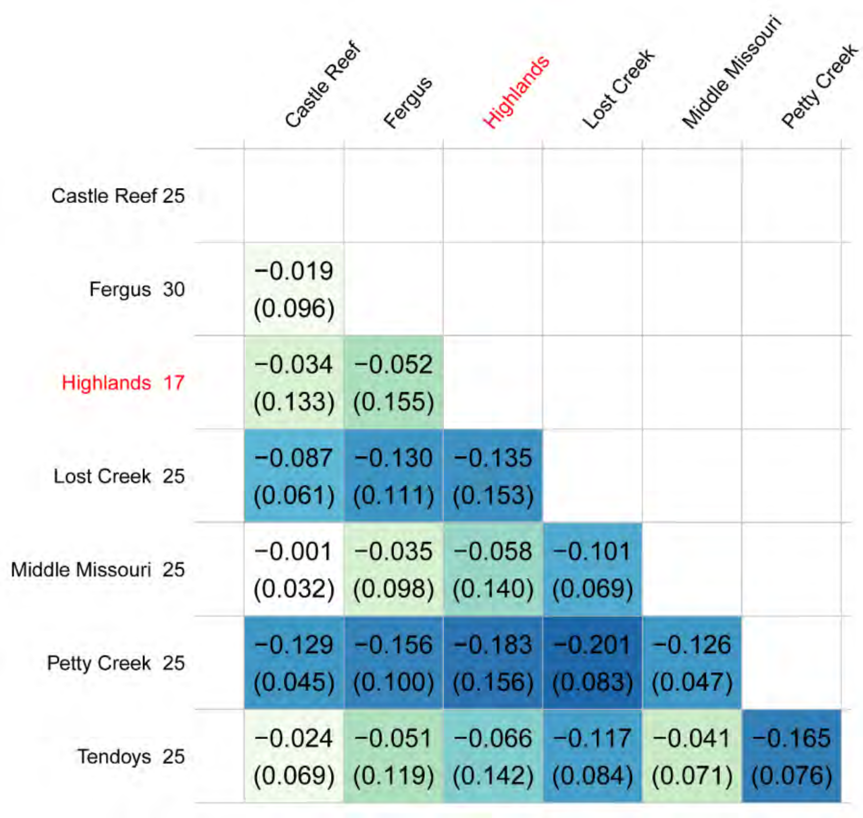
Augmentations provided an opportunity to assess the impact of specific gene flow events on fragmented population evolution and inform animal selection for future efforts. For translocation to be used for augmenting genetic diversity of small populations, managers need to 1) identify which populations need augmentation based on demographic attributes and levels of population fragmentation, genetic diversity and inbreeding, 2) identify source populations based on mean kinship, other direct measures of diversity, and other management concerns like disease history, 3) determine how many and which individuals should be moved, and 4) monitor recipient populations for realized genetic contribution, connectivity with nearby populations, genetic diversity, and demographic response, to determine if additional augmentations are needed (Johnson et al. 2010b, Adams et al. 2011, Harrisson et al. 2016, Frankham et al. 2017). Future research could evaluate the relationship between levels of inbreeding within populations and population performance to aid in determining which populations may require augmentation. Identifying which animals will contribute genetically to a recipient population includes considerations such as number, sex, age, adaptation, and disease status. Similar to reintroductions, matching environmental attributes of source/recipient areas (potential adaptation) for augmentations may not be a concern within the examined populations, as we observed successful augmentations across ecological regions defined by Montana Fish, Wildlife, and Parks and extensive latitudes from Whiskey Mountain to Dinosaur. Additional assessment evaluating hybrid fitness would be valuable (Olson et al. 2013). Five genetically detected translocations consisted of 18 to 57 animals, whereas 2 undetected augmentations each consisted of 2 males. During breeding season, adult male bighorn sheep can wander long distances between mountain ranges (Geist 1971) and potentially depart the augmentation destination, which may result in no genetic contribution to the intended recipient population. Since bighorn sheep are a polygynous species, a small number of dominant rams may competitively exclude translocated males due to female mate preference for residents or poor condition after transport/release, suggesting that translocating a greater proportion of females may be more effective for augmentation (Sigg et al. 2005, Mulder et al. 2017). After an augmentation of females, translocated ewe groups may socially segregate from the resident population, which was observed in Hilgard after augmentation from Lost Creek in 1989 (Roy and Irby 1994, Robinson et al. 2019). However, observed mixing of rams and ewes with different origins during breeding season or social mixing in later years resulted in hybrids descended from native and translocated animals after 24 years or 4 generations (Figure 45; Roy and Irby 1994). Future research could evaluate more specifically if translocated bighorn sheep males or females are more likely to reproduce successfully.

Our results also identified a case where multiple augmentations did not contribute genetically to the recipient population, suggesting translocated animals failed to survive and breed in the recipient population. We found evidence that 3 augmentations to Highlands from 3 sources not in our study may not have made a genetic contribution, as Highlands had high mean kinship with its founding and augmentation source Castle Reef. A Highlands epizootic event in the winter of 1994-1995 resulted in 90% population mortality. In an attempt to recover the population that numbered less than 100, managers implemented 7 augmentations of 140 total animals from 5 different sources during 2000-2014 (Montana Department of Fish, Wildlife and Parks 2010). Despite these efforts, Highlands is estimated to be approximately 150 animals (V. Boccadori, personal communication, March 30, 2018), further supporting the possibility for augmentation failures. One possible explanation for the lack of genetic contribution from augmentations could be that the translocated bighorn sheep were susceptible to resident pathogens in the recipient population and died prior to breeding. Alternatively, high mean kinship between the Highlands and its founding source may be due to the breeding success of 97 Castle Reef animals from 3 augmentations. The epizootic in the winter of 1994-1995 likely acted as a strong selective event on the local animals, so the struggle of the population after translocations during 2000-2014 may have been the result of genetic swamping and the disruption of local adaptation, which means the population lost adaptive alleles in subsequent generations that were in the outnumbered local survivors (Lenormand 2002). This is because more individuals were added in augmentations than the local population size, which does not meet genetic rescue guidelines (Frankham et al. 2017).

Augmentations are often promoted for genetic rescue (Tallmon et al. 2004, Hogg et al. 2006, Whiteley et al. 2015). To increase effectiveness of a genetic rescue, source and recipient populations should have been previously connected but recently isolated to allow differentiation over multiple generations in the past 500 generations. Many types of information should be evaluated to determine optimal sources for augmentation. Our results can provide guidance on selecting sources for genetic rescue augmentations by combining information on genetic differentiation and mean kinship. One possible approach, if managers want to maintain populations that are currently differentiated, would be to identify possible sources for future augmentations within clusters of populations in MDS or fastStructure analyses (Figure 43, Figure 44). Within identified clusters, managers can select a source population that has low mean kinship with the intended recipient population (Figure 46). Minimizing mean kinship between source and recipient populations would be an approach to retain genetic diversity and minimize inbreeding at the population level while still considering the possibility for local adaptation (Ballou and Lacy 1995, Frankham et al. 2017). For example, augmentations could be implemented within the cluster associated with Castle Reef (Figure 43), and mean kinship minimized between source and recipient by consulting a mean kinship table associated with that cluster (Figure 47). Following augmentation efforts, genetic diversity should optimally be monitored after 5-10 generations to determine if additional augmentations are needed, as the genetic diversity of similarly managed populations can vary due to many influences on their evolution (White et al. 2018).

There has been great concern that bighorn sheep augmentation efforts may spread respiratory pathogens novel to the recipient population or disrupt a stable relationship between resident pathogen and host, which could result in devastating epizootic events (Cunningham 1996, Aiello et al. 2014, Cassirer et al. 2017). Disease concerns have resulted in widespread testing of bighorn sheep populations for a variety of pathogen species and strains associated with epizootic events, but the results of these tests can be inconclusive due to imperfect detection probability and lack of understanding as to what individual test results imply for the overall population (Butler et al. 2017, 2018). Thus, when considering translocations between bighorn sheep populations, agencies could recognize the risk of mixing pathogens, consider how to mitigate this risk, and monitor outcomes for adaptive management. Future research to address this issue could evaluate genetic variation at the major histocompatibility complex (MHC) associated with immune response in bighorn sheep populations.

Our results provide insight regarding the population genomics of native and reintroduced populations, genetic contributions of past translocation efforts, and strategies for future bighorn sheep restoration efforts. This research serves as an example of how genomic analyses can provide information regarding the genetic outcomes of previous management approaches and inform future decisions. Successful genetic contribution of most reintroduction and augmentation efforts evaluated in this study in the context of the continued struggle of Rocky Mountain bighorn sheep conservation suggests there are multiple interacting influences on restoration success. Not only is there uncertainty regarding how genetic attributes affect population performance, but the specific drivers behind population trend and demography are also frequently unclear. Thus, genetic management should generally be integrated into conservation planning with other management considerations (IUCN/SSC 2013, Ralls et al. 2018). Assessment of multiple population attributes during restoration efforts,



	Castle Reef	Fergus	Highlands	Lost Creek	Middle Missouri	Petty Creek
Castle Reef 25						
Fergus 30	−0.019 (0.096)					
Highlands 17	−0.034 (0.133)	−0.052 (0.155)				
Lost Creek 25	−0.087 (0.061)	−0.130 (0.111)	−0.135 (0.153)			
Middle Missouri 25	−0.001 (0.032)	−0.035 (0.098)	−0.058 (0.140)	−0.101 (0.069)		
Petty Creek 25	−0.129 (0.045)	−0.156 (0.100)	−0.183 (0.156)	−0.201 (0.083)	−0.126 (0.047)	
Tendoys 25	−0.024 (0.069)	−0.051 (0.119)	−0.066 (0.142)	−0.117 (0.084)	−0.041 (0.071)	−0.165 (0.076)

Figure 47 - Mean kinship of all populations with the same fastStructure cluster as Castle Reef (K=6), to demonstrate translocation options for wildlife managers to maximize genetic diversity. Standard deviation from the mean is in parentheses. Smaller values indicate lower mean kinship. Sample size from each population is shown to the right of population name; populations with less than 20 samples are labelled in red. For example, to select an augmentation source for Middle Missouri, we would consider mean kinship values with populations in the same fastStructure cluster: Castle Reef (-0.001), Fergus (-0.035), Tendoys (-0.041), Highlands (-0.058), Lost Creek (-0.101) and Petty Creek (-0.126). Based on mean kinship, Petty Creek and Lost Creek would be optimal sources to maximize genetic diversity, as these populations have low mean kinship with Middle Missouri.

such as genetics, migratory patterns, mortality causes, and disease, can enable an adaptive management framework and improve the longevity of managed populations (IUCN/SSC 2013). Genomic data alone cannot dictate decisions concerning population and genetic management, but rather can be integrated with management judgements as to what population genetics are valuable to conserve and what should be built for the future. Our results demonstrate that genomic analyses are a tool for evaluating the genetic effects of translocations and planning future genetic management of small, fragmented populations.

GENETIC DIVERSITY & INBREEDING LEVELS OF HERDS

In this portion of the genetic study, we addressed the following objectives: 1) characterize genetic diversity of bighorn sheep populations with varied management histories, 2) compare genetic diversity at the population level with population origin, size, unassisted gene flow via dispersal (connectivity), and assisted gene flow via augmentations to determine if there is a correlation, and 3) evaluate if populations are experiencing inbreeding depression by comparing genetic diversity with juvenile survival at the population level. We expected that native origin, large population size, and greater gene flow via augmentations or natural dispersal would result in higher genetic diversity than reintroduced origin, small population size, and genetic isolation. We also predicted that the number of animals received in augmentations and origin would be the most important influences, due to observed effects of these variables on genomic similarity among populations (Flesch et al. 2020). Finally, we expected that populations with low genetic diversity would have lower juvenile survival, which would suggest the presence of inbreeding depression.

Study populations

For this analysis, we included nearly all of the wild populations used for our assessment of genetic uniqueness of populations reported above, except the Galton population was excluded due to insufficient number of samples (n=5). We aimed to sample at least 20-25 individuals per herd, based on sample size simulations that indicated lower sample sizes introduced an unacceptable level of uncertainty to mean kinship estimates (Flesch et al. 2018). While we did not meet the sample size goal for the Highland herd (n=17) we still included this herd in the analysis due to the limited number of herds in the study. In total, the analysis included genotype data for 488 individual bighorn sheep from 19 populations.

The data set included 10 native herds, composed of 7 small to moderately sized populations (70-227 animals) and 3 large populations (406-726 animals). Seven moderately sized and large native herds represented natural population structure prior to widespread fragmentation of the species range, as they are part of large, spatially-structured populations, defined as a collection of subpopulations that occupy distinct geographic areas but are linked by animal movement. These populations included 3 units in the Beartooth-Absaroka mountain ranges of Wyoming, 3 units in Glacier and Waterton Lakes National Parks, and Castle Reef in Montana. The Wyoming units are in the eastern front of the Greater Yellowstone Area and include Clark's Fork/Trout Peak, Wapiti Ridge, and Franc's Peak, with their boundaries defined by administrative units. The Glacier area was split into north, central, and south for our analyses, and the divisions were informed by previous genetic and movement analyses (T. Graves unpublished data; Flesch et al. 2020). Castle Reef is assumed to have connectivity across 4 administrative units in the Rocky Mountain Front of Montana (Montana Fish Wildlife and Parks 2010). Two of the remaining native herds were genetically isolated and 1 herd had some genetic connectivity with a neighboring population (Flesch et al. 2020). Nine reintroduced herds that were established by translocations within the past 75 years were also included in the data set. Reintroduced herds included 8 small to moderately sized populations (70-277 animals) and 1 large population (416 animals). Founder size for the reintroduced herds ranged from 8 to 53 animals. We considered a translocation to be part of the original founding group if it occurred within 3 years of the first reintroduction event to an unoccupied area for all populations except for Wild Horse Island. For this population, we considered 2 translocations 8 years apart as both founding events, because the first reintroduction effort moved only 2 animals. Five to 11 generations occurred since establishment of evaluated reintroduced herds, based on a generation time of 6 years.

Estimating genomic kinship and associations between kinship and herd attributes and management history

There are multiple metrics to calculate an index of inbreeding, and the optimal method may depend on the research question and dataset (Goudet et al. 2018). We choose to employ the kinship metric because it is used to make breeding decisions to minimize inbreeding in most, if not all, wildlife captive-breeding programs to support reintroductions, such as the black-footed ferret (*Mustela nigripes*) and California condor (*Gymnogyps californianus*; Ballou and Foose 1996, Ralls and Ballou 2004).

In addition, other studies have found that the kinship metric is effective for informing genetic rescue decisions in plants and animals to maximize genetic diversity and minimize inbreeding (Finger et al. 2011, Garbe et al. 2016). We calculated genomic kinship between all possible pairs of individuals within each population using KING v2.1.4 (Manichaikul et al. 2010). We calculated the average of all pairwise kinship values for sampled animals within each population to estimate the level of inbreeding in the next generation. The formal definition of mean kinship includes self-kinship values, but we excluded self-kinships in calculating a mean for each herd, because uneven sample sizes among populations would bias relative comparisons of mean kinship among herds with the inclusion of self-kinship values. Specifically, with the inclusion of self-kinship, mean kinship of herds with smaller sample sizes would be biased higher than that of herds with larger sample sizes. To distinguish our approach that excluded self-kinships, we termed our approach average pairwise kinship.

We used multiple linear regression to evaluate the relationship between average pairwise kinship and 4 predictor variables, including origin, historic minimum count, connectivity, and augmentation history. Population origin was defined as native (indigenous) or reintroduced and represented the potential for a recent founder effect within the past 75 years. The historic minimum count predictor was considered a metric of a past bottleneck. Agency staff and reports provided count data and we considered the raw counts to be an index for relative abundance across the evaluated populations. Historic minimum count represented the lowest credible count after 1980. Minimum counts could be a result of epizootic events, population growth following reintroduction, or other unknown factors. Most sampled herds experienced a documented all-age die-off suspected to be an epizootic event, so disease events were a predominant cause of documented bottlenecks. Connectivity represented the level of gene flow due to unassisted dispersal between the examined population and neighboring populations. Classification of herds into gene flow categories was based on input from agency biologists, previous research that evaluated genetic similarity and connectivity among populations, and GPS data (Flesch et al. 2020, T. Graves unpublished data, Lula et al. 2020, Lowrey et al. 2020). Herds were classified as “isolated” if there was no known recent gene flow, “some” if there was limited indication or some possibility for gene flow, and “high” if the population was a part of a continuous, spatially-structured population. Finally, we evaluated augmentation history of herds to account for assisted gene flow via translocations. Two documented augmentation events were excluded from the analysis because the translocations did not make a detectable genetic contribution in the recipient populations (Flesch et al. 2020). We included the total number of animals received in augmentations in the analysis, as augmentation recommendations are often based on the number of migrants per generation (Mills and Allendorf 1996).

