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The role of disease, habitat, individual condition, and herd attributes on bighorn sheep recruitment and population dynamics in Montana

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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 in the millions 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 pronghorn. 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 herd-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 herds as well as within each herd 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 entails using standardized methods to investigate demographic rates, body condition and nutrition, respiratory pathogens, movements, habitat use, and herd attributes across a diverse set of populations occupying a diverse set of landscapes (Figure 1). 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 strived to incorporate data from a companion MSU bighorn sheep study (Greater Yellowstone Area Mountain Ungulate Project), has worked with the MFWP Wildlife Health

Laboratory to incorporate data from additional populations captured for health monitoring purposes, and has collaborated with Wyoming Game & Fish Department (WGF) to develop sampling methods that are comparable across states. This study has and will continue to greatly benefit from inclusion of these parties in the research project. This annual report is the fifth produced by this research project. All findings reported herein should be considered preliminary, as data collection and analysis are ongoing.

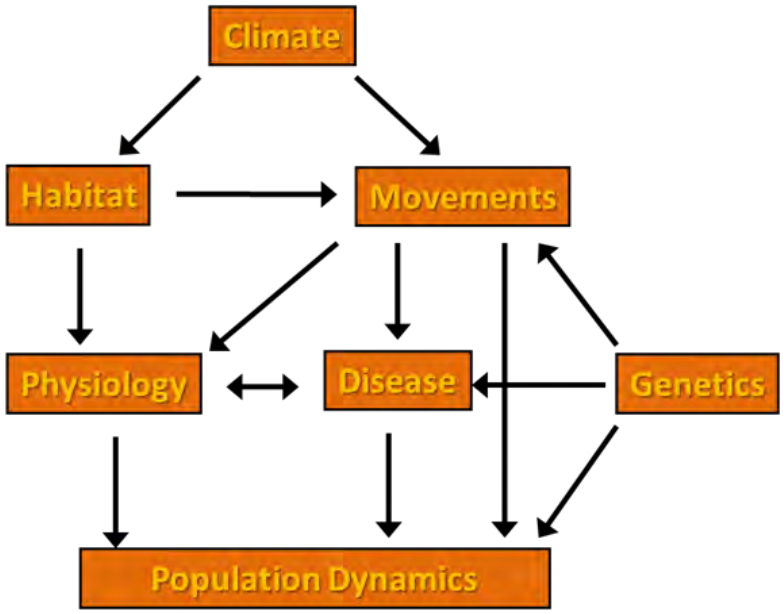


Figure 1. *Conceptual diagram of the integrated study design of the Montana bighorn sheep research program as well as the Greater Yellowstone Area Mountain Ungulate Project which is also led by the same core research team. Where appropriate the data from the both research programs will be combined to provide stronger inference.*

Locations

Research conducted under this grant is primarily focused within the range of eight distinct bighorn sheep populations across varying ecological settings in Montana (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. Populations and associated hunting districts (HD) included in the research program include Perma-Paradise (HD 124), Petty Creek/Grave Creek Range (HD 203), Lost Creek (HD 213), Taylor-Hilgard (HD 302), Castle Reef (HD 422), Fergus (HD 482), Stillwater (HD 500), and Middle Missouri Breaks/Larb Hills (HD 622). Data were also incorporated from ancillary populations to strengthen biological insights and enhance the utility of the study to inform management across all herds within the state. Ancillary Montana bighorn sheep herds 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 herds in Wyoming, Idaho, and Colorado.

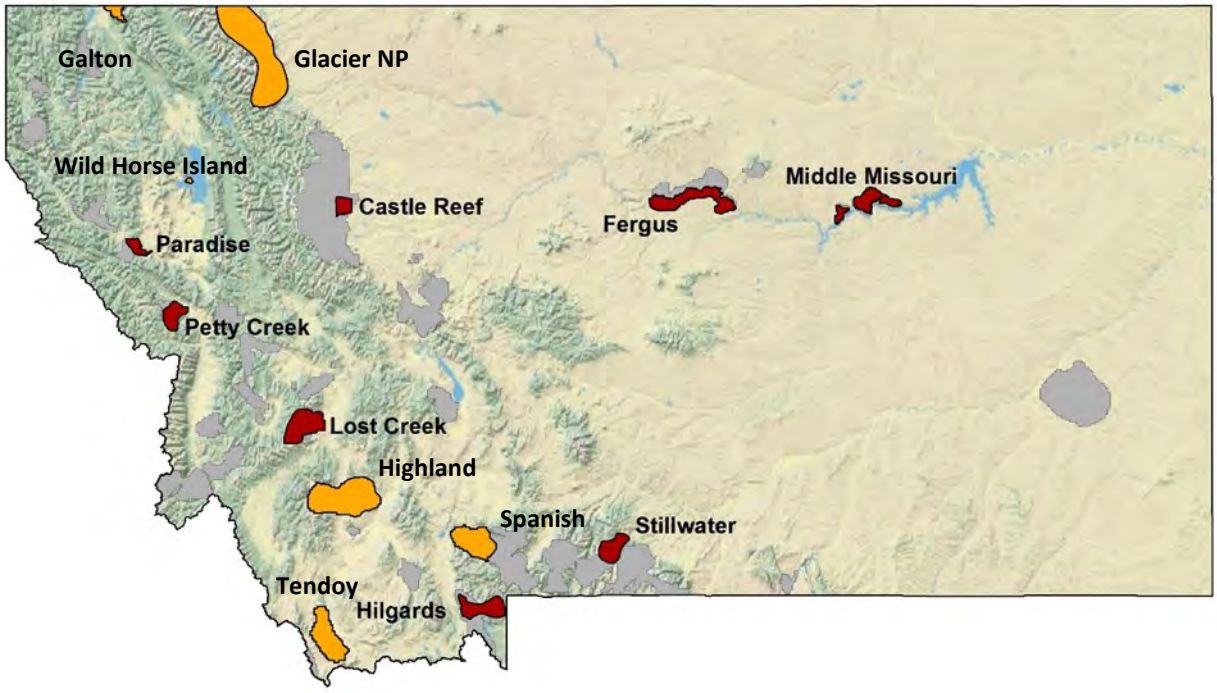


Figure 2. *Estimated distributions of bighorn sheep populations within Montana. Red polygons represent the eight Montana Bighorn Sheep Study populations. Polygons shaded in orange represent ancillary populations from which additional data are being incorporated into the statewide study to enhance biological insights. Polygons shaded in gray display ranges of the other bighorn sheep populations in Montana that are not part of this research effort.*

Study Objectives (Year 5 of 6-year study)

During the fifth year of this bighorn sheep research program, the primary objectives were:

1) Complete capture, sampling, and instrumentation of animals in all study populations
2) Assess respiratory pathogen communities and associations with demographic performance
3) Analyze GPS data to predict bighorn sheep habitat and evaluate movement strategies
4) Collect data to estimate demographic rates of each herd included in the statewide study
5) Collect and provide samples for a bighorn sheep genetics study and complete preliminary genomic analyses

<u>Objective # 1:</u> *Complete capture, sampling, and instrumentation of animals in all study populations*

1.1 Animal Capture and Sampling

1.1.1. Capture Methods

All captures were planned for winter months. Animals have been captured using three different capture 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 Montana State University Institutional Animal Care and Use Committee (Permit #2017-29).

Figure 3. *From left to right: MSU students, MFWP staff and volunteers collect samples from bighorn sheep captured via drop net on the Taylor-Hilgard January 2018, graduate student Ethan Lula collaring a chemically immobilized ewe in the Stillwater population, and Quicksilver Air Inc. staff with captured ewe in the Middle Missouri population.*

1.1.2 Sample Collection

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). 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. Additionally, weight and hind foot length (Zannése *et al.* 2006, Garel *et al.* 2010) were measured for all adult females.

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 conditions. 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 performance. Descriptions of the eight study populations, as relevant to the above characteristics, are outlined below along with sampling accomplishments in each to date.

Project sampling objectives were met in 2017, completing capture efforts for this research (Table 1).

Paradise:
This population, also known as Perma-Paradise, is located in northwestern Montana in the Northwest Montane ecoregion. The population was established with a reintroduction in 1979 and was never augmented. Currently the population numbers approximately 325 animals, 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.