Connectivity and augmentations were positively associated with average pairwise kinship

Average pairwise kinship per population ranged from 0.014 to 0.080 (Figure 48), and positive values suggested that all populations were in Hardy-Weinberg equilibrium. The most plausible model of average pairwise kinship contained connectivity and number of animals received in augmentations. This model was 1.7 times more likely than the next best approximating model, which contained the same predictors and minimum count. Populations with high connectivity had greater genetic diversity than isolated populations (Figure 51A), suggesting natural dispersal of breeders between populations was the most important influence in maximizing genetic diversity. This result is consistent with the observation that reduction of natural gene flow between bighorn sheep populations due to highways can result in decreased genetic diversity (Epps et al. 2005). When managing bighorn sheep populations, enabling natural gene flow may be of highest

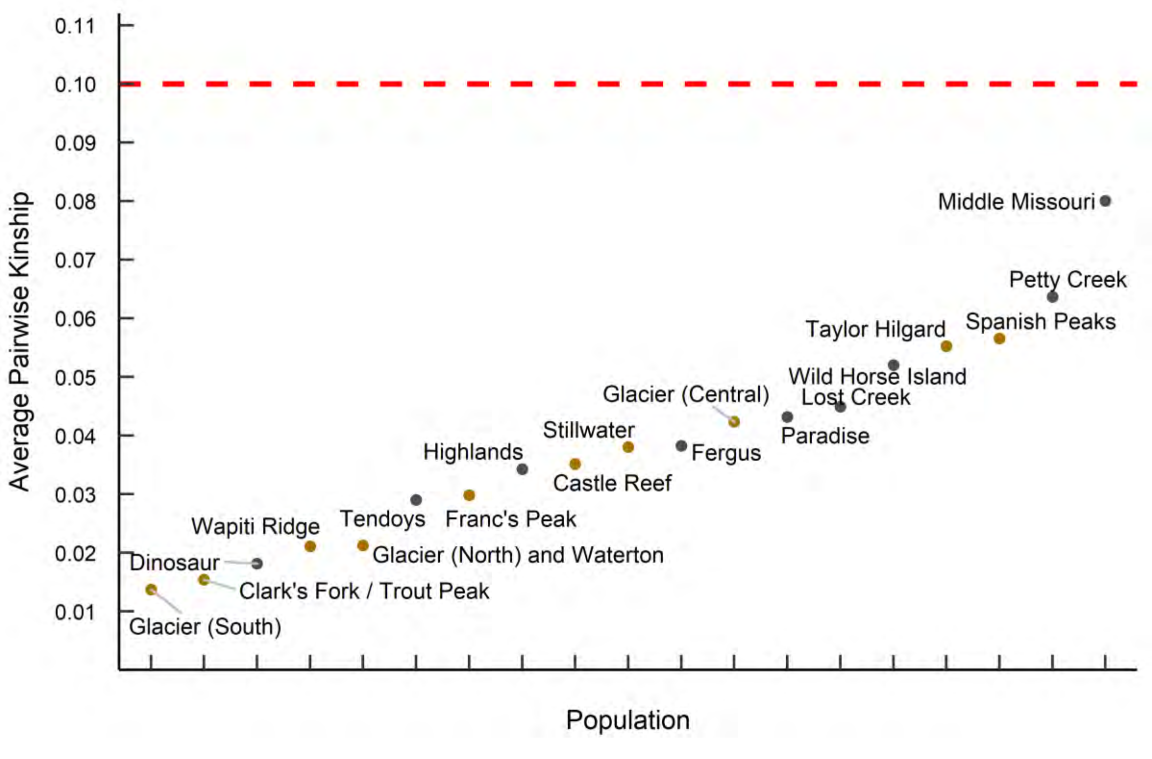


Figure 48 - Average pairwise kinship for 19 bighorn sheep (Ovis canadensis) populations located in Montana, Wyoming, Utah, Colorado, and Canada that were evaluated. Native populations are represented by dark brown points. Reintroduced populations are represented by black points. Average pairwise kinship values are sorted from lowest to highest. All examined populations had average pairwise kinships lower than the concerning mean kinship threshold of 0.1 (Frankham et al. 2017), indicated by a dotted red line.

priority to avoid negative impacts of inbreeding, when this approach does not conflict with other management considerations. Similar to increasing connectivity, increasing the number of animals received in augmentations was associated with a decrease in average pairwise kinship (i.e. increase in genetic diversity; Figure 51B). This result suggests that management intervention to supplement existing populations via augmentation was effective in increasing genetic diversity and minimizing inbreeding. A similar association with genetic diversity was observed in other bighorn sheep populations for the number of translocated animals and population sources (Jahner et al. 2018). This result is consistent with genetic management guidelines that suggest a certain number of migrants (i.e. 1-10 animals per generation) are needed to effectively maintain genetic variation (Mills and Allendorf 1996).

An isolated island population in our study (Wild Horse Island) provided an example of past augmentation serving to minimize average pairwise kinship. A small group of animals founded the population, including a male and female translocated to the island in 1939 and 6 animals (3 males and 3 females) translocated from a different source in 1947 (Ogren 1954). Following 6.6 generations of isolation (40 years), managers translocated 2 males from a third population source. Given the limited number of animals moved to the island, we expected to observe high average pairwise kinship for this population, but instead observed a moderate value of 0.05 (Figure 32). Mild climate and lack of predators on the island may have promoted robust population growth that enabled retention of the genetic diversity of the founding individuals. Productivity of the population can be demonstrated by the sustained removal of animals from the area. Over 10 generations (60 years from 1954 to 2014), managers moved 525 bighorn sheep from the island to other locations, including 29 different translocation events with an average of 2 years between each translocation (Montana Fish Wildlife and Parks 2010). Despite this history of removal, the population was composed of approximately 139 animals in 2019 and produced a 9 year old ram with the largest horns ever recorded for the species, according to the Boone and Crocket Club assessment

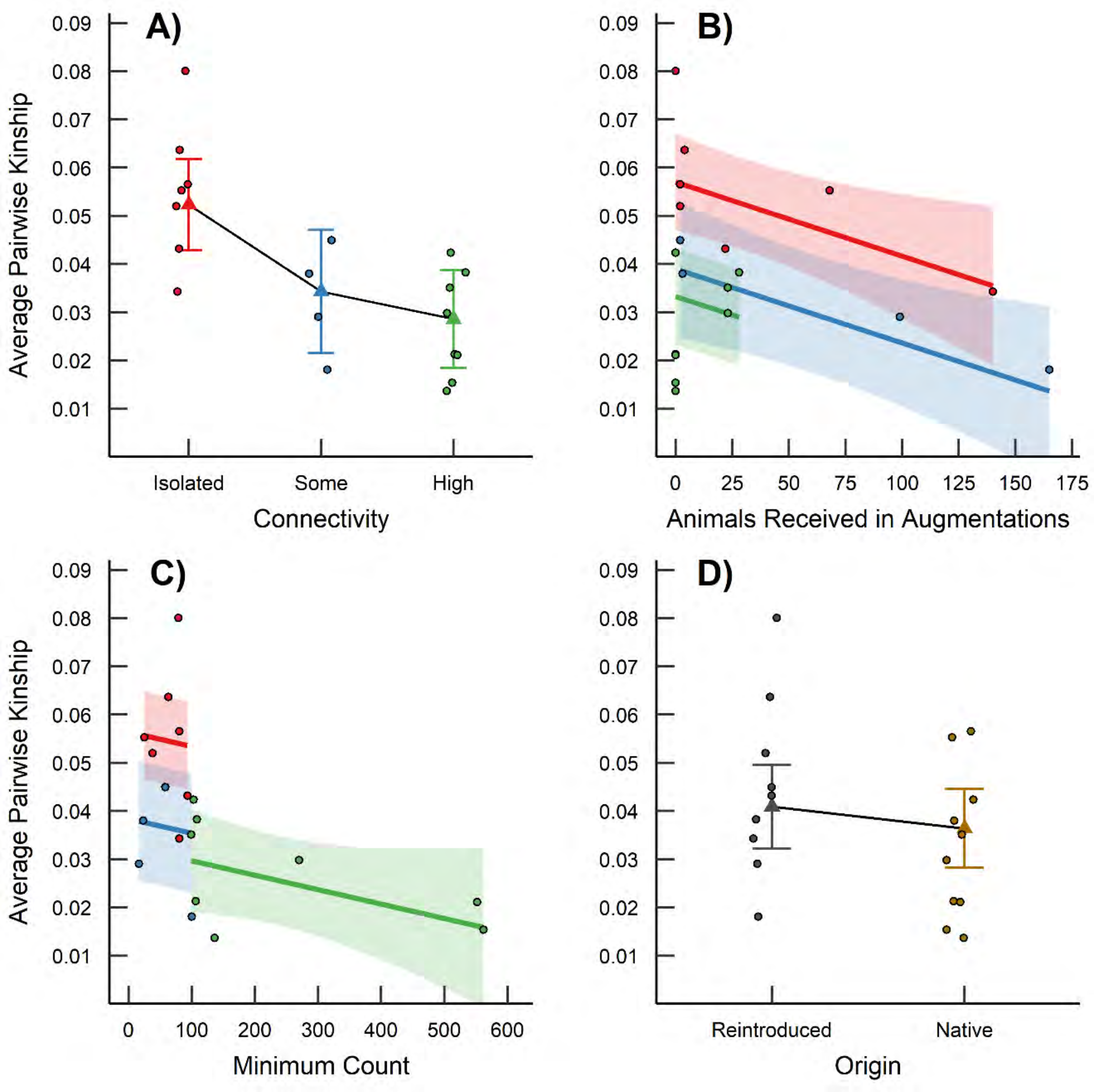


Figure 49 - Empirical linear model estimates of the relationship between average pairwise kinship for 19 bighorn sheep populations and connectivity (A), total number of animals received in augmentations by connectivity (B), minimum count by connectivity (C), and origin (D). High connectivity is represented by green, some connectivity is blue, and isolated is red. Whiskers and bands around coefficient estimates represent the 95% confidence interval for the full model. Observed data are shown as points.

(Scott 2018). The small number of founders and our results suggest that the augmentation of 2 rams from a different source population than the founding animals effectively served as genetic rescue to the population.

Minimum historic count and herd origin not strongly associated with average pairwise kinship

Most herds in this analysis experienced bottlenecks due to epizootic events, and these disease events can reduce genetic diversity of bighorn sheep populations (Ramey et al. 2000). However, we found only weak support for an association between mean pairwise kinship and minimum historic count and herd origin. The association, while weak, was in the predicted direction (Figure 51C), suggesting that bottlenecks consisting of smaller numbers of animals lowered genetic diversity. Although minimum count was not one of the most important predictors of average pairwise kinship, small population size or bottlenecks can interact with other influences that are genetic or not, such as predation, to negatively affect population trend.

Finally, origin was the least important predictor, and the model suggested that native herds may have lower average pairwise kinship and thus greater genetic diversity than reintroduced herds, but the confidence interval of this predictor overlapped zero (Figure 51D). This result was unexpected as previous research suggested reintroduced bighorn sheep herds generally have lower genetic diversity due to a founder effect (Fitzsimmons et al. 1997, Hedrick et al. 2001, Olson et al. 2013). Seven out of 9 reintroduced populations were established or augmented by sourcing from multiple populations, which may have mitigated a founder effect. Thus, our results suggest that gene flow and admixture (interbreeding between differentiated populations) after reintroduction can serve to minimize average pairwise kinship. Similarly, reintroduced bighorn sheep herds in Nevada, most with past augmentations sourced from different locations than that of the founders, generally did not have lower genetic diversity than native populations (Jahner et al. 2018). Thus, sourcing reintroductions and augmentations from different populations can be an effective management tool to maximize genetic diversity in reintroduced populations.

Previous bighorn sheep research has suggested sourcing only from native bighorn sheep populations for translocations due to higher success rates, which was speculated to be in part due to greater genetic diversity in native populations (Fitzsimmons et al. 1997, Singer et al. 2000c). In contrast, our research suggests that genetic diversity of reintroduced herds may not always be a concern to influence this decision, if reintroduced herds had gene flow to enhance genetic diversity. Considering reintroduced populations as potential sources for translocations would be helpful to translocation planning by providing a larger pool of candidate source populations.

Comparing average pairwise kinship with juvenile survival to evaluate inbreeding depression

Because juvenile survival can be affected by inbreeding depression more than adult survival (Ralls et al. 1979) and generally has a larger impact on population trend over other vital rates in large ungulates, including bighorn sheep (Gaillard et al. 1998), we compared average pairwise kinship with juvenile survival to evaluate the possibility for inbreeding depression and its effects on population growth. Juvenile survival data at the individual level were not available for the examined herds, as this information is logistically challenging to collect. Instead, annual population counts with age and sex classifications from agency monitoring efforts provided the best available data regarding juvenile survival. We input annual counts with classifications into an integrated population model using a Bayesian framework and known priors from telemetry research (described in Chapter 6). This approach produced vital rate estimates for each population, including juvenile survival. Juvenile survival estimates were available for 10 herds, and median juvenile survival with a 90% credible interval was calculated from the full time series that demographic data were available following the most recent augmentation. Poor recruitment due to lamb pneumonia can last from 3-5 years to decades following an epizootic event (Cassirer et al. 2013, Manlove et al. 2016), so we also compared median juvenile survival with population disease history. Population disease history was defined by 1) if the population had a known epizootic event since 1980, and 2) if *Mycoplasma ovipneumonia* was detected by PCR, as this pathogen has been associated with bighorn sheep epizootic events (Cassirer et al. 2018, Butler et al. 2018). *Mycoplasma ovipneumonia* was only recorded as absent from populations if the probability the pathogen was present was less than 0.06 (assuming 10% prevalence), given the number of swabs collected per animal, sample size, and detection probability (Butler et al. 2017, 2018, Paterson et al. 2020).

Inbreeding depression was not detected

Median juvenile survival at the population level ranged from 0.20 to 0.47 and the median was 0.34. Populations with greater levels of average pairwise kinship were not associated with lower juvenile survival (Figure 50), suggesting that inbreeding depression was not detected in the examined populations. Similarly, Johnson et al. (2011) used empirical data and simulations to suggest that inbreeding depression likely would not negatively impact population growth of Sierra Nevada bighorn sheep populations for eight bighorn sheep generations into the future (48 years) after genetic analysis, despite a history of reintroductions and bottlenecks. All examined populations included in our study had average pairwise kinships lower than the concerning mean kinship threshold of 0.1 (Frankham et al. 2017), suggesting that inbreeding in the observed populations was not high enough to negatively affect median juvenile survival. Alternatively, other influences on juvenile survival may have masked its relationship with average pairwise kinship. Many other variables such as maternal age, body weight, and predation are correlated with juvenile survival in mammals, and disease is of particular importance in bighorn sheep lamb survival. In the data set used in our analysis, there were 5 herds where *Mycoplasma ovipneumonia* was detected and an epizootic event was observed; 2 herds where *Mycoplasma ovipneumonia* was detected that did not have a known epizootic event, and 3 herds where *Mycoplasma ovipneumonia* was not detected that also did not have known epizootic events (Butler et al. 2018, Garrott et al. 2020). Herds that had documented all-age die-offs within the past 40 years generally had lower median lamb survival than those that did not (Figure 52). Thus, epizootic events were likely a more important influence on juvenile survival than average pairwise kinship, which is consistent with previous research that found poor recruitment after an epizootic event can last from 3-5 years to decades (Cassirer et al. 2013, Manlove et al. 2016). Thus, the effect of epizootics on juvenile survival may have masked any potential impact of inbreeding depression on juvenile survival. However, high genetic diversity in a population is still important as it enables evolution of more resilient populations to occur through natural selection of individuals that survive epizootic events (Alves et al. 2019).

Conclusions

Our study found that greater connectivity, number of animals received in augmentations, and minimum population count were correlated with higher genetic diversity at the population level, and origin was less important. Despite lack of evidence for inbreeding depression in the examined populations, the analyses highlight which herd attributes may result in inbreeding at concerning levels. For example, our results suggested that populations that are isolated with no natural connectivity or augmentations have the highest average pairwise kinship, regardless of origin. Thus, managers can prioritize herds of highest genetic concern based on kinship or population attributes ranked in importance by our analysis if genetic data are not available. This approach

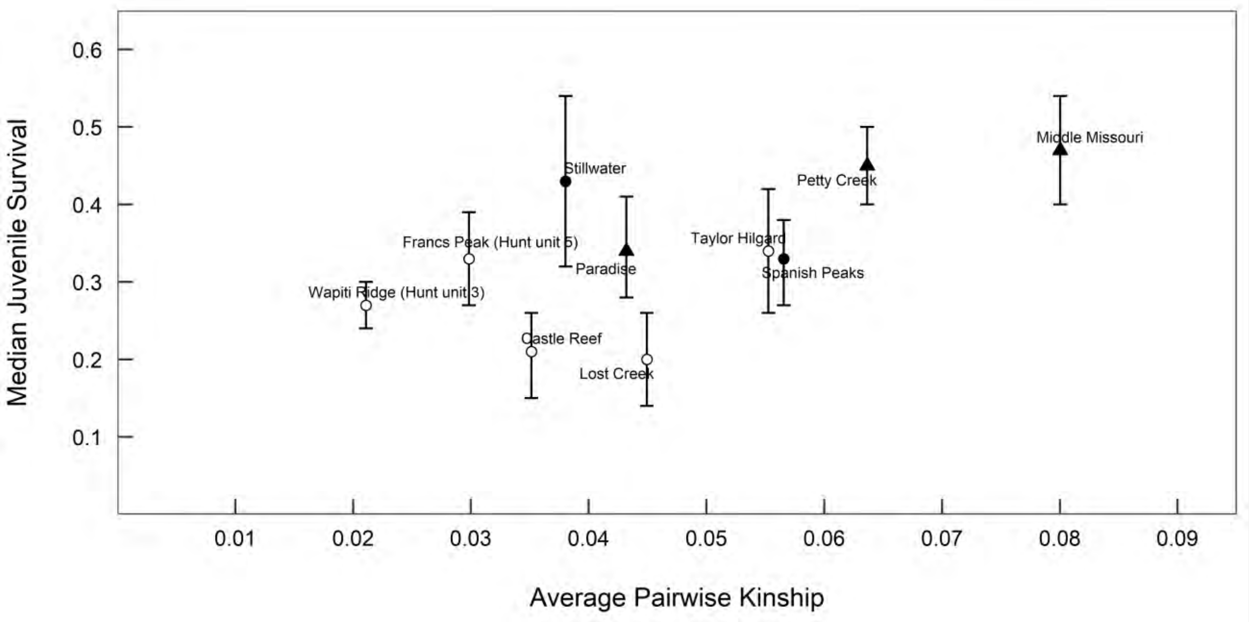


Figure 50 - Median estimated juvenile survival and average pairwise kinship for 10 bighorn sheep populations. Estimates for populations with Mycoplasma ovipneumoniae detected are circles; estimates for populations that were tested for Mycoplasma ovipneumoniae with negative results are shown as triangles (Butler et al. 2018, Garrott et al. 2020). Whiskers indicate the 90% credible interval for median juvenile survival. All median juvenile survival values were estimated using a time series of count data one year after the most recent augmentation of animals from another area. Estimates for populations that had at least one recorded all-age die-off in the past 40 years are white; estimates for populations without a known all-age die-off in the past 40 years are black.

would provide managers with additional information to weigh the costs and benefits of augmentation efforts.

An extensive history of augmentation efforts likely played a role in limiting inbreeding in the evaluated bighorn sheep populations, such that the observed range of average pairwise kinship values were not at concerning levels. However, there are new counter-balancing concerns that translocations could transmit novel pathogen species and genetic variants associated with respiratory disease from source to recipient populations, which could increase the potential for epizootic events and poor recruitment in populations after augmentation. These recent biological insights suggest that managers may need to be more cautious about translocating animals in the future. Our study suggests that it will be important to balance both disease and inbreeding concerns in translocation planning, especially when considering the establishment of new populations to enhance restoration of the species.

Chapter Summary

- We genotyped 511 bighorn sheep from 17 areas, including native and reintroduced populations that received 0-10 translocations, using a genomic technology that provided information on 6,155 to 33,289 genetic markers.
- We determined a sample size of 20-25 bighorn sheep per population is adequate for assessing mean kinship (a metric that describes level of genetic diversity) within and between populations.
- Sampled Montana populations were aggregated into 5 unique genomic groups primarily based on geographic proximity and translocation history.
- We detected the genetic signatures of 5 past augmentations that each consisted of 18 to 57 animals, indicating that the translocated animals survived and successfully contributed progeny to the recipient population. Two genetically undetected augmentations each consisted of 2 males.
- A single native population, Castle Reef, founded most of the reintroduced populations located across multiple ecological regions defined by Montana Fish, Wildlife and Parks, suggesting that matching environmental conditions was not required for populations to persist following reintroduction in the evaluated range of conditions.
- All examined populations included in our study had average pairwise kinship estimates lower than the concerning mean kinship threshold of 0.1, suggesting that inbreeding in these populations was not high enough to negatively affect population vigor.
- Greater gene flow, from population connectivity and animals received in augmentations, were more important predictors of genetic diversity than historic minimum count and origin.
- We did not observe a relationship between average pairwise kinship and median juvenile survival at the population level, so our results did not suggest that there was suppression of population growth in the examined populations due to inbreeding depression.

Peer-reviewed Science Products

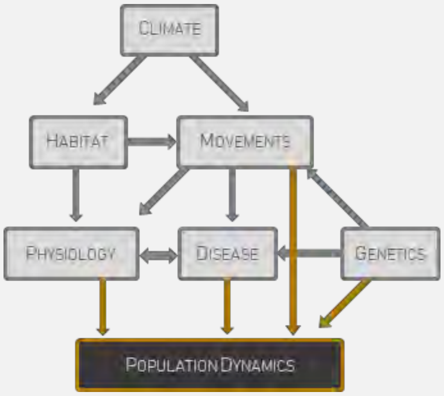
Flesch, E., J. Rotella, J. Thompson, T. Graves, & R. Garrott. 2018. Evaluating sample size to estimate genetic management metrics in the genomics era. Molecular Ecology Resources, http://doi.wiley.com/10.1111/1755-0998.12898.

Flesch, E. P., T. A. Graves, J. M. Thomson, K. M. Proffitt, P. J. White, T. R. Stephenson, and R. A. Garrott. 2020. Evaluating wildlife translocations using genomics: A bighorn sheep case study. Ecology and Evolution. https://doi.org/10.1002/ece3.6942.

Flesch, E. P., T. A. Graves, J. M. Thomson, K. M. Proffitt, and R. A. Garrott. In review. Genetic diversity of bighorn sheep populations is associated with connectivity, augmentation, and bottlenecks. Ecosphere.

CHAPTER 6

DEMOGRAPHIC RATES & CORRELATES



Objective Provide a summary of key demographic vital rates and their impact on population trajectories in the study populations, and the association between vital rates and covariates reflecting environmental, disease and predation processes, to better understand variable population dynamics and demonstrate the utility of a population modeling approach. The survey data from the study populations, combined with separately estimated adult survival and pregnancy rates, provided an opportunity to understand drivers of population growth rates that is relevant to improving the management of bighorn sheep.

ACCURATE ESTIMATES of population size and demographic vital rates of wildlife populations are fundamental to guiding management actions because they elucidate demographic health and can help inform the prediction of future population dynamics. Population growth is explicitly described by several vital rates: adult survival, fecundity, juvenile survival, immigration, and emigration. Reliable estimates of these vital rates allow for inferences regarding population growth or decline independently from the use of sequential population estimates (Eberhardt 2002, DeCesare et al. 2012). Knowledge of the relative contribution of different vital rates to dynamics of wildlife populations is imperative to identifying mechanistic drivers of population dynamics. Accordingly, accurate estimates of vital rates are fundamental for both effective research to gain ecological insight and for implementing management programs of wildlife populations. An important objective of the Montana Bighorn Sheep Study was to collaborate with area biologists to estimate population size, adult female survival and pregnancy rates, and annual recruitment. Below are presentations of our efforts to collect data on these important population parameters and use rigorous analytical techniques to estimate vital rates and quantify the uncertainty associated with each estimate. As a final product of this research program, we provide an analytical framework that integrates the individual data types and vital rate estimates into a comprehensive population model that can aid in gaining more ecological insight regarding processes that affect population dynamics of individual bighorn populations and assist in management decisions.

ADULT FEMALE MORTALITY

A total of 218 adult females from the 8 study populations were radio-collared and monitored via GPS and/or VHF radio for survival (Table 1). Of the 69 animals that died, causes of death included hunter-harvest (n=20), cougar predation (n=13), trauma (n=9), vehicle/train collision (n=5), drowning (n=1) and disease (n=2), however; the cause of most mortalities (n = 58) were undetermined (Table 11). In addition to the 8 populations that were part of the statewide bighorn sheep research project, we also report survival monitoring results for instrumented

Table 11 – Cause of death for mortalities of adult female bighorn sheep in the 8 study populations that were monitored from the start of the study in winter 2014/2015 until May 31, 2019.

CAUSE OF DEATH	STUDY POPULATION								TOTAL
	Fergus	*Paradise*	*Hilgard*	*Stillwater*	*Castle Reef*	*Lost Creek*	*Petty Creek*	*Middle Missouri*	
Hunter Harvest	6	1	1	-	-	-	5	7	20
Disease	-	-	1	-	-	1	-	-	2
Trauma/Accident	-	-	6	1	-	2	-	-	9
Roadkill/Train	-	2	2	1	-	-	-	-	5
Drowning	-	-	-	-	1	-	-	-	1
Predation	-	1	8	-	3	1	-	-	13
Undetermined	3	10	10	10	9	5	7	4	58
TOTAL	9	14	28	12	13	9	12	11	108

female bighorn sheep performed by area biologists in the Upper Yellowstone population complex, Highland, and Spanish Peaks populations. The Upper Yellowstone Complex was originally slated to be 1 of the core populations in the statewide study, but we failed to successfully instrument adequate numbers of animals to meet our research objectives and so an alternative population was selected for incorporation into the statewide studies. The animals that were instrumented in the Upper Yellowstone Complex (both GPS and VHF transmitters), however, were monitored and results are presented. The Highland population has a history of multiple augmentations over the past several decades following a severe respiratory disease epizootic. A sample of the translocated animals from a number of these augmentations were instrumented with VHF transmitters and monitored by Vanna Boccadori, the MFWP's area biologist, and these records were made available to the research team to include in the survival rate investigations. A sample of adult ewes was also instrumented with GPS collars in the Spanish Peaks population in conjunction with a population health assessment that was conducted independent of the statewide bighorn research project and monitoring records for these animals were made available for analysis by Julie Cunningham, the MFWP's area biologist.

ADULT FEMALE SURVIVAL

For survival analyses, a record was created for each instrumented animal that included the date the animal was initially released carrying a radio collar, last date the animal was known to be alive, first date the animal was known to be dead, and fate of the animal. Instrumented animals were monitored for mortality via ground, aircraft, and satellite tracking. Most animals that died were wearing functioning GPS collars, and date of death was defined as the first day that recorded locations indicated the animal had stopped moving with the last date known alive recorded as the previous day. For animals wearing only functioning VHF collars, we recorded all dates that survival monitoring occurred and whether the individual's radio signal was heard on alive or dead mode or if we failed to detect the signal. From these records, the date the radio signal was first heard on mortality mode was recorded as the first date the animal was known to be dead and the last date the radio signal was detected on the alive mode was recorded as the last day the animal was known to be alive. We terminated monitoring of all instrumented animals for this study May 31, 2019.

Our survival models needed to account for the staggered entry and exit of individuals as well as the uncertain fates of some individuals that died somewhere in the interval between the end of GPS collar monitoring and the day the VHF monitoring determined a mortality*.* All analyses were conducted using a Bayesian approach to accommodate the hierarchical structure in which individuals were nested within populations and years. We defined the biological year as June 1 to May 31, summer as June 1 to November 30, and winter as December 1 to May 31. We estimated summer and winter survival and combined the seasonal values to estimate annual survival. We modeled the fates of individuals *i* on each day *t* ($\mathrm{Fate}_{i,t}$; 0 = dead, 1 = alive) from the time they entered the study until the time of death or right-censoring (due to the end of the monitoring period) as a Bernoulli random variable:

$$Fate_{i,t} \sim Bernoulli\left(S_{ageclass_{i,t},season_{i,t},pop_{i,t},\,year_{i,t}}{}^{1/182.5}\right),$$

where $S_{\mathrm{ageclass}_{i,t},season_{i,t},pop_{i,t},\,year_{i,t}}$ was the seasonal survival rate for the age class (*ageclass*) in the season (*season),* population (*pop),* and year (*year)* corresponding to individual *i* on daily record *t.*

Survival rates were variable between seasons and among years and populations. Winter survival rate estimates were generally lower than estimates for the summer season, which is a common pattern in large ungulate populations occupying higher latitudes (Table 12, Table 13). Exceptions to this pattern were substantially lower summer survival estimates for the Fergus and Middle Missouri populations that experienced significant ewe harvest, which falls within the summer season (June – November). More modest reductions in summer survival estimates for Paradise, Hilgard, and Petty Creek populations were also realized due to a small number of harvested ewes in each population over the study period (Table 14).

Caution should be exercised in interpretation of all single season and annual survival estimates as the modest number of instrumented animals present in each population results in relatively wide confidence intervals on all estimates. Among-population comparisons are best made using survival estimates generated by pooling data across all years of monitoring. The pooled annual survival rates for the Petty Creek, Fergus, and Hilgard populations were relatively high, ranging from 0.90 to 0.97. Pooled survival estimates for Lost Creek, Stillwater, Middle Missouri, and Highland populations were intermediate, ranging between 0.86 and 0.88. Pooled survival rates were relatively low for the Paradise, Castle Reef, Spanish Peaks, and Upper Yellowstone populations, ranging between 0.71 to 0.82 (Table 12).

While survival estimates for the Spanish Peaks and Upper Yellowstone populations are relatively low, modest sample sizes resulted in considerable uncertainty in these estimates (as reflected in the associated confidence intervals), indicating caution in interpreting the point estimates. Sample sizes for the Paradise and Castle Reef populations, however, were better and the relatively low pooled survival estimates for these two populations, and the associated reasonably narrow confidence limits, suggest adult females in these 2 populations experience higher mortality rates which can result in weaker overall demographic performance.

Table 12 – Known-fate seasonal and annual survival estimates and associated 90% confidence intervals for radio-collared adult females in the 8 study populations. Collared animals that were harvested were excluded when calculating these estimates.

			ANNUAL		SUMMER		WINTER	
HERD	YEAR		EST.	90% CI	EST.	90% CI	EST.	90% CI
Paradise	2014-15						0.93	0.77-0.99
	2015-16		0.79	0.61-0.93	0.93	0.77-0.99	0.93	0.75-0.99
	2016-17		0.73	0.52-0.86	0.87	0.68-0.98	0.88	0.71-0.97
	2017-18		0.80	0.63-0.93	0.99	0.89-1.00	0.85	0.68-0.96
	2018-19		0.76	0.56-0.91	0.92	0.74-1.00	0.91	0.71-0.99
		Pooled	0.82	0.74-0.89	0.94	0.87-0.98	0.89	0.82-0.94
Petty Creek	2015-16						0.99	0.81-1.00
	2016-17		0.88	0.70-0.97	0.99	0.87-1.00	0.93	0.77-0.99
	2017-18		0.88	0.71-0.97	0.93	0.76-0.99	0.99	0.90-1.00
	2018-19		0.82	0.66-0.92	0.99	0.90-1.00	0.86	0.71-0.95
		Pooled	0.90	0.83-0.96	0.98	0.93-1.00	0.94	0.88-0.98
Lost Creek	2014-15						0.74	0.50-0.93
	2015-16		0.78	0.60-0.93	0.99	0.81-1.00	0.87	0.69-0.97
	2016-17		0.94	0.79-0.99	0.99	0.86-1.00	0.99	0.88-1.00
	2017-18		0.88	0.74-0.97	0.97	0.96-1.00	0.96	0.84-1.00
	2018-19		0.80	0.64-0.92	0.99	0.90-1.00	0.86	0.69-0.95
		Pooled	0.88	0.82-0.93	0.98	0.95-1.00	0.89	0.84-0.94
Hilgard	2011-12						0.98	0.62-1.00
	2012-13		0.82	0.51-0.97	0.98	0.68-1.00	0.98	0.68-1.00
	2013-14		0.85	0.60-0.97	0.98	0.68-1.00	0.95	0.83-1.00
	2014-15		0.91	0.79-0.98	0.99	0.90-1.00	0.95	0.84-1.00
	2015-16		0.89	0.76-0.96	0.92	0.82-0.98	0.99	0.91-1.00
	2016-17		0.89	0.77-0.96	0.99	0.90-1.00	0.93	0.82-0.98
	2017-18		0.84	0.70-0.93	0.91	0.80-0.98	0.95	0.84-1.00
	2018-19		0.83	0.67-0.93	0.91	0.78-0.98	0.95	0.83-1.00
		Pooled	0.90	0.86-0.94	0.95	0.91-0.98	0.95	0.92-0.98
Castle Reef	2014-15						0.91	0.73-0.91
	2015-16		0.71	0.52-0.86	0.99	0.87-1.00	0.76	0.57-0.90
	2016-17		0.80	0.64-0.94	0.92	0.74-0.99	0.95	0.82-0.99
	2017-18		0.72	0.55-0.86	0.99	0.90-1.00	0.75	0.59-0.89
	2018-19		0.70	0.42-0.91	0.85	0.57-0.98	0.98	0.68-1.00
		Pooled	0.80	0.71-0.87	0.95	0.90-0.99	0.84	0.77-0.91
Fergus	2014-15						1.00	0.93-1.00
	2015-16		0.97	0.87-1.00	1.00	0.92-1.00	1.00	0.92-1.00
	2016-17		0.96	0.88-1.00	1.00	0.92-1.00	1.00	0.92-1.00
	2017-18		0.92	0.83-0.98	0.96	0.87-1.00	1.00	0.92-1.00
	2018-19		0.94	0.84-0.98	0.98	0.90-1.00	0.99	0.90-1.00
		Pooled	0.97	0.94-0.99	0.98	0.95-1.00	1.00	0.98-1.00
Stillwater	2014-15						0.99	0.81-1.00
	2015-16		0.86	0.68-0.96	0.93	0.76-0.99	0.99	0.86-1.00
	2016-17		0.90	0.73-0.98	0.99	0.86-1.00	0.95	0.82-1.00
	2017-18		0.77	0.58-0.90	0.92	0.77-0.99	0.88	0.70-0.98
	2018-19		0.67	0.38-0.88	0.99	0.85-1.00	0.72	0.43-0.95
		Pooled	0.87	0.79-0.93	0.94	0.89-0.99	0.93	0.87-0.98
Middle Missouri	2016-17						0.99	0.88-1.00
	2017-18		0.81	0.62-0.93	0.94	0.80-0.99	0.92	0.74-0.99
	2018-19				0.88	0.62-1.00		
		Pooled	0.86	0.74-0.99	0.93	0.81-0.99	0.96	0.87-1.00

Table 13 – Known-fate seasonal and annual survival estimates and associated 90% confidence intervals for adult females from ancillary populations that were not part of the statewide study, however, data for collared animals associated with various management projects were made available by MFWP's area biologists to provide additional insights on survival of Montana bighorn sheep herds.