Original capture and sampling objectives were fully met at Paradise in December 2014 and helicopter operations successfully met resampling goals in December 2016. Sampling is complete for this population and all GPS collar data has been recovered.

Petty Creek:
Also known as the Grave Creek Range population, this population is located in western Montana in the Northwest Montane ecoregion. The population was established with an initial reintroduction in 1968 and received a small augmentation in 1985. The population is currently estimated at approximately 140 animals and is thought to be isolated from other populations. The population typically experiences strong annual recruitment rates and it is not known to have a respiratory disease history.

Attempts to attract animals at Petty Creek to drop-net sites in Winter 2014/2015 were unsuccessful. Accordingly, a helicopter contract was solicited and in February 2016 seventeen adult females were captured and sampled, with 15 pairs of GPS/VHF collars deployed via helicopter net-gunning. The population was resampled November 2017, and 21 animals were captured via net-gunning with 9 adult females instrumented with paired GPS/VHF collars. Sampling is complete for this population . Collection of final GPS collar data will occur during the summer of 2019.

Hilgard:
Also known as the Taylor-Hilgard population, this native population is located in southwestern Montana within the Mountain Foothills ecoregion. The population has been augmented on three occasions during the late 1980s and early 1990s due to concerns over low numbers after a respiratory disease even in 1987. A second major mortality event due to disease occurred in 1997, but the population experienced a robust recovery without management intervention. The population is believed to be isolated from other bighorn sheep populations and currently numbers at least 280 animals with strong annual recruitment in recent years.

Sampling and radio-collaring of the Hilgard population continues to be enhanced beyond the original research objectives. Just prior to the initiation of this study in winter 2011/12 the MFWP biologist responsible for the Hilgard population 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 herd 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 will contribute to the research program were collected from 50 of these animals. Ten of the translocated animals were also instrumented with Lotek LifeCycle™ GPS collars purchased with funds provided by the Montana Auction License Fund, allowing us to include this newly established population in our routine research monitoring.

Additional data from two supplementary translocations in 2016 (35 animals) and 2018 (32 animals) was also incorporated into the study, and this collaboration will undoubtedly improve insights that will be obtained from the research program. Resampling goals were successfully met in 2016 and 10 adult females were fitted with Iridium satellite-linked GPS collars. These collars transmit for approximately 5 years and provide location data to researchers and managers every two days, in addition to real time mortality alerts, via satellite transmission. This provides researchers and managers with a useful tool for improving population estimates, identifying causes

of mortality and understanding herd movement. Additional animals were captured February 2017 and January 2018 to redeploy Iridium collars collected from earlier mortalities. Sampling is complete for this population and all GPS collar data has been recovered.

Lost Creek:

This population is located in 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 and historically been of moderate population size. The population has experienced two significant respiratory disease outbreaks, the most recent occurring in 2010. The population currently numbers approximately 100 animals.

In Winter 2014/2015 seven animals (6 adult females and 1 adult male) were captured and sampled using a drop-net in January, and six adult females were captured and sampled using ground-based chemical immobilization throughout March. All 12 adult females were fit with paired GPS/VHF radio-collars, however 2 of these animals died before winter 2015/2016, leaving five sets of radio-collars to be deployed over winter 2015/2016. In December 2015, five adult females were captured via ground darting and sampled, all of which were instrumented with paired GPS/VHF radio-collars. An additional adult female was captured and collared via chemical immobilization 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. Sampling is complete for this population and all GPS collar data has been recovered.

Castle Reef:

This native population is located along the Rocky Mountain Front in the Prairie Mountain Foothills ecoregion of central Montana. The population received a single small augmentation in 1944 and has experienced three respiratory disease outbreaks between 1924 and 1936, a fourth outbreak in 1984, and the most recent outbreak in 2010. The population is currently estimated at approximately 160, but is part of a metapopulation complex along the Rocky Mountain Front representing an aggregate total of approximately 600 animals. Historically recruitment has been moderate to high, but since the most recent respiratory disease even, recruitment has been very low, but appears to be returning to "normal" levels over the past two years.

Twenty animals were captured and sampled using a drop net in December 2014 and January 2015 and three additional animals were captured and sampled using ground-based chemical immobilization in March 2015. Fifteen adult females were instrumented with paired GPS/VHF radio-collars and 1 was instrumented with a VHF radio-collar. An additional three animals were captured and sampled in December 2015 and four adult females were captured in March 2016, to redeploy two radio-collars from animals that had died. In 2016 animals were captured and sampled using a combination of helicopter net gunning (10 animals) and a drop net (17 animals) and seven Iridium linked GPS collars were deployed on adult females. Early 2017 ground darting and helicopter net-gunning efforts resulted in an additional 11 animals captured and sampled, 3 of which were fitted with Iridium collars and 1 with a paired VHF GPS collar. Sampling is complete for this population and all GPS collar data has been recovered.

Fergus:

This restored population is located in 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 three augmentations between 1959 and 1961, and the most recent augmentation occurring in 1980. This population consistently experiences very high recruitment rates and is the second largest bighorn sheep population in the state, numbering approximately 550 animals. There is free exchange of animals with the population on the north side of the Missouri River, creating a metapopulation of nearly 1000 animals with no known respiratory disease outbreaks since 1980.

Capture and sampling objectives were fully met and exceeded in December 2014. Collaboration and coordination between Montana State University, MFWP, and the Hells Canyon Initiative (another collaborative bighorn sheep research program) has 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 radio-collars were deployed on adult females in the Fergus population. 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 30 animals captured for translocation as were collected from the animals captured for the research project. Helicopter net gunning efforts in December 2016 successfully met recapture sampling and instrumentation objectives. In concert with this effort, 20 of the sampled animals were translocated to the Beartooth Wildlife Management Area. Sampling is complete for this population and all GPS collar data has been recovered.

Middle Missouri/Larb Hills:

This herd is located in the plains/Missouri River Breaks area of northeastern Montana and was established with the reintroduction of 28 bighorn sheep in 1980. The herd is composed of two distinct subpopulations thought to be linked by ram movement during the rutting season. The smaller portion of the herd 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 between 1997-2001. Cause of the decline was never determined, but disease and possibly poor nutrition were suspected. Since the die-off the population has recovered and currently numbers > 350 and experiences strong annual recruitment.

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

Capture and sampling objectives for this population were fully met in December 2016. Twenty adult females were captured via helicopter net-gunning, sampled and instrumented with paired GPS/VHF radio collars. Prior to integration with this study, this population was sampled during the winter of 2015/2016 as part of FWP disease monitoring (n=19) and data were incorporated into various aspects of the statewide research. Sampling is complete for this population and all GPS collar data has been recovered.

Stillwater:

This native population is located in south-central Montana within the Southern Mountains ecoregion. The population is believed to be relatively isolated, is small (~140 animals) and has moderate recruitment. There are no known respiratory events in the population in recent times, but the population has been augmented twice (1970, 1984).

Ground-based chemical immobilization was used throughout winter 2014/2015 to capture and sample 16 adult females, 15 of which were fit with paired GPS/VHF radio-collars. In order to more closely reach the capture and sampling objective and redeploy a pair of GPS/VHF radio-collars, which were originally deployed on an animal that died, three additional adult females were captured and sampled using chemical immobilization 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 herd to capture and sample an additional 15 animals with 5 adult ewes fitted with paired GPS/VHF collars. From

November, 2016 to March, 2017, 11 animals were sampled via ground darting, and all 5 pairs of collars successfully deployed. Two of these animals were subsequently recaptured to reprogram faulty collars, and an additional animal was captured Jan, 2018 for genetic sampling. Sampling is complete for this population and all

Table 1. *Sampling accomplishments to date in each of the eight study populations. Increased sampling in the Hilgard and Fergus populations resulted from coordination with MFWP during translocation captures. The increased number of radio-collars deployed in the Hilgard population also resulted from coordination with MFWP and the increased number of deployed radio-collars in the Fergus population resulted from collaboration with the Hell's Canyon Initiative.*