			ANNUAL		SUMMER		WINTER	
HERD	YEAR		EST.	90% CI	EST.	90% CI	EST.	90% CI
Highland*	2006-07						0.98	0.68-1.00
	2007-08		0.82	0.58-0.95	0.86	0.63-0.98	0.99	0.89-1.00
	2008-09		0.67	0.50-0.81	0.72	0.55-0.86	0.99	0.86-1.00
	2009-10		0.92	0.73-0.99	0.99	0.84-1.00	0.99	0.83-1.00
	2010-11		0.84	0.61-0.95	0.99	0.83-1.00	0.90	0.70-0.99
	2011-12		0.89	0.66-0.99	0.99	0.80-1.00	0.99	0.78-1.00
	2012-13		0.75	0.39-0.95	0.98	0.63-1.00	0.97	0.56-1.00
	2013-14		0.70	0.35-0.95	0.97	0.56-1.00	0.97	0.56-1.00
	2014-15		0.88	0.49-0.99	0.97	0.53-1.00	0.99	0.87-1.00
	2015-16		0.80	0.63-0.93	0.86	0.70-0.97	0.99	0.85-1.00
	2016-17		0.90	0.72-0.99	0.98	0.80-1.00	0.99	0.85-1.00
	2017-18		0.75	0.53-0.91	0.99	0.80-1.00	0.83	0.62-1.00
	2018-19		0.67	0.43-0.87	0.98	0.71-1.00	0.79	0.57-0.95
		Pooled	0.86	0.81-0.91	0.89	0.84-0.94	0.96	0.93-0.99
Spanish Peaks	2017-18						0.99	0.75-1.00
	2018-19		0.78	0.58-0.92	0.92	0.75-0.99	0.92	0.73-0.99
		Pooled	0.79	0.62-0.93	0.88	0.73-0.99	0.91	0.81-0.99
Upper Yellowstone	2011-12						0.96	0.41-1.00
	2012-13		0.81	0.45-0.97	0.97	0.56-1.00	0.98	0.73-1.00
	2013-14		0.81	0.57-0.95	0.98	0.75-1.00	0.92	0.72-0.99
	2014-15		0.68	0.46-0.88	0.90	0.69-0.99	0.88	0.63-0.98
	2015-16		0.08	0.00-0.38	0.62	0.30-0.86	0.19	0.02-0.62
		Pooled	0.71	0.58-0.84	0.86	0.74-0.96	0.84	0.73-0.94

** All collared Highland animals used to estimate survival rates were translocated into the population from other populations in MT.*

Table 14 – Known-fate seasonal and annual survival estimates and associated 90% confidence intervals for radio-collared adult females in each of the 4 study populations in the statewide study where significant numbers of radio-collared females were legally harvested by hunters. The table compares survival estimates when hunting mortalities were censored ("No harvest") and included ("With harvest") in analyses.

		ANNUAL		SUMMER		WINTER	
		EST.	90% CI	EST.	90% CI	EST.	90% CI
Fergus	No harvest	0.97	0.94-0.99	0.98	0.95-1.00	1.00	0.98-1.00
	With harvest	0.93	0.89-0.96	0.94	0.90-0.97		
Middle Missouri	No harvest	0.86	0.74-0.94	0.93	0.81-0.99	0.96	0.87-1.00
	With harvest	0.64	0.50-0.77	0.70	0.54-0.81		
Paradise	No harvest	0.82	0.74-0.89	0.94	0.87-0.98	0.89	0.82-0.94
	With harvest	0.81	0.73-0.88	0.92	0.85- 0.97		
Petty Creek	No harvest	0.90	0.83-0.96	0.98	0.93-1.00	0.94	0.88-0.98
	With harvest	0.87	0.79-0.93	0.94	0.87-0.98		

PREGNANCY

Pregnancy rates of adult female animals (>1.5 years old) in the study populations were assessed using serum assays that measure serum concentrations of pregnancy specific protein "B" (PSPB) and progesterone (P4). PSPB concentrations indicate whether an animal is or recently was pregnant; however, this assay requires up to a month following fertilization to reliably indicate pregnancy. P4 concentrations indicate whether the animal is cycling (reproductively active) and capable of becoming pregnant (if sampled during the breeding season) or is pregnant (if sampled after the breeding season). For animals sampled in December (near the end of the breeding season) PSPB cannot reliably assess pregnancy and P4 can reliably indicate whether or not an animal is cycling, but not whether it has been successfully bred. There is little indication in the literature that cycling ungulates fail to conceive if populations maintain adequate ratios of adult males to females and all bighorn populations in this study have excellent male to female ratios, hence, we assume that any animals sampled in December who's P4 level indicated cycling was in early stages of pregnancy, or would have become pregnant after they were sampled, and were reported accordingly. Asymmetric binomial 90% confidence intervals were calculated for all point estimates for pregnancy rates.

Estimated pregnancy rates for most populations were very high, generally >0.90 (Figure 51). This pattern of high pregnancy rates corroborates findings from previous studies that bighorn sheep pregnancy rates are consistently high and not likely an important factor limiting lamb recruitment (Singer et al. 2000d, Cassirer and Sinclair 2007, Stephenson et al. 2011). Despite the evidence for overall high pregnancy rates, our sampling has produced some results that indicate potentially lower pregnancy rates occur in some populations and in some years that could have the potential to dampen demographic performance of populations. For example, pregnancy rate estimates for the Galton and Highland populations, 2 populations

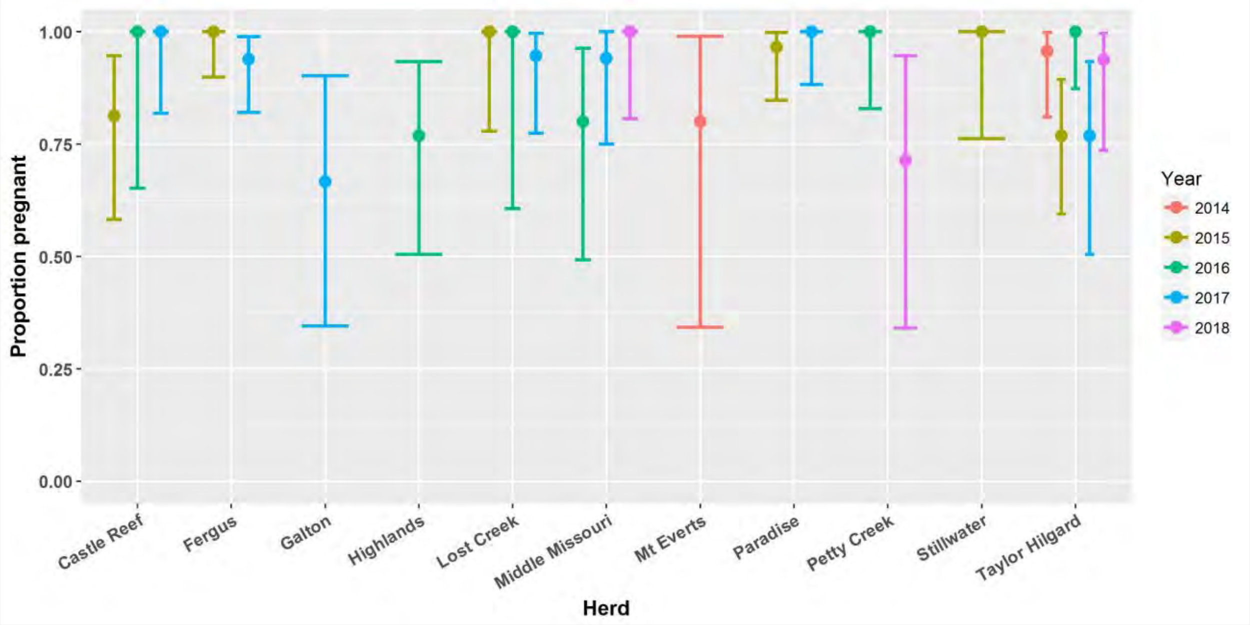


Figure 51 - Estimated pregnancy rates of the 8 study populations in the statewide study, 2 populations (Galton, Highland) that were sampled as part of MFWP's herd health program, and a herd located in the upper Yellowstone River drainage within Yellowstone National Park (Mt Everts) that was sampled as part of the GYA Mountain Ungulate Research Program.

sampled as part of MFWP's herd health program, were 0.67 and 0.77, respectively. The Galton population is located in the wet and heavily forested ecoregion of northwestern Montana along the Canadian border, which may represent a poor-quality environment for bighorn sheep which are primarily grazers. The Highland population has experienced very poor demographic performance that has generally been attributed to poor lamb recruitment since a catastrophic respiratory disease die-off during the winter of 1994-95. Low recruitment rates after respiratory disease die-offs have been commonly documented and are generally attributed to high summer lamb mortality rates due to chronic pneumonia (Cassirer et al. 2017); however, our results suggest low pregnancy rates may also be contributing to the poor demographic performance of this population. We also found some evidence for significant annual variation in pregnancy rates for 2 of the 3 populations (Castle Reef, Hilgard) that have been sampled for 3 - 5 consecutive years. Inter-annual variation in pregnancy rates in ungulates has generally been associated with variability in precipitation and temperature experienced during the summer influencing productivity and phenology of plant communities which, in turn, influences nutrition and body condition of females entering the breeding season in the fall (Parker et al. 2009, Cook et al. 2013).

The low pregnancy rate for the Mt. Evert, Galton, and Petty Creek-2018 populations should also be interpreted with caution as there is considerable uncertainty in these estimates, as reflected in the wide confidence intervals (Figure 51), due to the small number of animals sampled (< 10). While estimated pregnancy rates for Castle Reef-2016 and Lost Creek-2016 were high, sample sizes for these estimates were also < 10.

RECRUITMENT

Recruitment rates are indexed by lamb:ewe ratios obtained by area biologists as part of their routine population monitoring surveys. These sex-age classification surveys are generally conducted in late winter or early spring just prior to the lambing season and, hence, are interpreted as an index of the lambs surviving their 1st year of life to become recruited into the adult population. Populations where sex-age classification surveys are routinely conducted at the optimal time to index recruitment (April to early-May) include Castle Reef, Hilgard, Lost Creek, Paradise, and Petty Creek. Classification surveys for the 2 prairie bighorn populations in the statewide study (Fergus, Middle Missouri), as well as the Stillwater population that winters in a rugged mountainous valley with dense conifer, are normally conducted mid-winter due to better observability of animals. Lamb:ewe ratios derived from these surveys are likely overestimates of actual annual recruitment as the vast majority of overwinter mortality of young-of-the-year ungulates occur in late winter to early spring.

As is typical for large ungulate populations, the age-sex classification surveys documented substantial annual variation in recruitment rates for all populations included in the statewide study over the past decade (Figure 52). The 3 populations with the most pronounced annual variation are the Taylor-Hilard, Castle Reef, and Lost Creek populations. The latter 2 populations experienced a pneumonia epizootic during the winter of 2010. Subsequent to the disease-related die-offs in these populations lamb:ewe ratios were depressed (0.03 - 0.12) for 4 to 5 years, which is a pattern routinely observed in bighorn sheep populations after pneumonia events (Cassirer et al. 2018). However, lamb:ewe ratios in both of these populations have improved in recent years (> 0.30), suggesting recruitment in both populations may be returning to more typical rates experienced in the populations prior to the disease events. The substantial annual variation in Taylor-Higard lamb:ewe ratios is likely, at least partially, due to variability in when surveys were conducted and how data from multiple ground-based surveys were aggregated to estimate annual ratios.

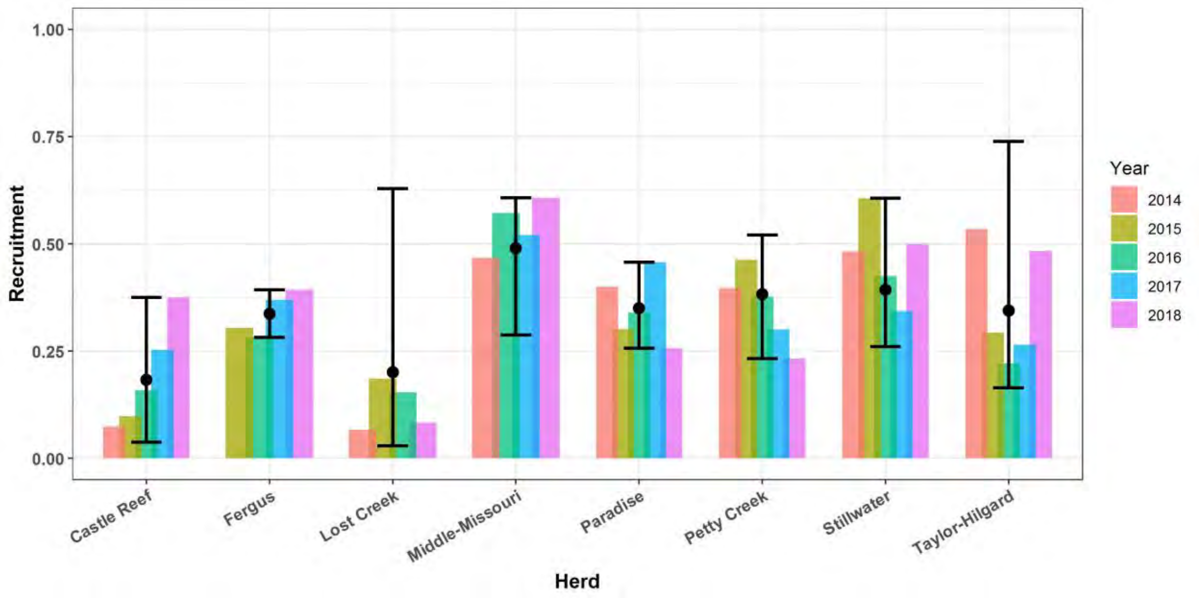


Figure 52 - Annual variation in lamb:ewe ratios determined from routine population monitoring surveys conducted by area biologists responsible for managing each of the research populations in the statewide study. For most populations the surveys were conducted in April-May and can be considered reasonable indices of annual recruitment. The points represent the mean lamb:ewe ratio for the most recent 10 years, with the lines representing the range (minimum and maximum) of annual ratios recorded.

INTEGRATED POPULATION MODELING

Population dynamics are the integrated result of variation in multiple underlying demographic vital rates such as the probabilities of pregnancy, and adult and offspring survival (Caswell 2001). Although the established paradigm of ungulate population dynamics suggests that growth rates are most sensitive to variation in offspring survival, the dynamics of small, declining and/or threatened populations subject to stochastic epizootics and harvest are more nuanced such that it is not always clear which vital rate to target for restoration efforts (Gaillard et al. 1998, Johnson et al. 2010a). Understanding both the total variability in demographic vital rates and how they integrate to shape population dynamics, in particular which vital rates are the predominate drivers of changes in growth rates, can therefore be valuable for informing management decisions as well as helping to plan future monitoring efforts. Combined, the management survey data from the bighorn sheep populations in the Montana statewide studies with similar data from populations incorporated into the companion greater Yellowstone area mountain ungulate studies provided a unique opportunity to describe the distribution of underlying probabilities of adult and offspring survival, their influence on population growth rates and correlates of lamb survival in regional bighorn sheep populations, while demonstrating the power and utility of population models.

The survey data were comprised of routine count and classification data for 17 bighorn sheep populations (Figure 53), with the longest time series from 1983 to 2018. Ideally, during each survey a total count of the total number of individuals in all age/sex classes was combined with a classification of a sample of that count into lambs, adult ewes, and adult rams. However, nearly every population had some years missing a count (but had classification data), some years missing classification data (but had a count), some years missing all data, and some years with both count and classification data (complete; Figure 54). We left-truncated the time series for each population at the 1st year for which there was a representative count.

The most comprehensive assessment to date suggests that all of these populations host *Pasteurellaceae* bacterial pathogens, and all but Paradise, Petty Creek, Targhee, and Middle Missouri host *Mycoplasma ovipneumoniae* (Chapter 2; Butler et al. 2018). All 17 populations had a management history that included at least some removals from the population due to translocations of animals to other populations or harvest. The management histories indicate that 9 of the populations (Castle Reef, Dubois, Francs Peak, Jackson, Lost Creek, Wapiti Ridge, Whiskey Mtn-East, Whiskey Mtn-West and Younts Peak) experienced all-age die-off events at least once.

We had 3 goals for our research of the survey data: 1) to describe the distributions of lamb survival, adult ewe survival, and adult ram survival rates, and the consequent distribution of population growth rates, 2) the drivers of changes in population trajectories, and 3) to assess the strength of evidence for associations between the key vital rate of lamb survival and covariates that indexed environmental variation, disease processes, and predation. To do so, we used a modified version of a previously published population model that was explicitly designed to work with such routine survey data (Paterson et al. 2019). This model merged a biological model, wherein the abundances of each age and sex class through time were connected by the relevant probabilities of reproduction and survival, to an observation model wherein the total count and classification data were a function of the underlying expected biology and a stochastic observation process. By monitoring the

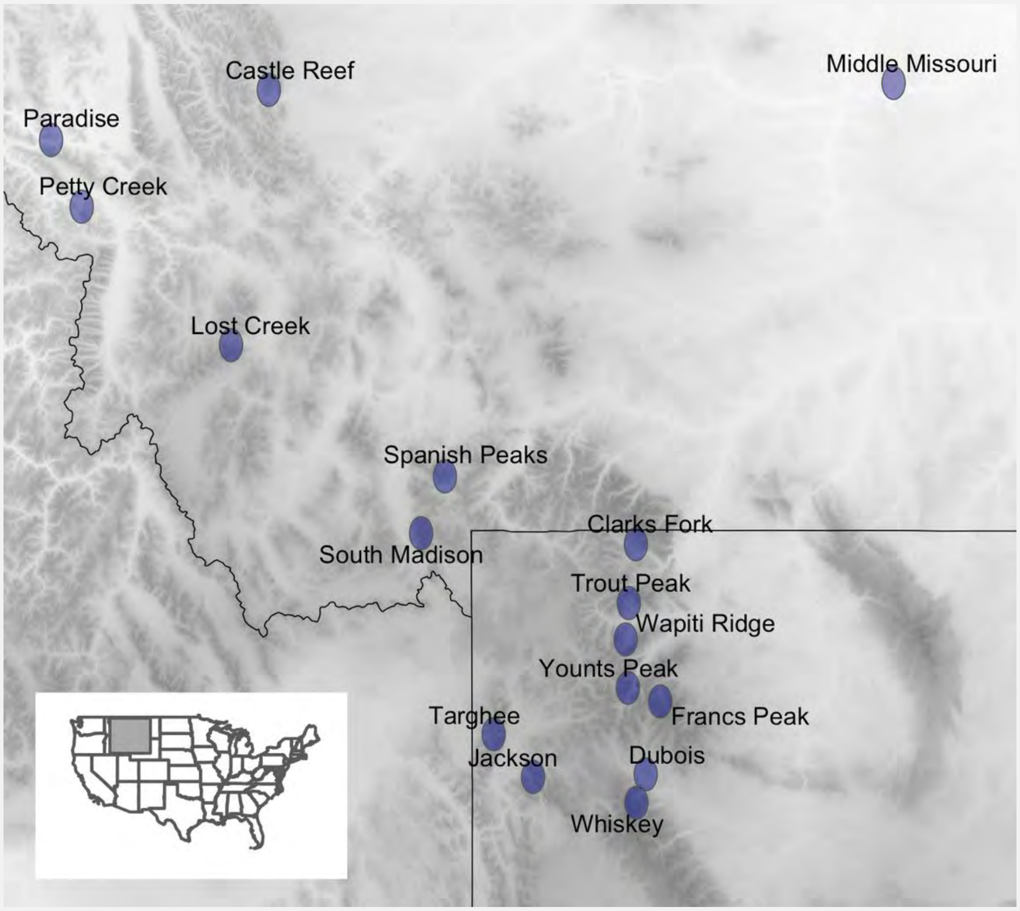


Figure 53 - Study area map showing the approximate locations of the 17 populations used in this study (Whiskey is composed of 2 separate populations: Whiskey Mtn-East and Whiskey Mtn-West).

abundances in each age/sex class in the biological model, we were able to derive population growth rates that were corrected from observation error.

Distributions of Vital Rates

Populations of bighorn sheep suffered significant declines near the turn of the 20th century in response to a series of pressures including disease and over-harvest (Buechner 1960, Berger 1990), and restoration efforts have demonstrated mixed success (Singer et al. 2000d, Picton and Lonner 2008, Hedrick 2014). The major areas of research have been delineated for decades (Buechner 1960), and a substantial body of work has developed in the intervening years characterizing the vital rates that are important for bighorn sheep population growth. For non-harvested populations, adult survival is high with limited among-year variation (Festa-Bianchet 1989, Jorgenson et al. 1997, Festa-Bianchet et al. 1997, Parr et al. 2018), which reflects a canalization of vital rates important to population growth rates (Gaillard et al. 1998). However, a recent comprehensive assessment of adult ewe survival rates for many of the populations in this study (Proffitt et al. *in press*) demonstrated that annual survival rates for non-harvested populations showed variation sufficient to impact population growth rates. Pregnancy rates for bighorn sheep are high and can display significantly greater among-year variation than survival rates (Festa-Bianchet 1988a, Singer et al. 2000d), given that pregnancy reflects a complex set of metabolic processes that integrate environmental variation and state processes such as previous year's reproductive success (Parker et al. 2009). Lamb survival rates in bighorn sheep are highly variable and have been shown to vary in response to a diverse set of drivers including seasonal environmental conditions, pathogen communities, and predation (Portier et al. 1998, Manlove et al. 2014, Smith et al. 2014). Despite the considerable volume of work describing separate vital rates in disparate populations, little is known about region-wide distributions of vital rates and population growth rates for bighorn sheep populations. Given the challenges associated with bighorn sheep restoration, a comprehensive evaluation of vital rates and population growth rates across populations can facilitate an understanding of the scale of the problem(s) required for informed management.

We sought to provide wildlife managers with an assessment of vital rates and population growth rates derived from routinely collected survey data, based on a population modeling approach. We used a hierarchical Bayesian model that provides multiple advantages for estimating a realistic population model: 1) the ability to incorporate previously estimated adult survival rates as an informed prior for sex- and year-specific survival rates, 2) the ability to incorporate previously estimated probabilities of pregnancy as informed priors for yearling- and adult ewe-specific pregnancy rates, 3) the ability to include a 'hidden' yearling class with lower probabilities of pregnancy but unobservable during the survey process, 4) the ability to deal with missing data in the time series, and 5) the ability to easily estimate population growth rates as a derived parameter based on the underlying biological model. Importantly, we were able to estimate vital rates separately for each population, i.e., we did not share information across populations. We evaluated the goodness-of-fit of our model using posterior predictive checks to ensure that our model could adequately reproduce the variation seen in the total count data as well as the variation seen in lamb:ewe ratios.

Our results indicated substantial variation in the key vital rates of lamb survival, adult ewe survival, adult ram survival, and the population growth rates (Figure 54). The population-specific medians of estimated ewe survival ranged from a minimum of 0.78 for Lost Creek to 0.88 for Wapiti Ridge. The variation in ewe survival within and among the populations was substantial: the within-population difference

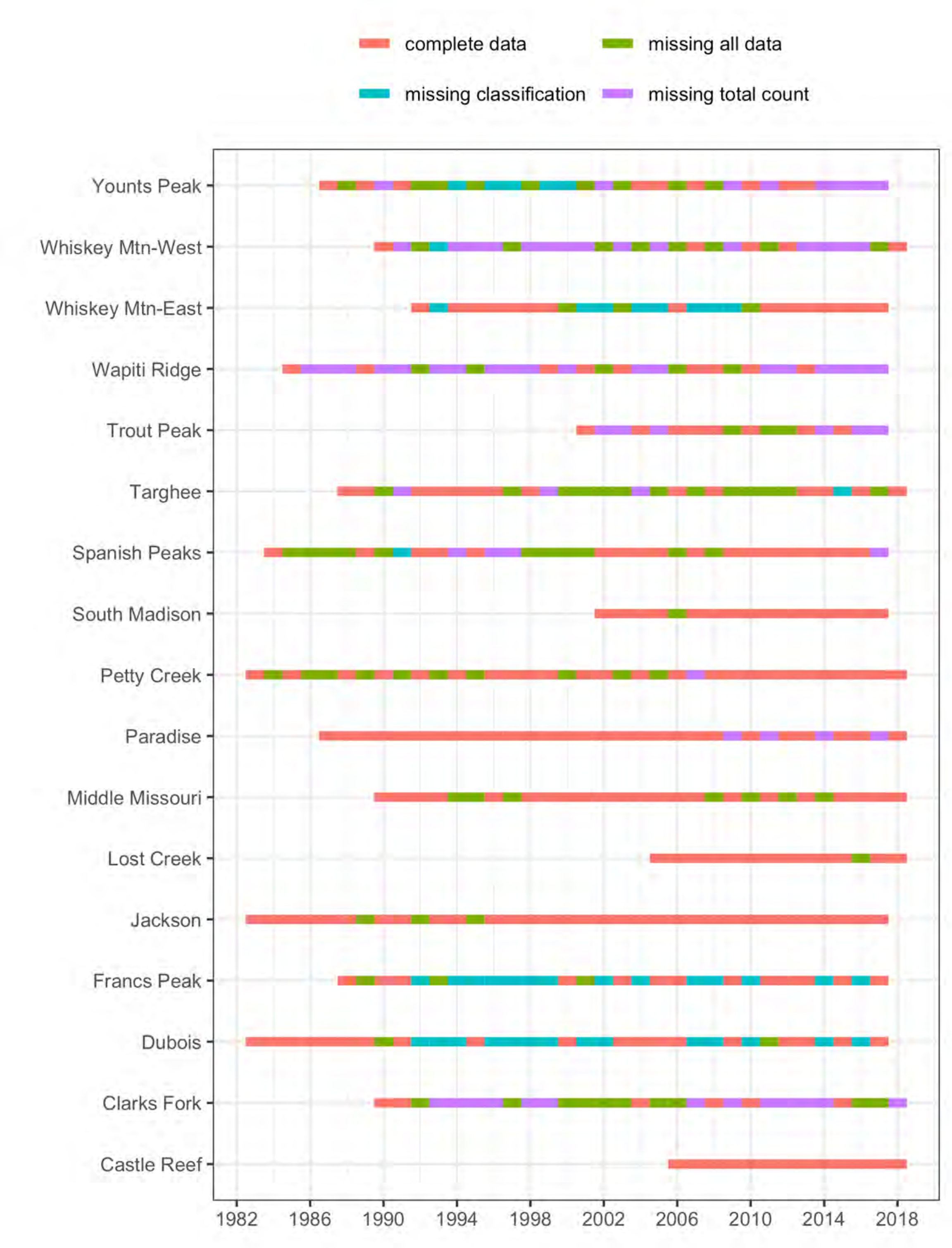


Figure 54 - Structure of the survey data. The survey data are largely discontinuous time-series of data, where missing data in any year can be: missing both total count data and classification data (missing all data), missing classification data, or missing total count data.

between the maximum and minimum estimated ewe survival values varied from 0.08 in Younts Peak to 0.24 in Middle Missouri. Estimated ram survival rates tended to be lower than those for ewes and display more variation. The population-specific medians of estimated ram survival varied from a minimum of 0.71 for Clarks Fork to 0.82 for Paradise, and within-population differences between the maximum and minimum estimated values ranging from 0.11 in Middle Missouri to 0.45 in Jackson. Lamb survival was lower and more variable than adult survival, with a range of median lamb survival values within a population from a low of 0.17 for Whiskey Mtn-East to a high of 0.47 for Middle Missouri. Within-population differences between the maximum and minimum estimated values of lamb survival ranged from 0.27 for Targhee to 0.64 for Paradise.

Unsurprisingly, this variation in vital rates translated into significant variation in population growth rates (Figure 55). We estimated the geometric mean growth rate for each population, and found that out of the 11 populations for which the 90% credible interval for the geometric mean did not include zero, 9 had geometric means less than 1 (Castle Reef, Clarks Fork, Francs Peak, Lost Creek, Targhee, Trout Peak, Wapiti Ridge, Whiskey Mtn-East and Younts Peak), and only 2 had estimated geometric means greater than 1 (Middle Missouri and South Madison). We compared the characteristics of population-years with growth rates less than 1 to those with growth rates greater than 1 and, unsurprisingly, found substantial differences in population vital rates: years with $\lambda \geq 1$ had overall higher survival rates (median ewe survival = 0.88; median ram survival = 0.80; median lamb survival = 0.47) than years with $\lambda < 1$ (median ewe survival = 0.82; median ram survival = 0.73; median lamb survival = 0.24).

In contrast to the perspective that population growth rates are dominated by variation in offspring survival, the 2nd pattern we found evidence for more complex constraining relationships between lamb survival, ewe survival and the population growth rate for the female component of the population (λf) (Figure 56). We found that the association between these vital rates depended on their values, such that the impact of lamb or ewe survival on λ^f depended on the value of the other rate (Figure 56, panel (b)). For example, at values of ewe survival greater than or equal to 0.90 we found that $\lambda^f \geq 1$ over a wide distribution of lamb survival values. Once ewe survival dropped to less than or equal to 0.80, however, we found that $\lambda^f \geq 1$ over only a narrow and high distribution of lamb survival values. If ewe survival dropped below 0.75 there was no value of lamb survival that resulted in $\lambda^f \geq 1$. Conversely, when lamb survival rates were greater than or equal to 0.40 we found $\lambda^f \geq 1$ over a wide distribution of ewe survival rates. When lamb survival rates were less than or equal to 0.20, no value of ewe survival resulted in $\lambda^f \geq 1$.

A particular strength of our approach was that it allowed population-specific inference, and facilitated the comparison of each individual population to the larger data set (Figure 57, Figure 58). In doing so, it allows managers to assess how the distribution of vital rates for the local population compared to all populations and, in doing so, identify potentially limiting factors, e.g., if lamb survival is lower than the region-wide distribution.

Our results demonstrate significant variation in vital rates and population growth rates, and suggest that most of these populations have a geometric mean growth rate less than 1. For all populations (even for populations with geometric mean growth rates greater than 1) our results indicate that population growth rates are routinely marginal and that sustained periods of population growth are uncommon. Moreover, these results strongly suggest that population growth rates can be driven by a combination of lamb survival and ewe survival; however, when either rate is particularly low the population growth rate is constrained be less than 1 regardless of the other survival rate.

Drivers of Population Trajectories

Our 2nd major goal was to understand how variation in vital rates translated into variation

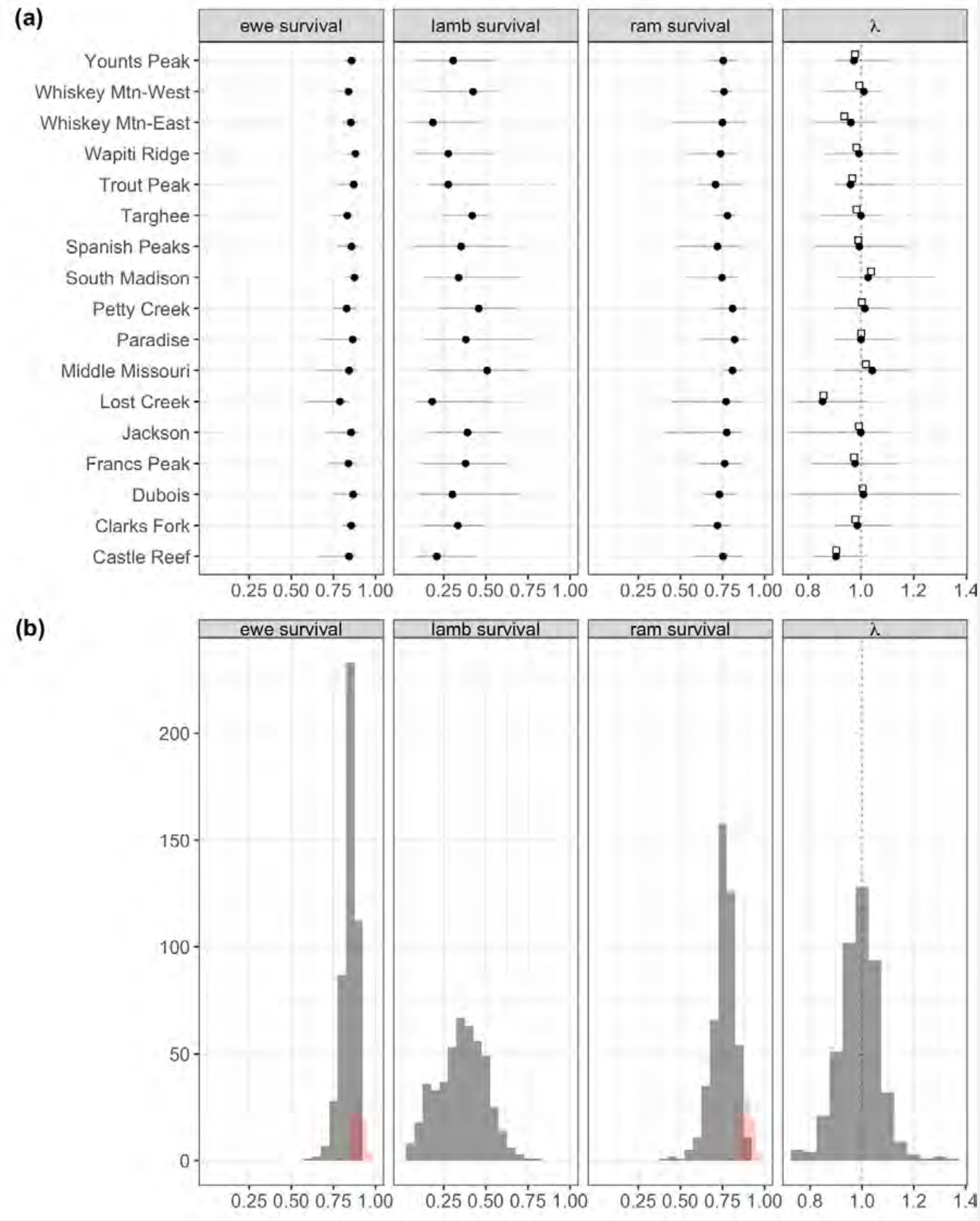


Figure 55 - Distribution of estimated yearly vital rates for the 17 populations in the study (a), and all population-years combined (b). The dot denotes the median estimated value in the time series, and the black line the 90% credible interval. For estimates of the population growth rate, the open square indicates the geometric mean (vertically jittered). For panel (b), the light red corresponds to the distribution of adult female annual survival rates taken from Proffitt et al. in review.