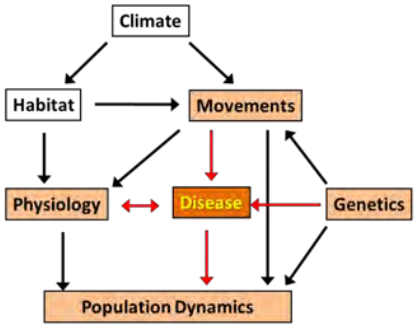
	BIGHORN SHEEP SAMPLED						RADIO-COLLARED EWES	
	2013/ 2014	2014/ 2015	2015/ 2016	2016/ 2017	2017/ 2018	TOTAL	TOTAL COLLARED	CURRENTLY ON AIR
Paradise		30	0	30	0	**60**	25	13
Petty Creek		0	17	0	21	**38**	24	20
Lost Creek		13	6	24	0	**43**	27	21
Hilgard *	29	50	35	31	32	**177**	32	20
Castle Reef		23	7	38	0	**68**	29	16
Fergus		60	0	30	0	**90**	40	31
Stillwater		16	3	11	1	**31**	21	15
Middle Missouri			19	20	0	**39**	20	11
TOTAL	**29**	**192**	**87**	**184**	**54**	**546**	**218**	**147**

* Collar total does not include 5 rams captured 2012/2013 or 27 ewes collared as part of FWP translocations

Adrian Sanchez-Gonzalez

Figure 4. *Bighorn sheep captured beneath drop net during the Hilgard sampling and translocation capture, January 2018.*

Objective # 2: *Assess variation in respiratory pathogen communities and associations with 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 (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 of poor recruitment to decades of poor recruitment (Plowright et al., 2013). In numerous cases, local populations have gone extinct or are depopulated after many years of chronically poor performance following respiratory disease epizootics (MFWP, 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 as 98% of bighorn sheep commingled with healthy domestic sheep in captive studies have developed respiratory disease and died (Besser et al., 2013). 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 and frequent close interactions 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) *Pasteureallaceae* organisms, including strains of *Mannheimia haemolytica* and *Bibersteinia trehalosi,* which cause respiratory disease in captive bighorn sheep, but not in domestic sheep (Bavananthasivam et al., 2012; Dassanayake et al., 2013; Dassanayake et al., 2010; Dassanayake et al., 2009; Lawrence et al., 2010). 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., 2012a, 2012b, 2008) and that transmission of *Mycoplasma 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 (Edwards et al., 2010; Festa-Bianchet, 1988; Ryder et al., 1992) 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 (Besser et al., 2013; Miller et al., 2012, 2011; 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 two hypothesized mechanisms are very different.

Over the first four years of the 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 across Montana. 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 funding of the Montana bighorn project we extended our collaborations to include personnel associated with Montana Fish Wildlife and Parks' 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 herds throughout Montana and Wyoming.

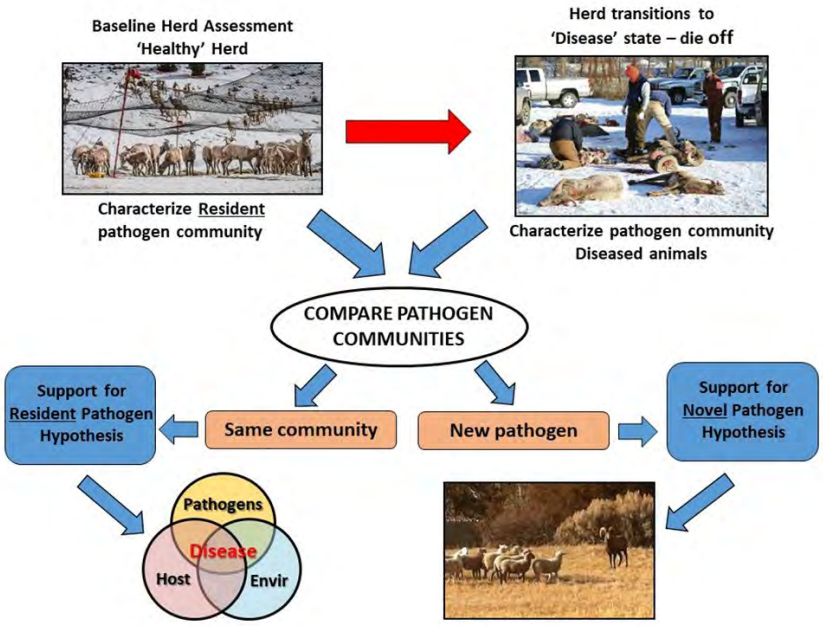


Figure 5. *A conceptual diagram of how rigorous assessments of the resident respiratory pathogen communities in bighorn sheep herds can help provide insight into competing ideas regarding the role of resident pathogens in epizootics (die-offs) as opposed to novel pathogens being introduced (spillover event) via interactions with*

The first 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 5). The second 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.

2.1 Assessing Pathogen Detection Probability and Insights for Sampling

A total of 2093 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). 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 (Safaee et al. 2006, Wild and Miller 1994). Nevertheless, this is a limitation whenever samples must be shipped to a laboratory for culture tests.

2) The PCR-based diagnostic 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., Bibersteinia trehalosi,* and *Pasteurella multocida* of 95%, 96%, and 83%, respectively. Estimated detection probability for *Mannheimia 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 *Mycoplasma 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 *Mycoplasma ovipneumoniae* on two 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% (0.27 x 0.27) would not test positive either time, and ~40% would test positive for one of the two 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. 2012, 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 hosted.

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 two or three tonsil swabs from each live-sampled animal.

3) The presence of *Pasteurella 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 herd.

5) The use of a single nasal swab to assess presence of *Mycoplasma 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 herd (given an adequate number of animals from the herd 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 herd 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 herd 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 herd, but remained undetected.

9) If prevalence of a pathogen in a sampled herd 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 herd- and individual-level evaluations of resident pathogen communities and pathogen prevalence estimates.

2.2 Characterizing Respiratory Pathogen Communities and Demographic Attributes of Diverse Bighorn Sheep Populations

Coordinated efforts were used 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, assess whether presence of any specific pathogen or combination of pathogens was associated with differences in recruitment, and determine the extent to which populations hosting different respiratory pathogens maintained satisfactory recruitment rates. Little or no association between demographic performance and presence of suspected respiratory pathogens was hypothesized. Given the long history of domestic sheep grazing in the two states and the translocation of bighorn sheep to establish new populations and augment struggling populations, it was 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 of each year from 2012–2017 (Figure 6). Four of the five 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 one Wyoming study population and two 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, despite concerted efforts to prevent contact between the species, 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 have faced and will continue to confront when attempting to craft management strategies to reduce the occurrence of respiratory disease die-offs and advance bighorn sheep restoration.

Respiratory Pathogen Communities and Recruitment

Mean lamb:ewe ratios of individual populations where any specific pathogen was detected ranged from <0.20 to > 040. For each pathogen species, there were at least four 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 7.

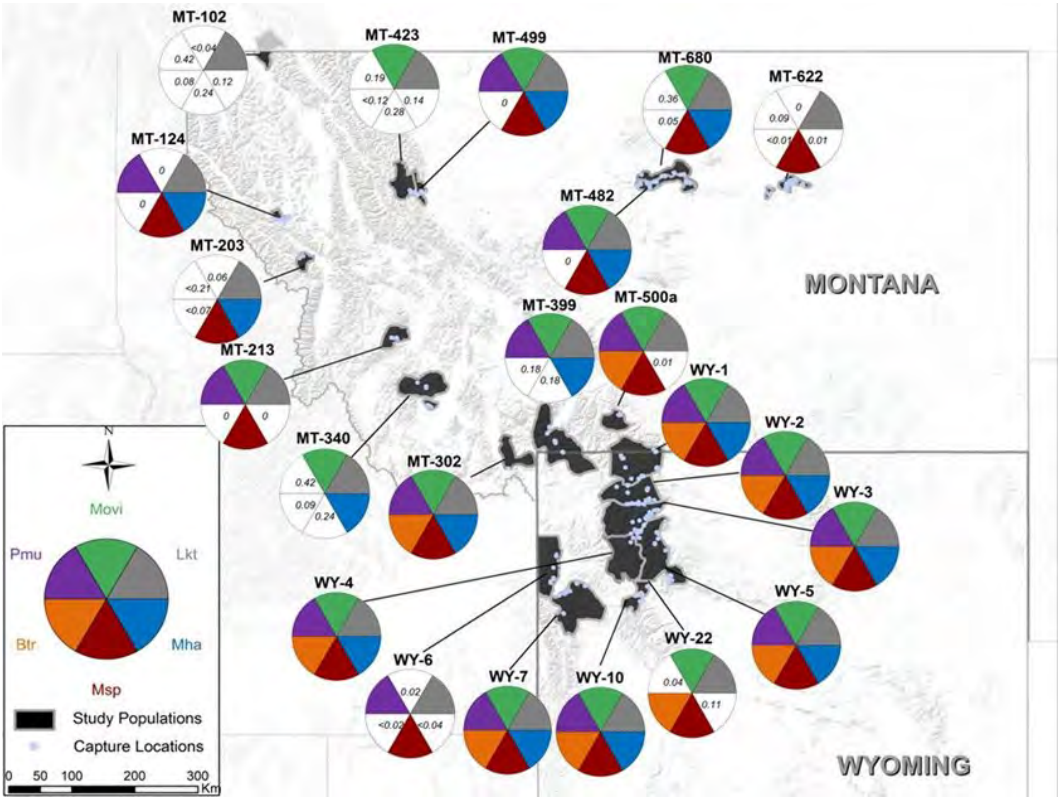


Figure 6. *Map of 22 bighorn sheep study populations and detected respiratory pathogen communities. All sections of the pie-charts are fixed to equal size and represent whether the respective pathogens were detected in the study population. The key for pathogen abbreviations are as follows: Movi= Mycoplasma 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.*

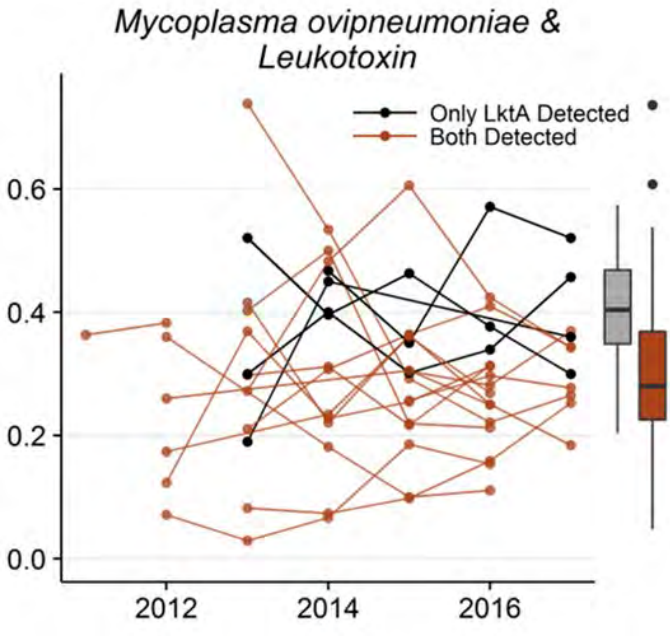


Figure 7. *Lamb:ewe ratios of 14 bighorn sheep populations in Montana and Wyoming where both M. ovipneumoniae and leukotoxigenic Pasteurellaceae were detected and populations where only leukotoxigenic Pasteurellaceae was detected.*

Although both *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae* were detected in most (n=16) bighorn sheep 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 six 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 successfully managed 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 *Pasteurellacea.* 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. And finally, 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 5).

2.3 Development of a Tool to Aid Wildlife Management Agencies in Interpreting Respiratory Pathogen Test Results

The primary data collected by managers when evaluating herds regarding translocation and augmentation decisions, as well as evaluating risk of disease die-offs, is the capture and 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.

The development of 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, however, provides 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 herd. Given positive test results, the models estimate the true prevalence of a pathogen in a herd and provide appropriate confidence limits. 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 6) and is now being used by FWP's Wildlife Health Lab personnel to interpret the pathogen test results for the ongoing health assessments of all bighorn sheep populations in the state (Figure 8).

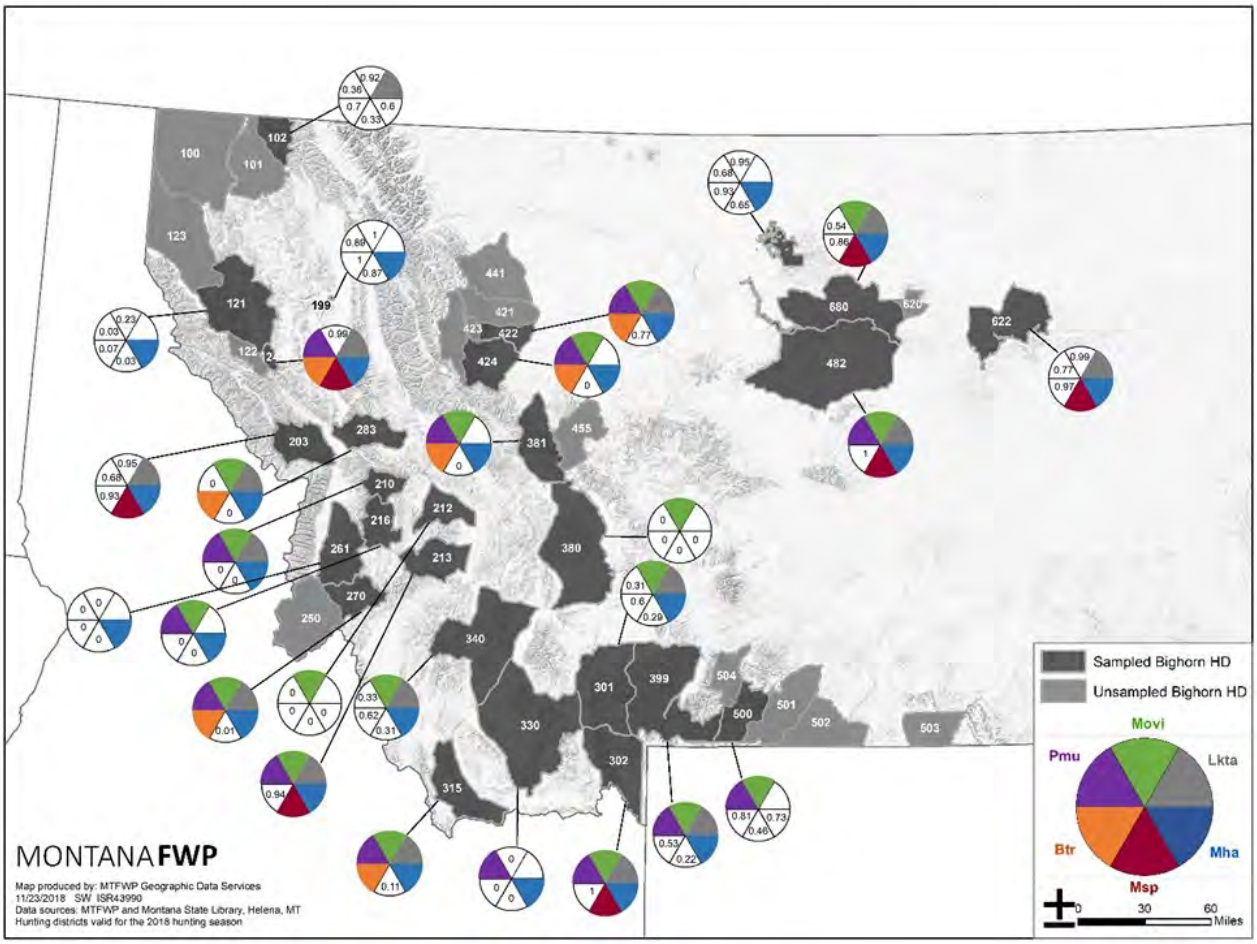


Figure 8. *Map of 27 bighorn sheep study populations in Montana that have been sampled to assess presence of resident respiratory pathogen communities. All sections of the pie-charts are fixed to equal size and represent whether the respective pathogens were detected in the study population. The key 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. The large number of unfilled pie sections with large probabilities illustrates the difficulty of sampling herds intensively enough, given the limitations of current commercially available diagnostic protocols, to have confidence in assessments of resident pathogen communities.*

The analytical procedure to accomplish this rigorous interpretation of pathogen testing results is not trivial and while we intend to make the code publicly available, in all likelihood few managers and wildlife health professionals would have the expertise to execute the analyses. Thus, in order to assure that the broader community can benefit from the results of our work, we have developed an easy-to-use web-based tool to assist wildlife health professionals in the rigorous interpretation of herd-level respiratory pathogen assessments that specifically accounts for imperfect detection of diagnostic protocols as well as the intensity of the sampling performed in each herd (Figure 9). 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 (Figure 10), or prevalence in the event of as least one positive test (Figure 11). 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 assess the probability of pathogen presence/absence in wild populations. The web-app is currently in beta testing and a manuscript describing the app is being prepared with plans to make the app publicly available at the time the manuscript is submitted for publication.