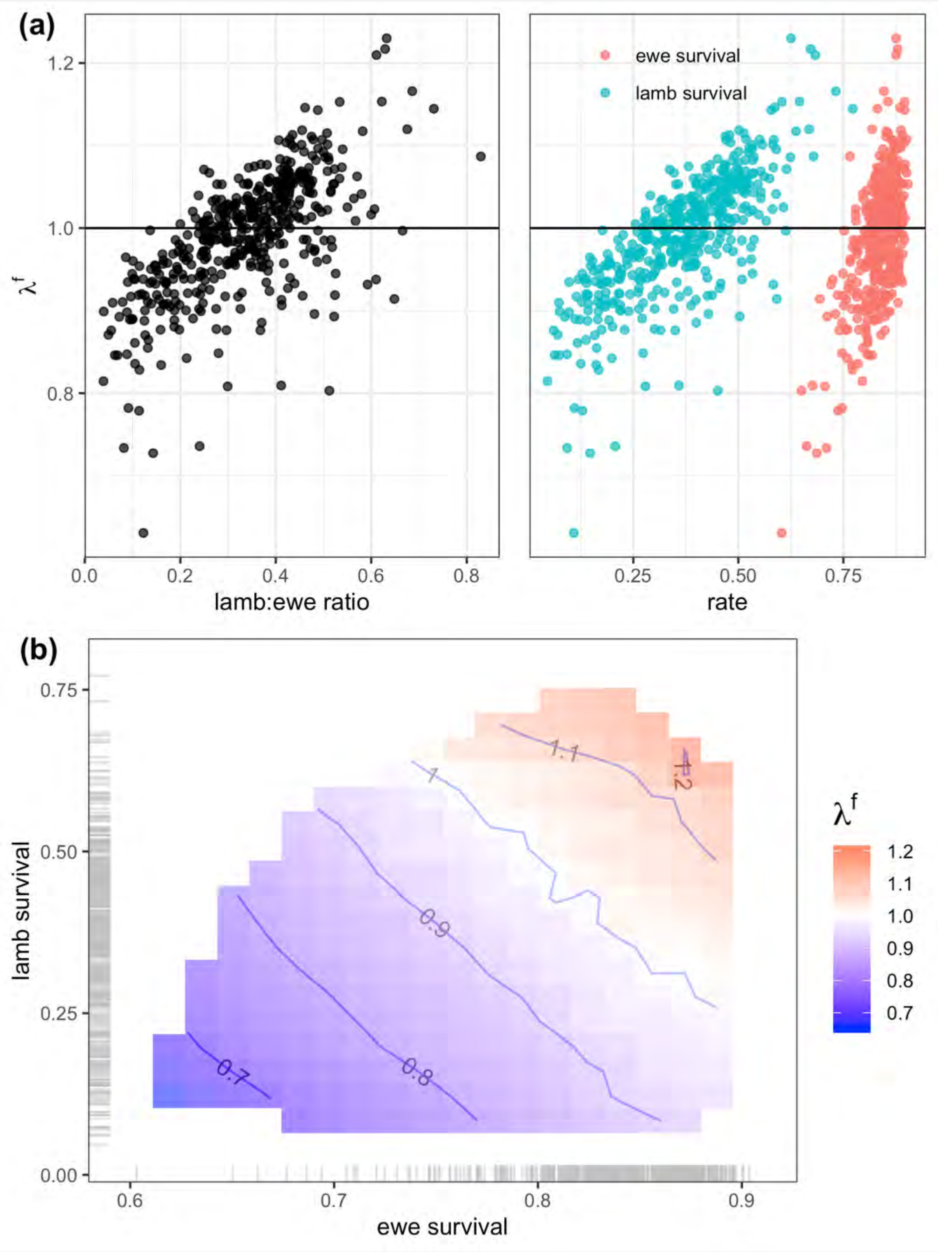


Figure 56 - Relationship between key vital rates and the growth rates of the female component of the population (λ^f). Panel (a) represents the univariate relationships between the lamb:ewe ratios and λ^f on the left side, and the relationships between two of the rates that underlie lamb:ewe ratios (lamb survival, ewe survival) and λ^f on the right. Panel (b) represents the multivariate relationship between these 2 key underlying vital rates and λ^f. For panel (a), the dots are the estimated median of ratios and rates. For panel (b), the colored and contoured surface represents a simple linear interpolation of the underlying estimated values (shown by the rug on the x- and y-axes).

in population growth rates. Although it is generally thought that population growth rates are most sensitive to variation in adult survival, it is unclear how simultaneous variation in multiple vital rates (particularly variation induced by harvest or epizootics) combine to impact population trajectories. Previous work has demonstrated that the drivers of population growth can substantially differ among populations of bighorn sheep: whereas some population-specific growth rates are driven by variation in adult survival, others align with the general ungulate life-history expectations and are driven by recruitment (Johnson et al. 2010a). Critically, this implies that informed management of bighorn sheep populations may need to be population-specific.

We sought to understand how patterns of variation in population growth rates were associated with changes in underlying vital rates. Here, we used the results from the same hierarchical Bayesian model used to estimate vital rates described in the previous section. Rather than a prospective analysis such as life-stage simulation analysis (Wisdom et al. 2000) that estimates how hypothetical changes in vital rates may impact population growth rates, we decomposed the observed variation in population growth rates into contributions from the vital rates using life-table response experiments (LTREs). LTREs are a powerful tool to investigate these drivers of observed variation in population growth rates and we took advantage of recent work that derived a series of transient LTREs that do not assume a stationary environment (Cooch et al. 2001, Koons et al. 2016, 2017). In this study, we were more interested in the drivers of changes in λ_t between successive time steps (e.g., $\Delta\lambda_t$ and what may have contributed to an abrupt decline in the population between year t and year t+1). This technique decomposes $\Delta\lambda_t$ into the contributions from underlying lamb survival, adult ewe survival, and changes in age structure. For example, if $\Delta\lambda_t = -0.2$ (a decline in population growth rate of 0.2), we could attribute -0.18 of that decline to changes in ewe survival, -0.015 of that decline to changes in lamb survival, and -0.005 to changes in the age structure of the population. For this portion of our analysis, we assumed that the size of the male population was not limiting to population growth and focused on the population growth rate of the female component of the population, λ_t^f.

Our results indicate that changes in population trajectories (e.g., from $\lambda_t^f < 1$ to $\lambda_t^f \geq 1$, a reversal from a declining population to an increasing one) can be driven by a diverse set of changes in underlying vital rates (Figure 59). However, we found some commonalities that should be helpful for informed management of these populations. In cases where the trajectory of a population changed from increasing or stable ($\lambda_t^f \geq 1$) to decreasing ($\lambda_{t+1}^f < 1$; n = 63, upper right quadrant of Figure 59), declines in lamb survival were involved in 54 (86%) of the cases and declines in ewe survival were involved in 31 (41%) of the cases. Notably, in 32 of the 54 cases associated with declines in lamb survival (59%), the population trajectory reversed despite increases in ewe survival. In 9 of the 31 cases associated with declines in ewe survival (29%), the trajectory was reversed despite increases in lamb survival. When the population's trajectory changed from decreasing ($\lambda_t^f < 1$) to increasing or stable ($\lambda_{t+1}^f \geq 1$; n = 60, lower left quadrant of Figure 59), increases in lamb survival were involved in 57 (95%) of the cases and increases in ewe survival were involved in 40 (67%) of the cases. A small number (3, or 5%) of cases involved increases in ewe survival coupled to decreases in lamb survival, whereas 20 (33%) involved increases in lamb survival and decreases in ewe survival.

We found strong evidence that the drivers of population trajectories were different between the populations (Figure 60). For positive reversals of negative population trajectories ($\lambda_t^f < 1$, $\lambda_{t+1}^f \geq 1$), increases in lamb survival were the single, most common cause in all populations. In contrast, negative reversals ($\lambda_t^f \geq 1$, $\lambda_{t+1}^f < 1$) were associated with more diverse circumstances. In 3 populations (Clarks Fork, South Madison, and Younts Peak), decreased ewe survival was the the only factor involved. In 8 others (Dubois, Francs Peak,

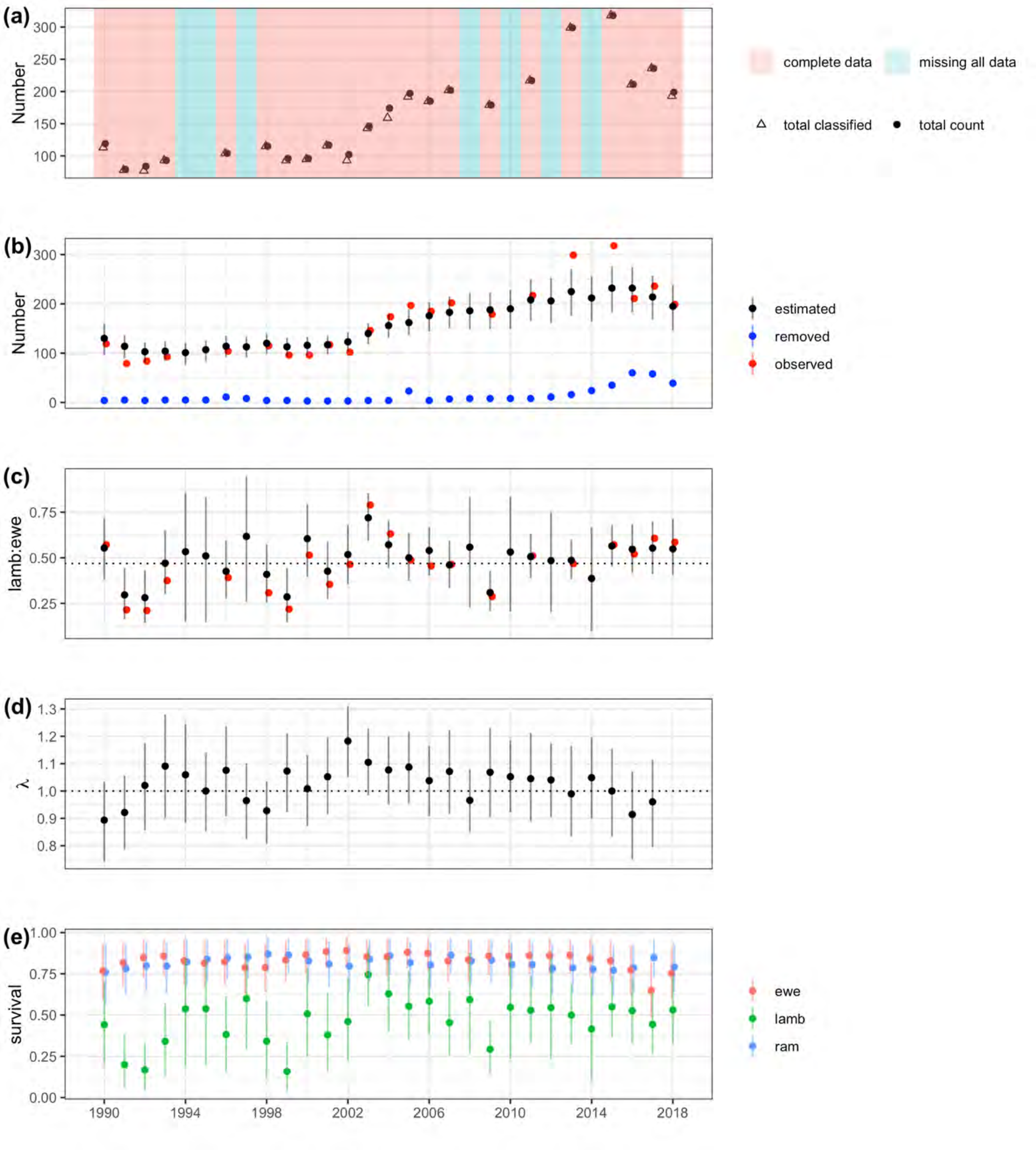


Figure 57 - Example of population-level inference for the Middle Missouri population. Panel (a) shows the distribution of the data (total count and total classified each year) and indicates the type of data missing. Panel (b) shows how the total count (red) is related to the estimated population size (black) and the number removed (blue; via harvest or translocation). Panel (c) shows how the observed lamb:ewe ratio (red) compares to the estimated lamb:ratio (black). Panel (d) shows the estimated population growth rate for the entire population. Panel (e) shows estimated ewe, ram and lamb survival. For all estimated quantities, the dot denotes the median and the line denotes the 90% credible interval.

Middle Missouri, Spanish Peaks, Trout Peak, Wapiti Ridge, Whiskey Mtn-West and Whiskey Mtn-East) decreased ewe survival was the dominant driver, with minority contributions from lamb survival or the combination of ewe and lamb survival. In 2 populations (Paradise and Targhee), decreased lamb survival was the dominant driver with minority contributions from ewe survival or the combination of lamb and ewe survival. In the remaining populations (Jackson, Lost Creek, Petty Creek) no clear dominant cause for the population declines was evident. These results suggest that the mechanisms for changes in population trajectories can differ among populations.

Correlates of Lamb Survival

Both the established model for ungulate population dynamics as well as our results in the previous sections underscore the importance of lamb survival in shaping population trajectories. Our final goal was to understand how lamb survival varied in response to variation in indices of environmental, disease and predation processes. We used an alternative formulation of our hierarchical, Bayesian population model that allowed us to assess the strength of evidence for these covariates separately for each of the populations in the study.

We used the entire time series for each population to assess the association between lamb survival and environmental variation. Our results suggested that the most common association was a negative relationship between lamb survival and the cumulative precipitation over winter range (PRECwinter), although this relationship was weak or poorly estimated for several populations (Figure 60). We found conflicting signals for the relationship between lamb survival and early/late season precipitation on summer range (PRECearly and PREClate), as well as early/late season integrated NDVI (NDVIearly and NDVIlate; Figure

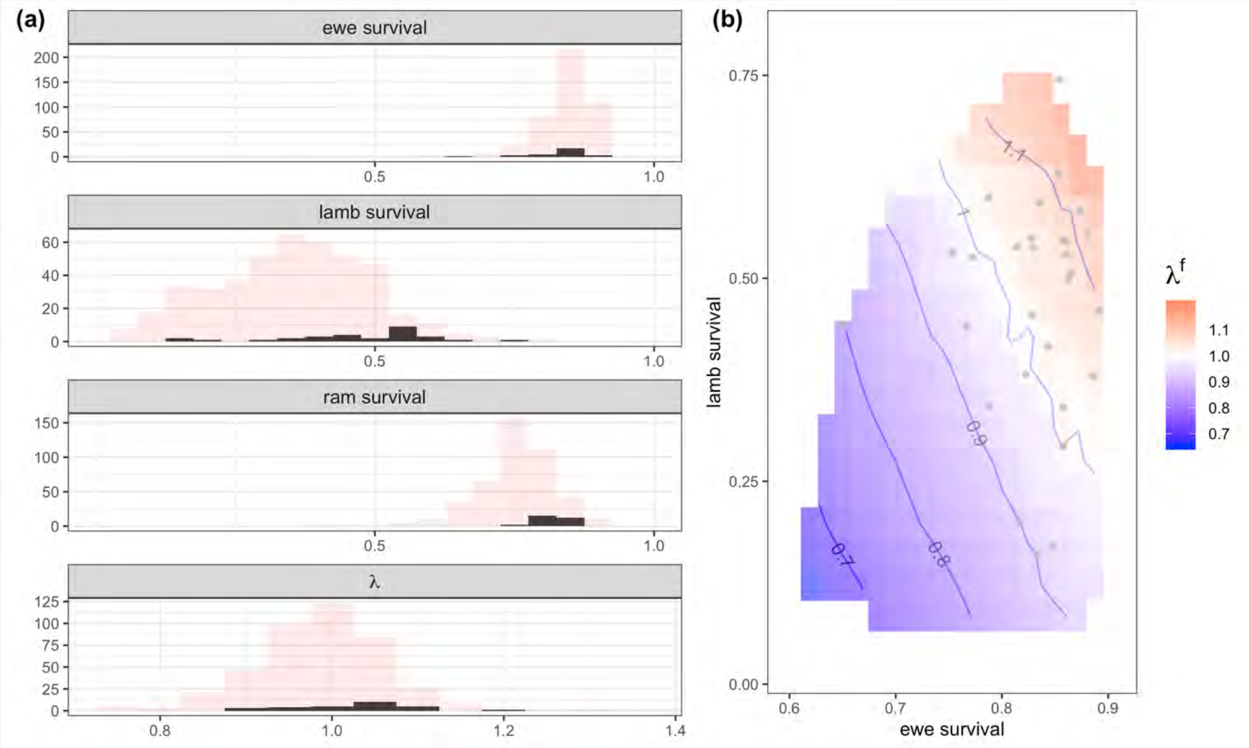


Figure 58 - Distributions of vital rates and their associations with population growth rates for the Middle Missouri population. Panel (a) depicts the distribution of estimated vital rates for all population-years (light red), and the population-specific distribution (black). Panel (b) shows the relationship between ewe survival, lamb survival and λ^f *for all population-years, with the annual population-specific vital rates displayed as dark grey*

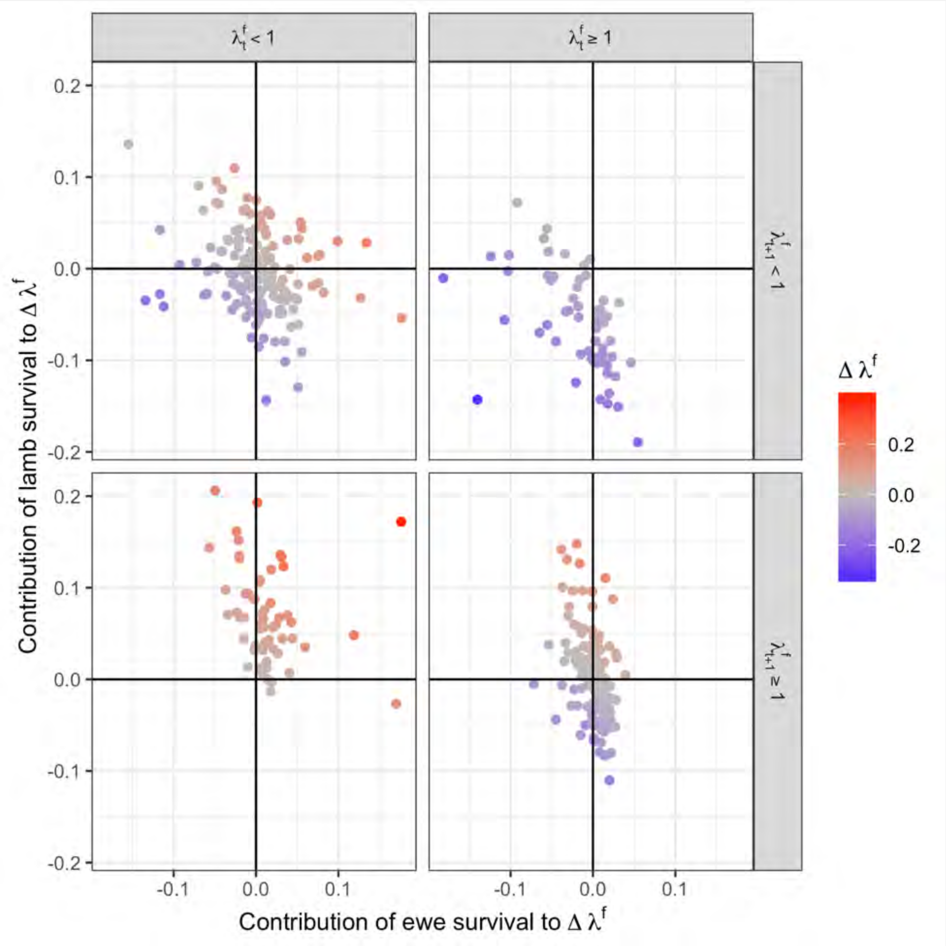


Figure 59 - Across-population results of the life-table response experiment analysis of the relationship between vital rates and the population growth rate of the female component of the population (λ^f). The columns represent λ^f in year t (less than one or greater than 1), the rows represent λ^f in year t+1, the x- and y-axes represent the contribution of changes in ewe survival and lamb survival to changes in λ^f ($\Delta\lambda^f$), and the color represents $\Delta\lambda^f$. For example, the upper right quadrant is the case where the population was increasing in year t and declining in year t + 1, and the dots denote how ewe survival and lamb survival contributed to that change in λ^f.

61). A simple post-hoc investigation weakly suggests that these conflicting signals may be the result of a functional response between a covariate and lamb survival (Figure 61), i.e., the direction and strength of the response was related to the range of covariates experienced by a population. Regardless of the lack of clarity as to the origin of the apparently conflicting signals, it is important to note that the estimated relationships corresponded to substantial predicted changes in lamb survival (Figure 63, Figure 64).

Our model results indicate that environmental variation can strongly influence lamb survival, adding to a small but growing number of studies demonstrating sources of variation in this important driver of population dynamics. However, the weak evidence of a functional response between lamb survival and environmental variation suggests that at least some care needs to be taken when trying to generalize results across populations. The management implication is that understanding the role of the environment as a limiting factor in lamb survival may require high quality population-specific data and analyses.

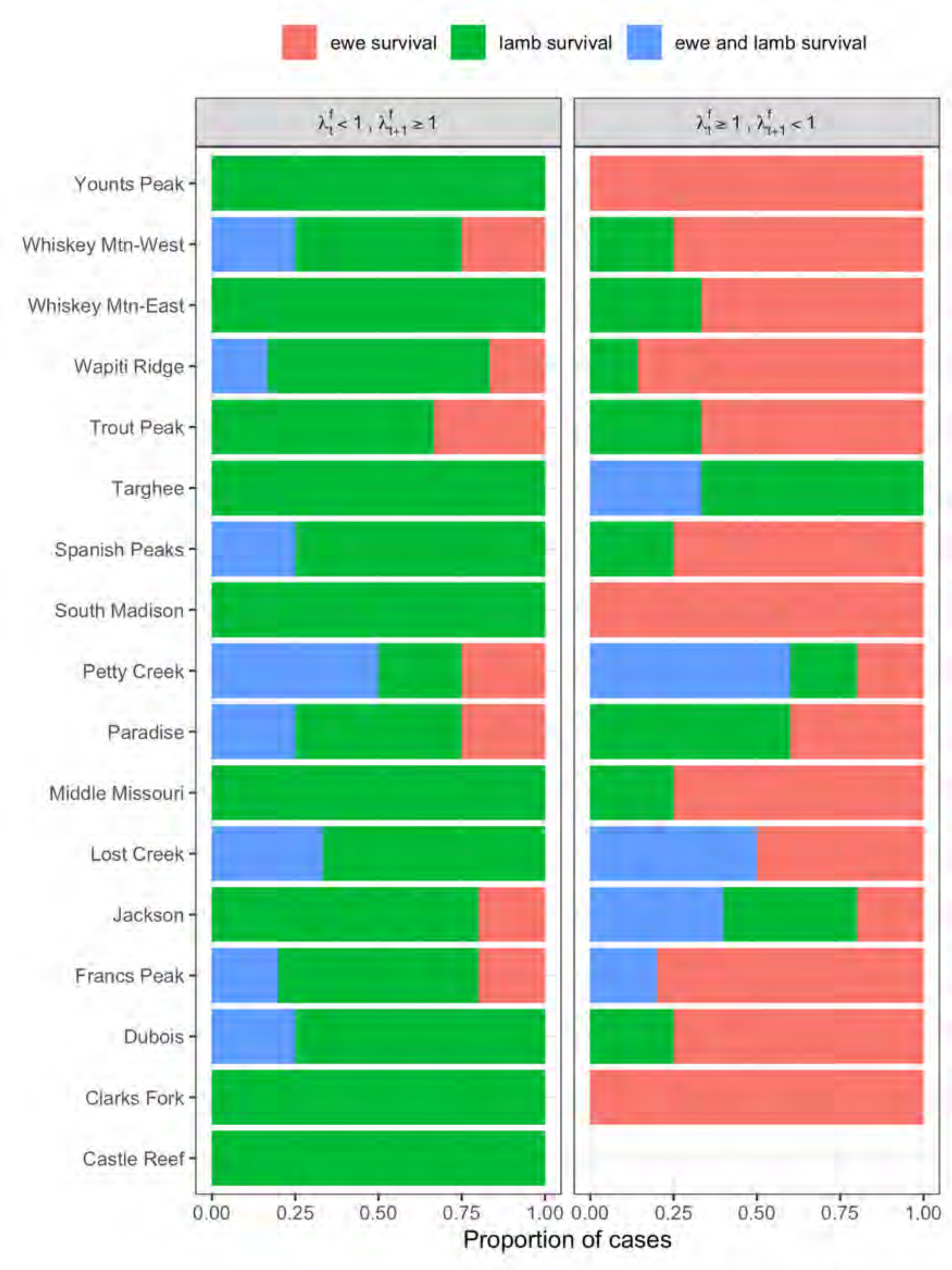


Figure 60 - Within-population drivers of changes in population trajectories. For the 2 cases in which population trajectories reversed ($\lambda_t^f < 1$, $\lambda_{t+1}^f \geq 1$ and $\lambda_t^f \geq 1$, $\lambda_{t+1}^f < 1$), we evaluated whether that reversal was due to changes in ewe survival, lamb survival, or whether the contributions from ewe and lamb survival were roughly equal.

To explore the potential role of disease as a limiting factor in lamb survival, we assessed the strength of evidence for a relationship between pathogen communities in these populations and lamb survival. Using the results of the most comprehensive study to date on the composition of communities of pathogens associated with respiratory disease in bighorn sheep (Butler et al. 2018), we compared the distributions of lamb survival for those populations that hosted both *Mycoplasma ovipneumoniae* and Pasteurellaceae bacteria (*Bibersteinia trehalosi*, Pasteurella *multocida*, and *Mannheimia haemolytica*) to those populations that only hosted Pasteurellaceae (i.e., all study populations hosted Pasteurellaceae). We used the last 10 years of estimates of lamb survival for each population from the unpooled version of our model (where lamb survival was estimated independently each year), and estimated the median lamb survival for all years for both groups. Our results indicate that populations that host both *M. ovipneumoniae* and *Pasteurellaceae* had a lower median lamb survival (0.24) than those that hosted Pasteurellaceae alone (0.41) (Figure 65, panel (a)).

These results strongly support previous results that relied on lamb:ewe ratios rather than lamb survival to evaluate associations between pathogen(s) detected in a herd and demographic vigor (Butler et al. 2018), which was unsurprising given the strong correlation between the two. Combined with our previous result demonstrating that population growth rates were sensitive to low values of lamb survival, this result suggests that the presence of *M. ovipneumoniae* may be limiting in some cases when lamb survival interacts with environmental factors to further decrease this key vital rate. However, we note that although median lamb survival was lower in populations that hosted both *M. ovipneumoniae* and *Pasteurellaceae* compared to those that hosted *Pasteurellaceae* alone, both groups of populations experienced a wide range of lamb survival values (Figure 65, panel(b)). Importantly for management, the wide variation in lamb survival regardless of pathogen presence suggests that the mere presence of specific pathogens is not sufficient as a limiting factor; rather, it likely serves to predispose populations to declining growth rates when disease processes interact with environmental drivers as demonstrated above.

Predation on bighorn sheep lambs has been shown to be a significant factor in limiting lamb survival and, by extension, population growth rates (Smith et al. 2014). Future work is needed to better understand the role of predation as a potential limiting factor for bighorn sheep populations. Given the diverse suite of predators to which these populations are now exposed as predator populations recover in much of this study area, we further suggest that future work should be designed to evaluate potentially complicated interactions between ungulates and multiple populations of predators.

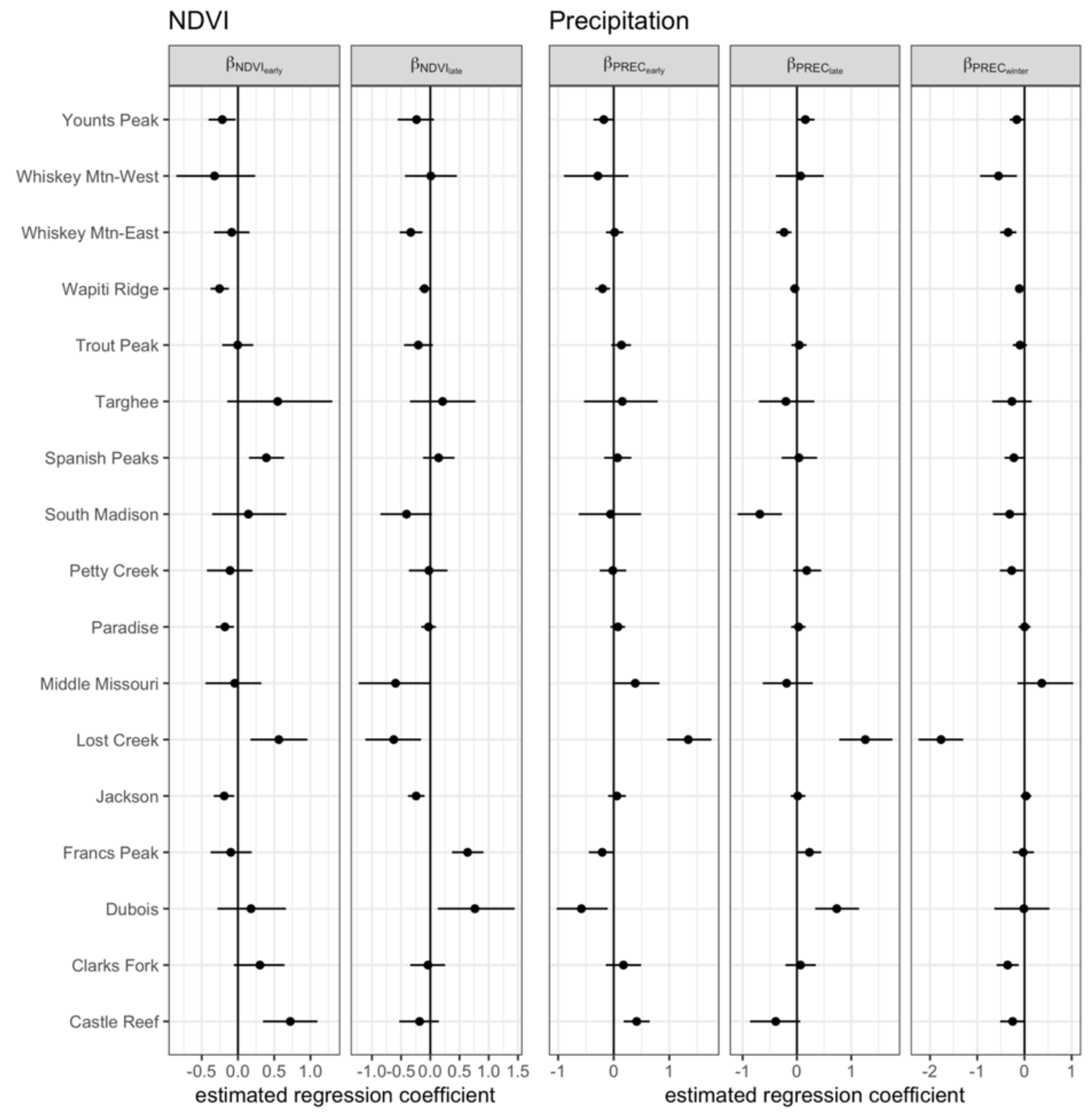


Figure 61 - Estimated regression coefficients describing the relationships between environmental conditions during the 1st year of life and lamb survival. We indexed growing conditions using 2 metrics (NDVI and precipitation, PREC) during 2 periods (early (spring) and late (summer), and indexed winter severity using precipitation (PREC). The dot represents the median of the estimated regression coefficients, and the line the 90% credible interval.

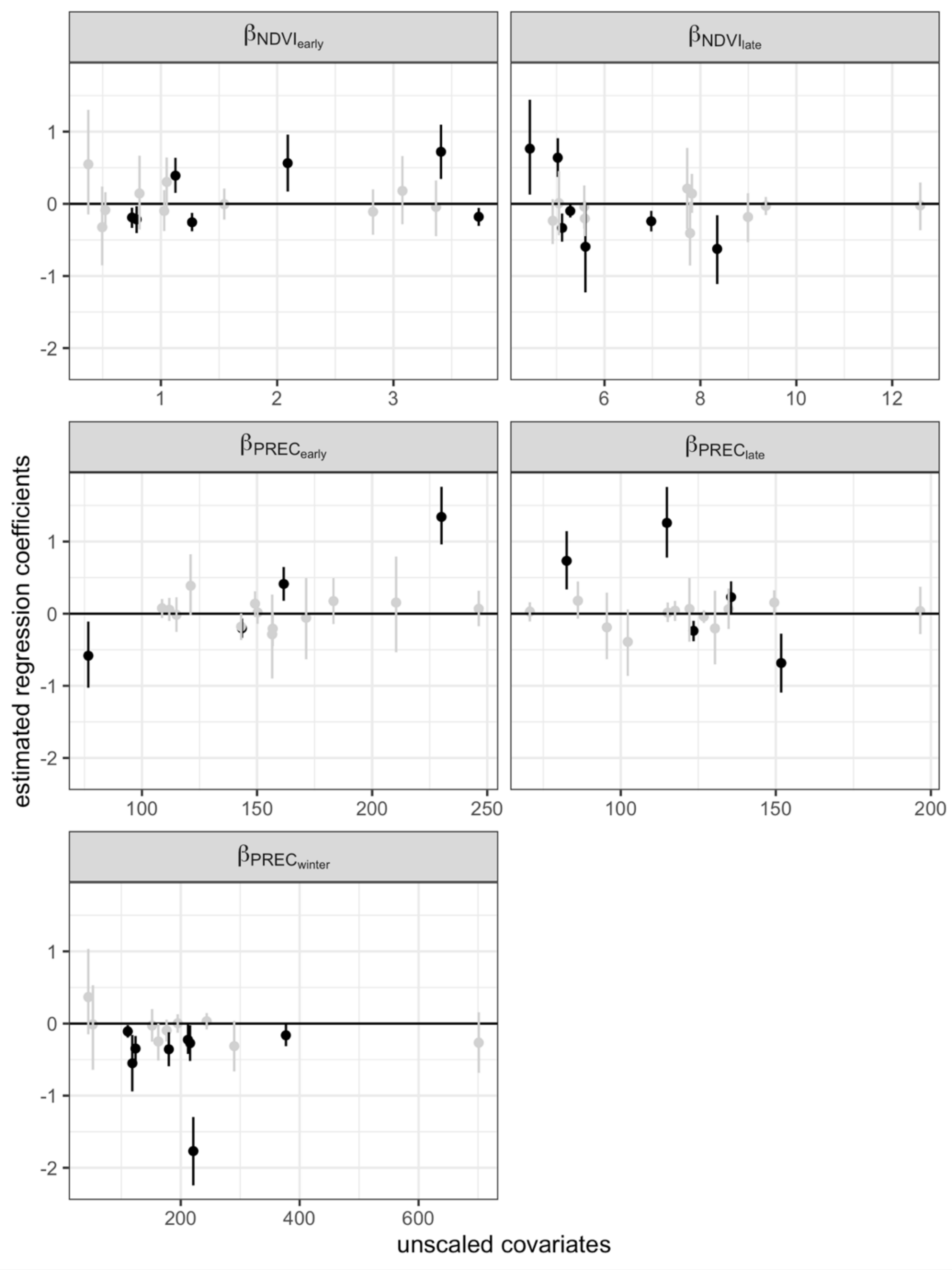


Figure 62 - Relationships between estimated regression coefficients for NDVI and PREC covariates and the median value of each covariate for each population. For each of our 5 covariates, we graphed the median value through time for each population (x-axis) against the estimated regression coefficient (y-axis). Estimated regression coefficients whose 90% credible interval did not overlap zero are in black; the remainder are in grey. The dot denotes the median and the line the 90% credible interval.