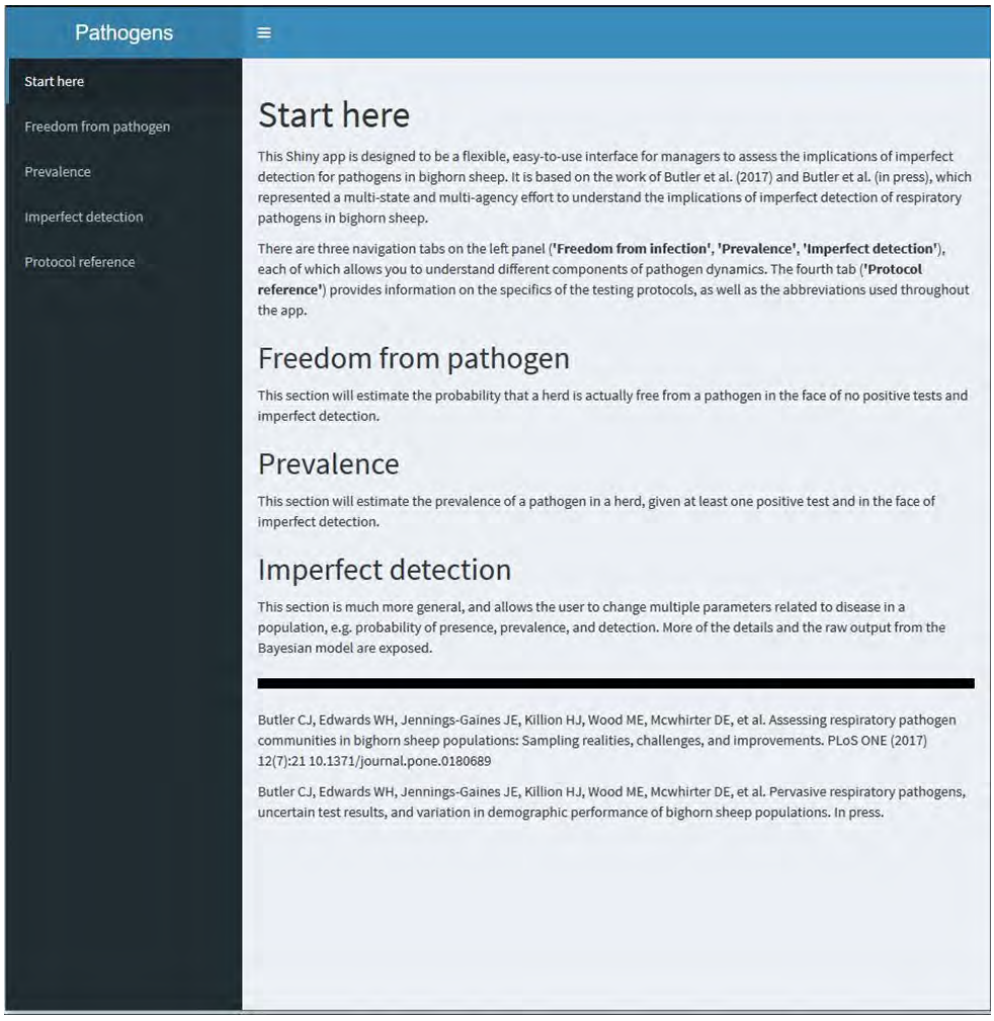


Figure 9. *A image of the opening screen of a web-based software application that provides an easy-to-use interface for managers to enter sampling information and pathogen testing results and obtain a rigorous analysis of the results that incorporates the estimates of detection probability of common pathogen diagnostic protocols as well as the number of animals sampled from a population.*

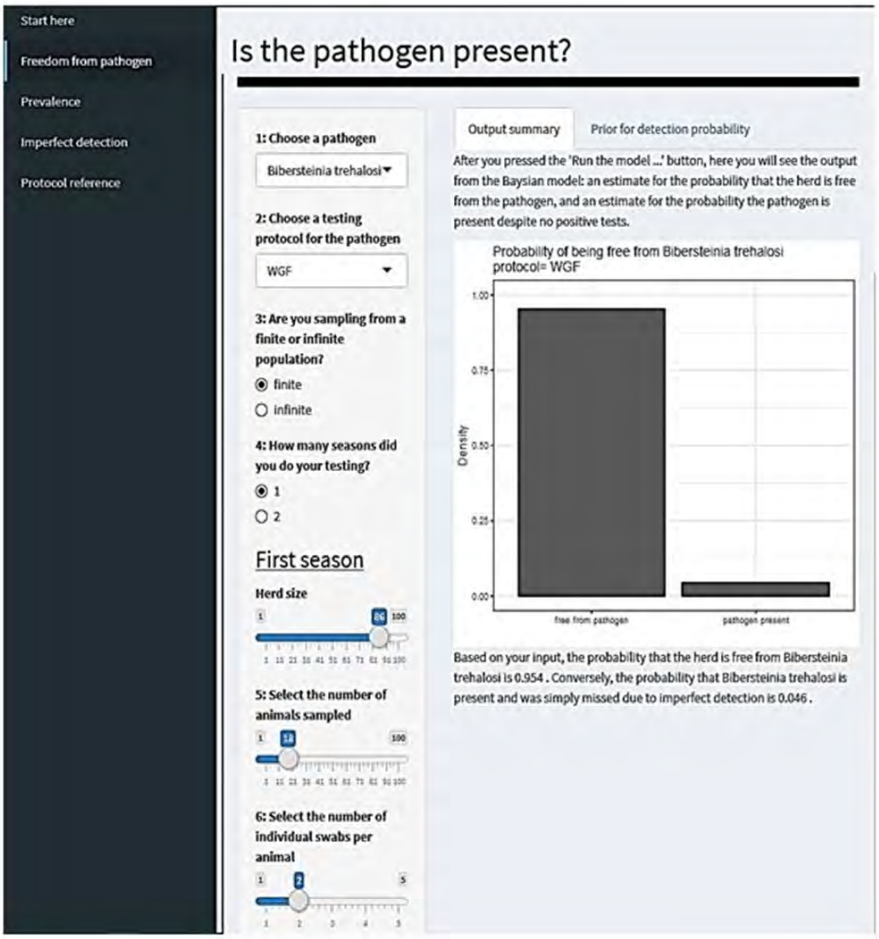


Figure 10. *An image of the screen that prompts the user to enter the sampling information for a pathogen that was not detected and the results of estimating the probability that the pathogen is present in the population.*