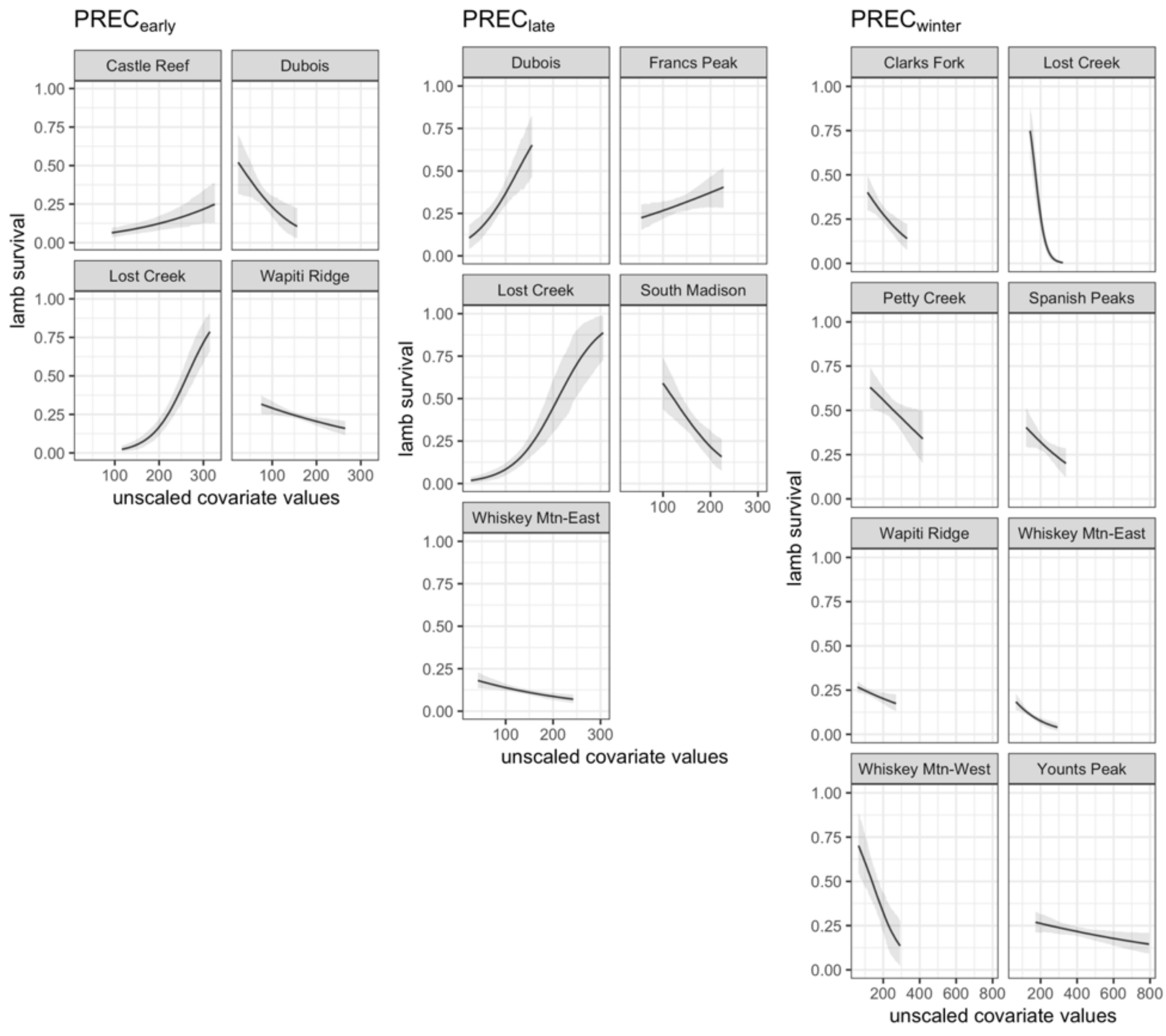


Figure 63 - Population-specific predicted relationships between precipitation and lamb survival. All predictions were constructed by holding other covariates to their mean value. The black line represents the median of the estimated response to change in that covariate (the grey ribbon represents the 90% credible interval).

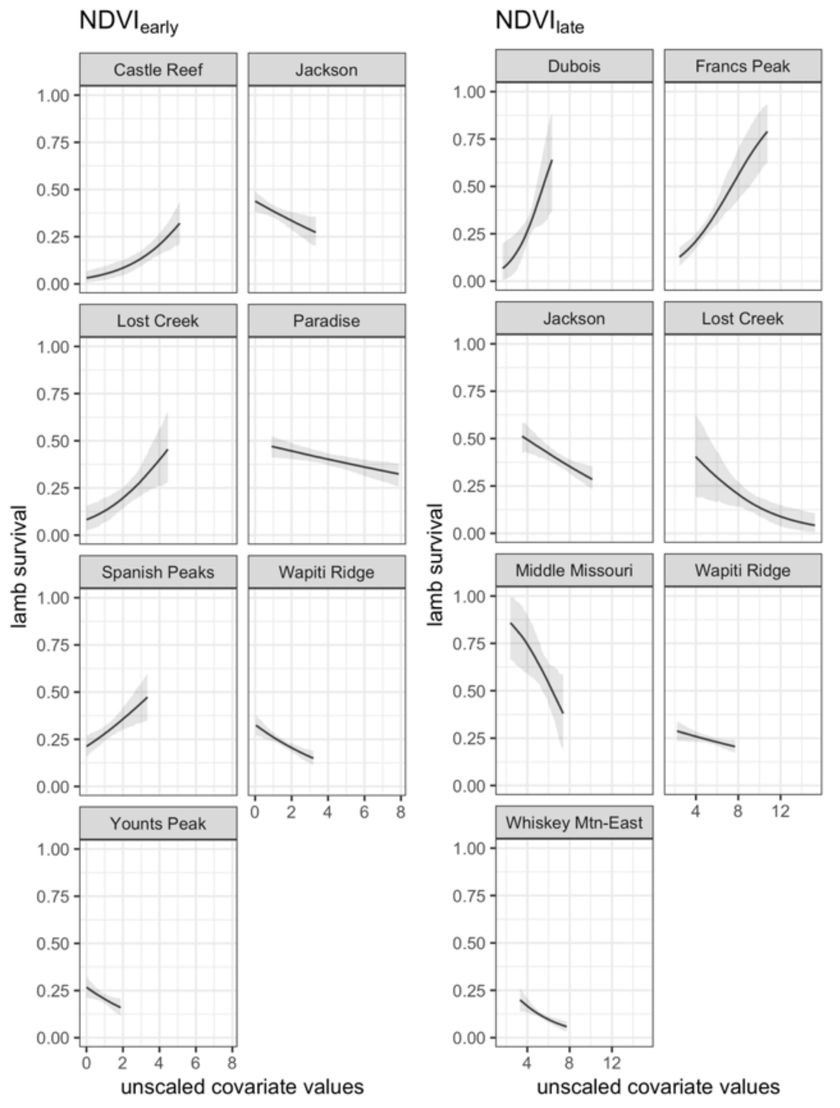


Figure 64 - Population-specific predicted relationships between NDVI and lamb survival. All predictions were constructed by holding other covariates to their mean value. The black line represents the median of the estimated response to change in that covariate (the grey ribbon represents the 90% credible interval).

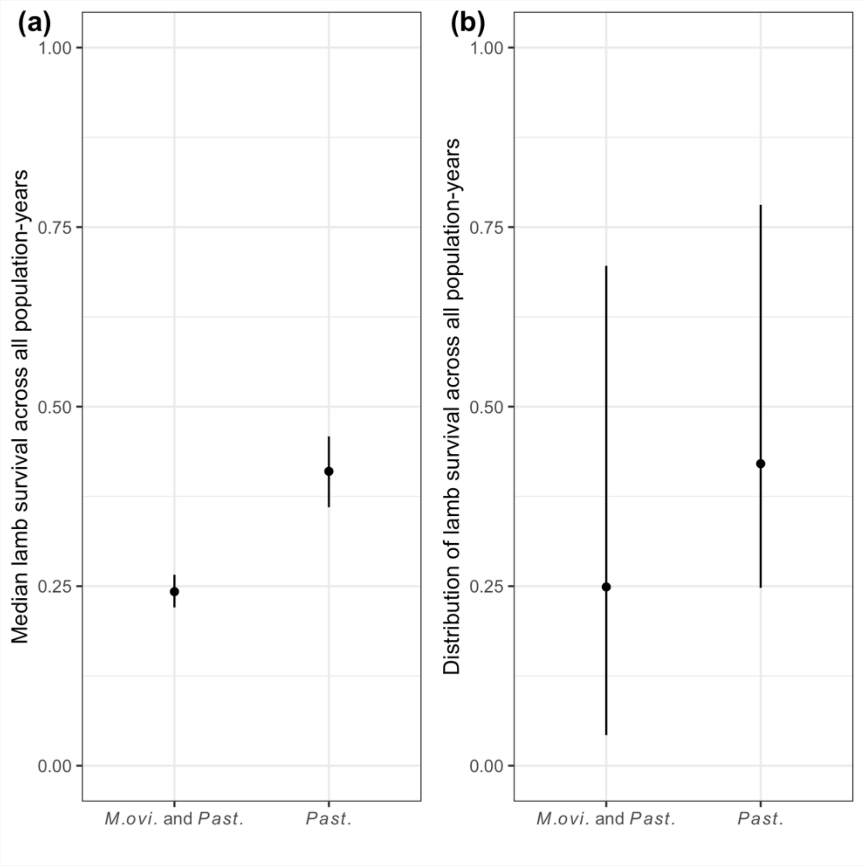


Figure 65 - Comparison of lamb survival for populations that host both Mycoplasma ovipneumoniae and Pasteurellaceae bacteria (M. ovi and Past.) and populations that host Pasteurellaceae alone (Past.). Panel (a) represents the estimated median lamb survival across all population years for both groups, indicating a lower average survival for populations that host both (dot = median, line = 90% credible interval). Panel (b) depicts the distribution of all estimated lamb survival values for both groups (dot = median, line = minimum lamb survival to maximum lamb survival).

Chapter Summary

- The monitoring of radio-collared adult bighorn ewes demonstrated that annual survival varied substantially among years and populations with data pooled across years providing herd-specific survival estimates ranging from approximately 0.80 to 0.97. Given the sensitivity of population growth rates to adult female survival, these results suggest that mortality factors operating on ewes have important impacts on demographic vigor of some bighorn sheep populations.
- Estimated pregnancy rates for most study populations were very high, generally >0.90, corroborating findings from previous studies that bighorn sheep pregnancy rates are likely not an important factor limiting lamb recruitment. Our sampling, however, produced some results that indicated lower pregnancy rates occurred in some populations and in some years that could have the potential to dampen demographic performance.
- As is typical for large ungulate populations, the age-sex classification surveys documented substantial annual variation in recruitment rates for all populations included in the statewide study. The 2 populations with the lowest recruitment rates experienced a pneumonia epizootic during the winter of 2010; however, lamb:ewe ratios in both of these populations have improved in recent years (>0.30), suggesting recruitment in both populations may be returning to more typical rates experienced in the populations prior to the disease events
- We developed a hierarchical population model that connects biological processes to an observation model and integrates routine survey and classification data with other sources of data on bighorn sheep vital rates. The model has important advantages for the informed management of populations of bighorn sheep, including the ability to incorporate the best-available information from other studies, the ability to estimate and understand the drivers of population growth rates, and the ability to assess sources of variation in vital rates.
- Vital rates of bighorn sheep varied widely across population-years, and their integrated result translated into similar variation in population growth rates. Model results indicated the majority of populations in this study had low growth rates, and we identified key relationships between lamb and adult ewe survival that may be limiting growth rates.
- The drivers of changes in population trajectories demonstrated substantial variation such that it is difficult to generalize which vital rate should best be targeted by management interventions to enhance demographic vigor of bighorn sheep populations.
- We demonstrated the substantial role that environmental conditions can play in driving lamb survival but the relationships between environmental covariates and lamb survival varied among populations.

Chapter Summary (continued)

- Although we demonstrated an association between pathogen communities and lamb survival, this association alone was insufficient to explain the substantial variation in survival rates. Rather, we suggest that the impact of respiratory disease on lamb survival is the result of the interactions of resident pathogen communities with ecological and physiological processes and environmental factors (the epidemiological triad).

Peer-reviewed Science Products

Proffitt, K., A. Courtemanch, S. Dewey, B. Lowrey, D. McWhirter, K. Monteith, J. T. Paterson, J. Rotella, P. White, and R. Garrott. In press. Regional variability in Rocky Mountain bighorn sheep pregnancy and survival rates. Ecosphere.

Paterson, J.T., K. Proffitt, J. Rotella, R. Garrott. In review. Variation in the population dynamics of bighorn sheep. Ecosphere.

Chapter 7

Deliverables

Final Reports

Garrott, R., K. Proffitt, J. Rotella, E. Flesch, E. Lula, C. Butler, B. Lowrey, J. T. Paterson, & J. DeVoe. **2020**. The role of disease, habitat, individual condition, & population attributes on bighorn sheep recruitment & population dynamics in Montana. Final Report for Federal Aid in Wildlife Restoration Grant #W-159-R. Montana Fish, Wildlife & Parks, Helena, Montana

Garrott, R., K. Proffitt, J. Rotella, E. Flesch, E. Lula, C. Butler, B. Lowrey, J. T. Paterson, J. DeVoe, & E. Grusing. **2021**. Bighorn sheep ecology: an integrated science project to support restoration and management. Final Report for Federal Aid in Wildlife Restoration Grant #W-159-R. Montana Fish, Wildlife & Parks, Helena, Montana

Annual Reports

Garrott, R., K. Proffitt, J. Rotella, & C. Butler. **2014**. The role of disease, habitat, individual condition, and herd attributes on bighorn sheep recruitment and population dynamics. Annual Reports, Federal Aid in Wildlife Restoration Grant W-159-R1.

Garrott, R., K. Proffitt, J. Rotella, & C. Butler. **2015**. The role of disease, habitat, individual condition, and herd attributes on bighorn sheep recruitment and population dynamics. Annual Reports, Federal Aid in Wildlife Restoration Grant W-159-R1.

Garrott, R., K. Proffitt, J. Rotella, J. Berardinelli, J. Thompson, C. Butler, E. Lula, E. Flesch, & R. Lambert. **2016**. The role of disease, habitat, individual condition, and herd attributes on bighorn sheep recruitment and population dynamics. Annual Reports, Federal Aid in Wildlife Restoration Grant W-159-R1.

Garrott, R., K. Proffitt, J. Rotella, J. Berardinelli, J. Thompson, C. Butler, E. Lula, E. Flesch, & R. Lambert. **2017**. The role of disease, habitat, individual condition, and herd attributes on bighorn sheep recruitment and population dynamics. Annual Reports, Federal Aid in Wildlife Restoration Grant W-159-R1.

Garrott, R., K. Proffitt, J. Rotella, E. Lula, E. Flesch, B. Lowrey, C. Butler, J. T. Paterson, & E. Almberg. **2018**. The role of disease, habitat, individual condition, and herd attributes on bighorn sheep recruitment and population dynamics. Annual Reports, Federal Aid in Wildlife Restoration Grant W-159-R1.

Graduate Theses & Dissertations

Butler, C. **2017**. Assessing respiratory pathogen communities and demographic performance of bighorn sheep populations: a framework to develop management strategies for respiratory disease. M.S. thesis, Montana State University, Bozeman. https://scholarworks.montana.edu/xmlui/handle/1/14894.

Lowrey, B. **2018.** Spatial ecology of mountain ungulates in the northern Rocky Mountains: range expansion, habitat characteristics, niche overlap, and migratory diversity. Ph.D. dissertation. Montana State University, Bozeman. https://scholarworks.montana.edu/xmlui/handle/1/15091.

Lula, E. **2019**. Is habitat constraining bighorn sheep distribution and restoration? A case study in the greater Yellowstone ecosystem. M.S. thesis, Montana State University, Bozeman.

Flesch, E. **2020**. Population structure, gene flow, and genetic diversity of Rocky Mountain bighorn sheep informed by genomic analysis. Ph.D. dissertation. Montana State University, Bozeman.

PEER-REVIEWED PUBLICATIONS

Butler, C., W. Edwards, J. Jennings-Gaines, H. Killion, D. McWhirter, M. Wood, J. T. Paterson, K. Proffitt, E. Almberg, J. Ramsey, P. White, J. Rotella, & R. Garrott. **2017**. Assessing respiratory pathogen communities in bighorn sheep populations: sampling realities, challenges, and improvements. PLoS One. https://doi.org/10.1371/journal.pone.0180689.

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Lowrey, B., K. Proffitt, D. McWhirter, P. White, A. Courtemanch, S. Dewey, H. Miyasaki, K. Monteith, J. Mao, J. Grigg, C. Butler, E. Lula, & R. Garrott. **2019**. Characterizing population and individual migration patterns among native and restored bighorn sheep (*Ovis canadensis*). Ecology and Evolution. https://doi.org/10.1002/ece3.5435.

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DeVoe, J., B. Lowrey, K. Proffitt, & R. Garrott. **2020.** Restoration potential of bighorn sheep in a prairie region. Journal of Wildlife Management 84:1256-1267. http://doi.wiley.com/10.1002/jwmg.21922.

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Garrott, R., K. Proffitt, & J. DeVoe. **In preparation**. Maximizing inference with modest investment: monitoring strategies for bighorn sheep. Wildlife Society Bulletin.

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Butler, C., R. Garrott, & J. Rotella. **2014**. Correlates of recruitment in Montana bighorn sheep populations. Montana Chapter of The Wildlife Society Annual Conference, Bozeman, MT.

Garrott, R., J. Rotella, K. Proffitt, J. Ramsey, & C. Butler. **2014**. Montana's new statewide bighorn sheep research initiative. Montana Chapter of The Wildlife Society Annual Conference, Bozeman, MT.

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Flesch, E., J. Thomson, R. Garrott, & T. Graves. **2016**. An initial assessment of the potential of genomic analysis to help inform bighorn sheep management. Montana Chapter of The Wildlife Society Annual Conference, Missoula, MT.

Herrygers, M., J. White, J. Thomson, C. Butler, D. McWhirter, W. Edwards, K. Monteith, R. Garrott, & J. Berardinelli. **2016**. Pregnancy rates, metabolites and metabolic hormones in bighorn sheep during and after the breeding season. Montana Chapter of The Wildlife Society Annual Conference, Missoula, MT.

White, J., M. Herrygers, J. Thomson, V. Copie, B. Tripet, C. Butler, D. McWhirter, K. Monteith, R. Garrott, & J. Berardinelli. **2016**. Developing physiological profiles using nuclear magnetic resonance spectroscopy to inform bighorn sheep (Ovis canadensis) management. Montana Chapter of The Wildlife Society Annual Conference, Missoula, MT.

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