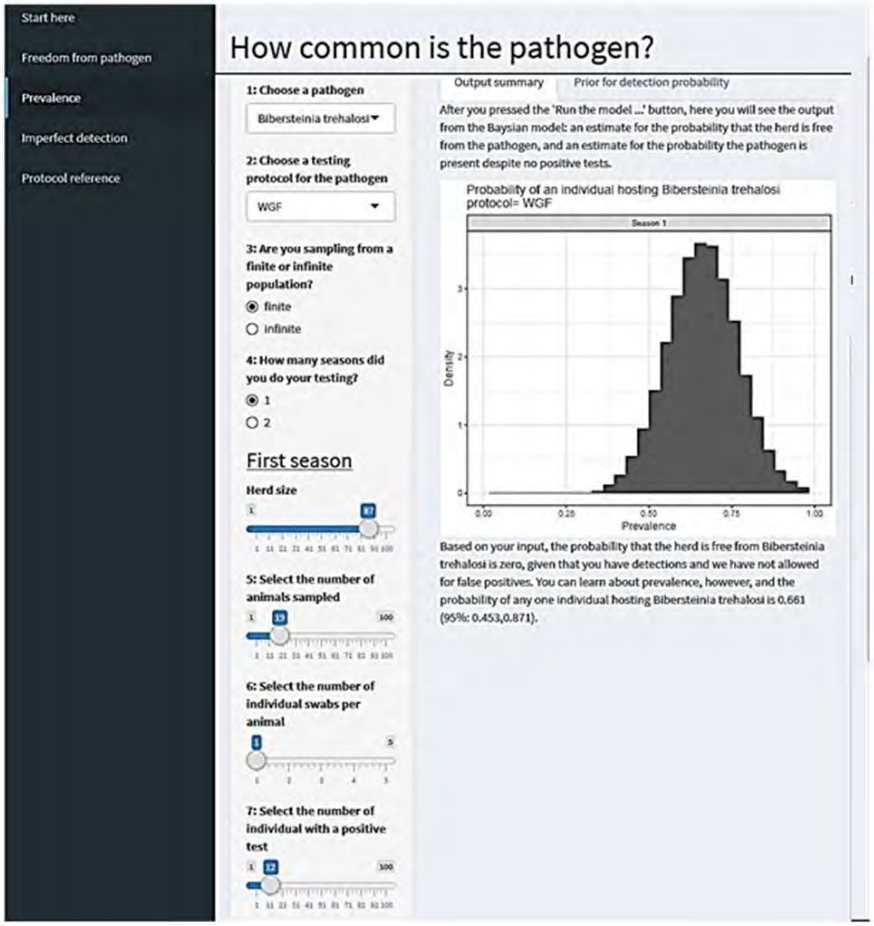


Figure 11. *An image of the screen that prompts the user to enter the sampling information and test results for a pathogen that was detected and the results of estimating the prevalence of the pathogen in the population.*

2.4 Assessing Temporal Variation in Respiratory Pathogen Communities

Evaluating the presence and prevalence of respiratory pathogens in bighorn sheep herds requires considerable effort and expense to coordinate the logistics of capture operations, contract with wildlife helicopter capture companies, and pay for diagnostics tests. Cost can often exceed $500/animal, hence, sampling bighorn herds is infrequent and the results of a testing event must be used for extended periods of time to inform management decisions before a herd may be retested. Given these realities, it would be useful for managers and wildlife health professionals to have an understanding of how dynamic pathogen test results may be from one year to the next. The literature, however, provides little insight on the apparent temporal dynamics of pathogen communities in bighorn sheep as herds are seldom intensively sampled in consecutive years.

The capture and sampling regime for the statewide bighorn sheep studies dictated a goal of capturing approximately 30 animals the year the herd entered the study and capturing and sampling another 30 animals two years later to bolster the number of radio collared animals for monitoring and repeat pathogen testing to evaluate temporal variation in pathogen presence and prevalence. In addition to the scheduled research captures for the Taylor-Hilgard herd this population was captured for three additional years for an intra-mountain translocation experiment aimed at expanding the distribution of bighorn sheep in the Madison Mountain range. All animals captured during the five consecutive years were sampled for respiratory pathogens using our research protocol, thus, providing a unique opportunity to evaluate temporal variation in bighorn respiratory pathogen communities.

All five bacterial pathogens were detected in each of the five years of sampling but the estimated prevalence (proportion of population) of each pathogen was markedly variable among years with coefficients of variation for each pathogen ranging from 33% to 69% (Figure 13). Estimated prevalence of *Mycoplasma ovipneumoniae* ranged from 0.09 to 0.94, *Bibersteinia trehalosies* prevalence ranged from 0.04 to 0.10, *Mannheimia haemolytica/glucosida* prevalence ranged from 0.19 to 0.87, *Mannheimia spp.*prevalence ranged from 0.18 to 0.62, and estimated prevalence of *Pasteurella multocida* ranged from 0.17 to 0.83.Confidence intervals for most prevalence estimates were relatively narrow due to the fact that generally >10% of the estimated population was sampled in each of the five years.

The five years of intensive sampling of this herd for respiratory pathogens was also coupled with a number of demographic monitoring surveys that included evaluation of pregnancy rates using serum hormone analyses, known-fate survival estimates derived from monitoring of radio collared adult ewes, and replicate ground-based mark-resight surveys that provided data for Lincoln-Petersen population estimates and lamb:ewe ratios. These data will provide an opportunity in the final year of this project to explore possible correlations between annual variation in estimate prevalence of the various respiratory pathogens and annual variation in demographic attributes.

Figure 12. *MFWP disease ecologist Emily Almberg collects a throat swab from a captured bighorn on the Taylor-Hilgard population 2018.*

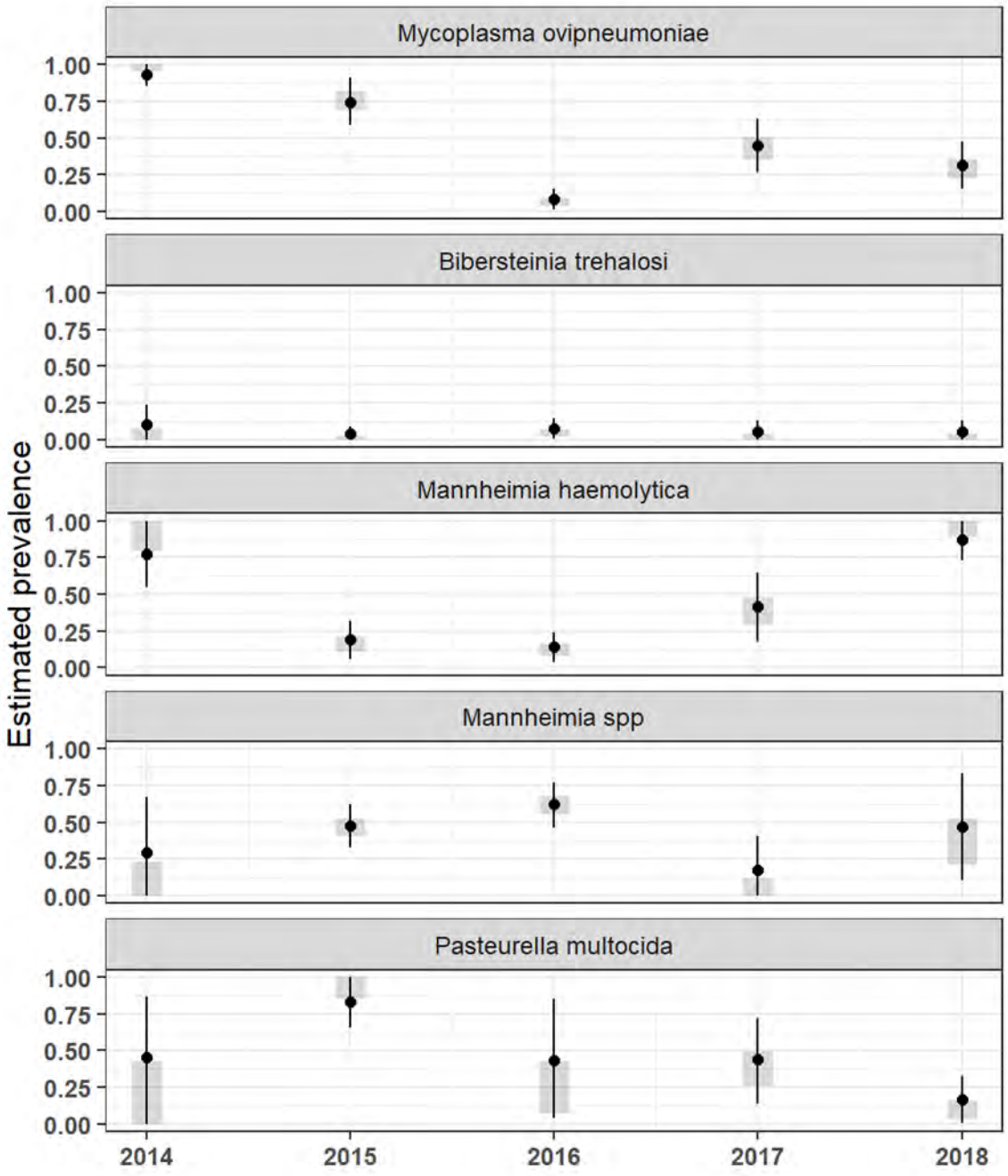
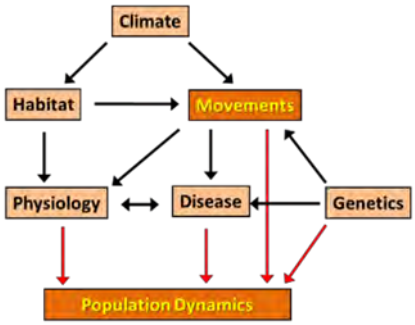


Figure 13. *Temporal variation in estimated prevalence of respiratory pathogens in the Taylor-Hilgard bighorn sheep population over 5 consecutive years. For each estimate, the black dot represents the mean, the thin line represents the 90% highest posterior density interval (HPDI), and the light gray box represents the 50% HPDI.*

Objective #3:

Analyze GPS data to predict bighorn sheep habitat and evaluate movement strategies.



How animals move across a landscape and utilize components of their habitat can have direct influence on vital rates and demographic performance (Manley et al. 2004). Advances in radio telemetry using global positioning system (GPS) now allow for the collection of temporally and spatially fine scale location data that greatly enhance ecological insights. By incorporating GPS radio collar data from multiple bighorn sheep populations, we can not only accurately describe the movement and habitat selection of specific populations, but also compare these attributes among populations to potentially identify environmental factors associated with bighorn sheep demographic performance.

4.1 Collection of GPS Data

Initial sampling objectives for each study population included the instrumentation of 15 adult female bighorn sheep with paired GPS and VHF radio collars equipped with mortality sensors (Models: TGW4400 [GPS] and MOD400 [VHF], Telonics Inc, Mesa, Arizona). Subsequent 2015/2016 resampling objectives included the instrumentation of an additional subset of 10 females per study population. The GPS collars were programmed to transmit a VHF signal and record location information every 4-h for a period of approximately 21 months, storing the data internally. These collars were further equipped with a CR-2A release mechanism, programmed to release the collar from the animal on a scheduled date. At the time of release, the paired VHF collar began transmitting, so as to continue survival monitoring for an additional 3-5 years. Field crews, using telemetry navigated to the dropped GPS collar and retrieve the stored data for analysis.

A total of 197 adult female bighorn sheep have been instrumented with GPS radio collars (Table 2). Of these, data has been retrieved from 161, and 6 have either failed or are considered unrecoverable. Eight remaining collars are scheduled to release in the Petty Creek population during the summer of 2019 concluding all GPS data collection. Twenty-two animals in the Castle Reef and Hilgard populations were instrumented with Iridium satellite linked radio collars and their location and survival continue to be monitored in real time.

Figure 14. *MFWP area biologist Bruce Sterling with a GPS collar recovered from the Perma-Paradise herd during the summer of 2018.*

Table 2. *The number and current status of GPS collars deployed among study populations since 2014.*

Study Population	Total	Recovered	Failed/ Unrecov-erable	Iridium	Remaining
Castle Reef	28	17	1	10	0
Fergus	25	25			0
Lost Creek	27	26	1		0
Middle Missouri	20	19	1		0
Paradise	25	24	1		0
Petty Creek	24	15	1		8
Hilgard	27	15		12	0
Stillwater	21	20	1		0
TOTAL	**197**	**161**	**6**	**22**	**8**

Among all study populations, a total of 596,647 GPS locations have been collected, averaging about 69,000 locations per population (excluding Middle Missouri). Location data were censored for imprecise GPS locations by removing failed fixes and locations with poor spatial accuracy (Table 3). All study populations experienced good overall GPS precision, specified as being ≥75% successful fixes. The Stillwater population experienced the highest proportion of censored locations (12.6%). After censoring, a total of 568,310 locations are available for spatial analysis. GPS data from the 8 unrecovered collars will be incorporated in future analyses, and used to validate initial results.

Table 3. *Summary of collected bighorn sheep GPS locations for the eight study populations.*

Study Population	Number of Individuals	GPS Location Data			GPS Fix Success
		Good	Censored	Total	
Castle Reef	27	67,944	3,884	71,828	94.6
Fergus	25	95,366	2,880	98,246	97.1
Lost Creek	26	84,579	2,120	86,699	97.6
Middle Missouri	18	55,273	1,467	56,740	97.4
Paradise	24	83,466	3,751	87,217	95.6
Petty Creek	14	48,316	3,289	51,605	93.6
Hilgard	27	68,377	2,694	71,071	96.2
Stillwater	20	64,989	8,252	73,241	88.7
TOTAL	**181**	**568,310**	**28,337**	**596,647**	

Figure 10. *GPS locations displayed for the four non- migratory study populations.*

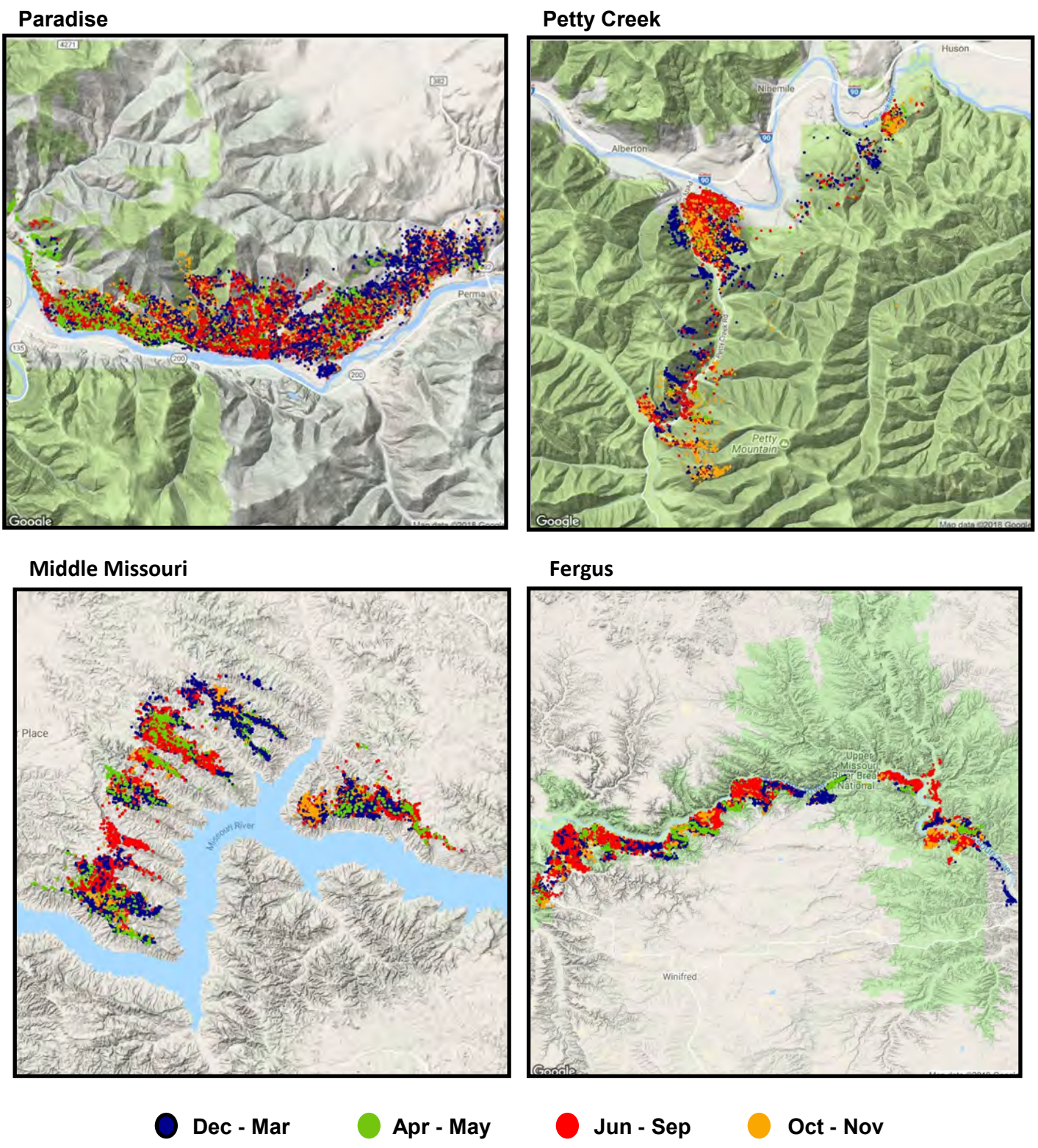
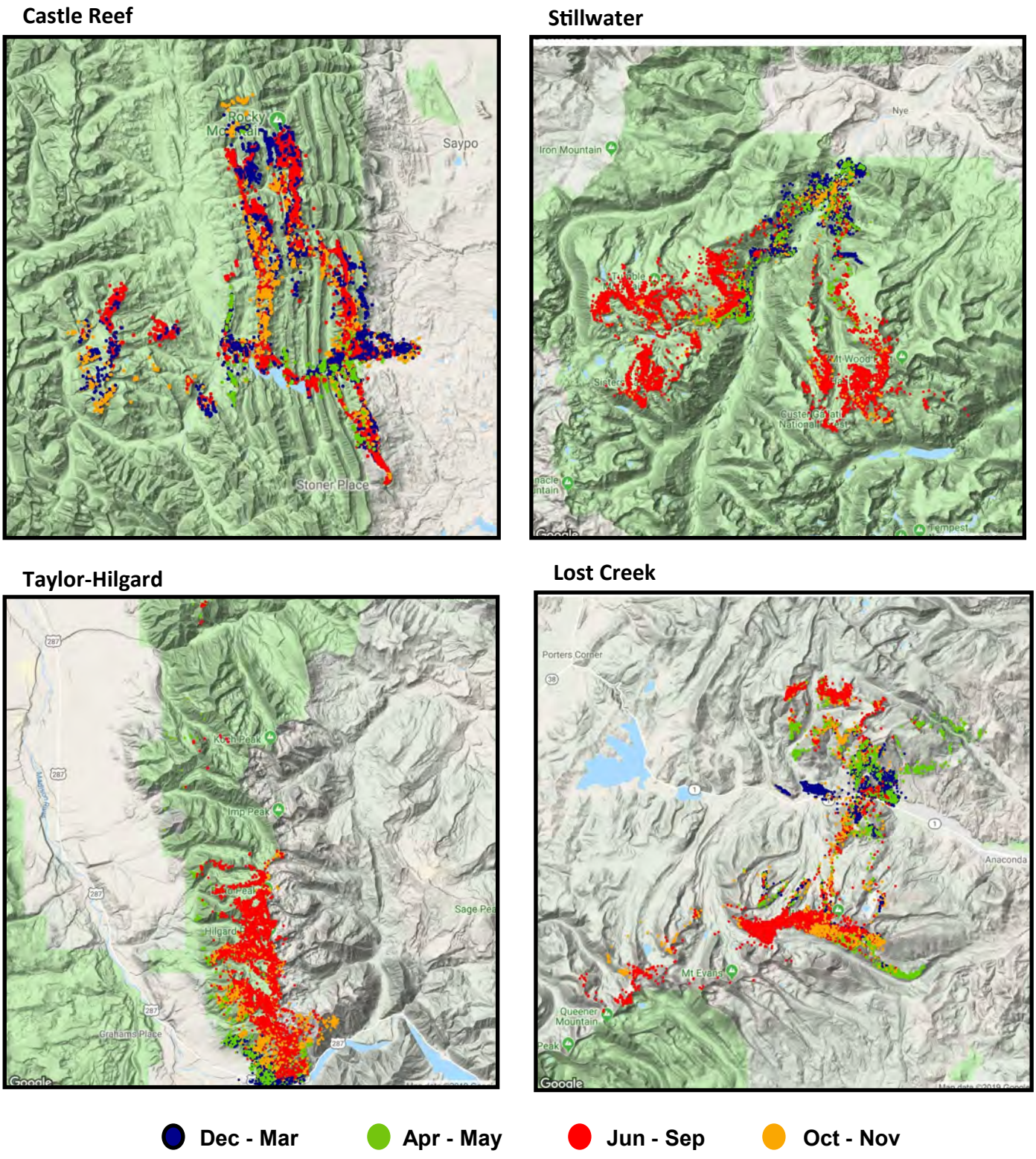


Figure 11. *GPS locations displayed for the four migratory study populations.*



4.2 Madison Range Resource Selection

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 herds with discrete seasonal ranges (Demarchi et al. 2000, Singer et al. 2000*a*). 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 1988*b*, Enk et al 2001).

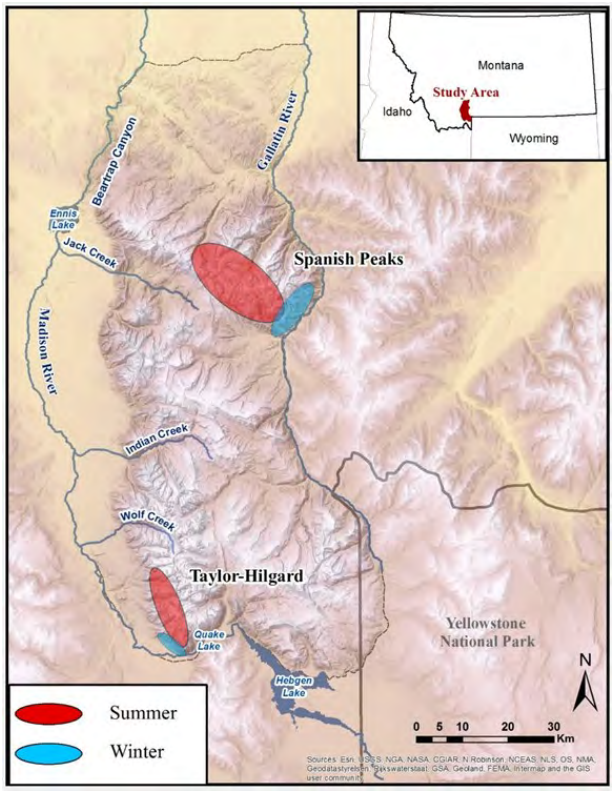


Figure 17. *Madison Mountains study area with seasonal ranges for the Spanish Peaks and Taylor-Hilgard bighorn sheep populations displayed.*

The Madison Mountain Range (Figure 17), is located along the western edge of the Greater Yellowstone Ecosystem and although historical accounts suggest that bighorn sheep occurred throughout the range, only two populations are recognized by management agencies today, the Taylor-Hilgard and the Spanish Peaks (MFWP 2010). Both populations are considered isolated from each other and have seen little expansion into surrounding habitat during their eight-decade management history (MFWP 2013). In particular the Taylor-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

Table 4. *Covariate descriptions and hypothesized seasonal relationships with the relative probability of use for bighorn sheep in the Taylor-Hilgard population Montana, 2013-2016.*

Covariate	Description	Functional form	Spatial Resolution (grain)	Predicted Effect (summer, winter)
ELV	Elevation (m)	Ln, Sq	30	pos, neg
SLP	Slope (°)	Ln, Sq	30, 100, 500, 1,000	pos, pos
DST	Distance to steep terrain (m)	Ln, Ps	30	neg, neg
CRV	Landscape curvature	Ln, Sq	30	pos, pos
SLPvar	Slope variance	Ln, Ps	30, 100, 500, 1,000	pos, pos
VRM	Vector ruggedness measure	Ln, Ps	30, 100, 500, 1,000	pos,pos
ASPC	The inverse cosine of aspect -35°	Ln	30	neg, pos
CANCO	Canopy cover (%)	Ln	30, 100, 500, 1,000	neg, neg
NDVItin	Time-integrated NDVI: Mean daily integrated NDVI above baseline for duration of growing season (2014-2016)	Ln	30, 100, 500, 1,000	pos,pos
NDVIamp	NDVI_amplitude: The mean difference between max and baseline NDVI at beginning of growing season (2014-2016)	Ln	250, 500, 1,000	pos, pos
SNOW	Average proportion of winter covered in snow (2013-2015)	Ln, Ps	500	na, neg

Note: Ln, linear; Sq, quadratic; Ps, Psuedothroeshold (natural log).

limiting the distributions of bighorn sheep within the Madison Range and, if not, evaluate the potential for restoration.

Using GPS data from 15 bighorn sheep captured on Taylor-Hilgard winter range in 2013, and full suite of habitat covariates expected to influence bighorn sheep habitat selection (Table 4), 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 said resources within a defined extent, thus linking a species to a set of habitat characteristics (Boyce and McDonald 2002, Manley et al. 2002). For our analysis, we defined the extent of availability as that of the Taylor-Hilgard annual range (Figure 18).

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 (linear, quadratic, pseudothreshold) and spatial grains (Meyer and Thuiller 2006, Laforge et al. 2015) for appropriate covariates and compared similar landscape covariates (Table 4) bringing forward the most explanatory covariates in our top summer and winter models.

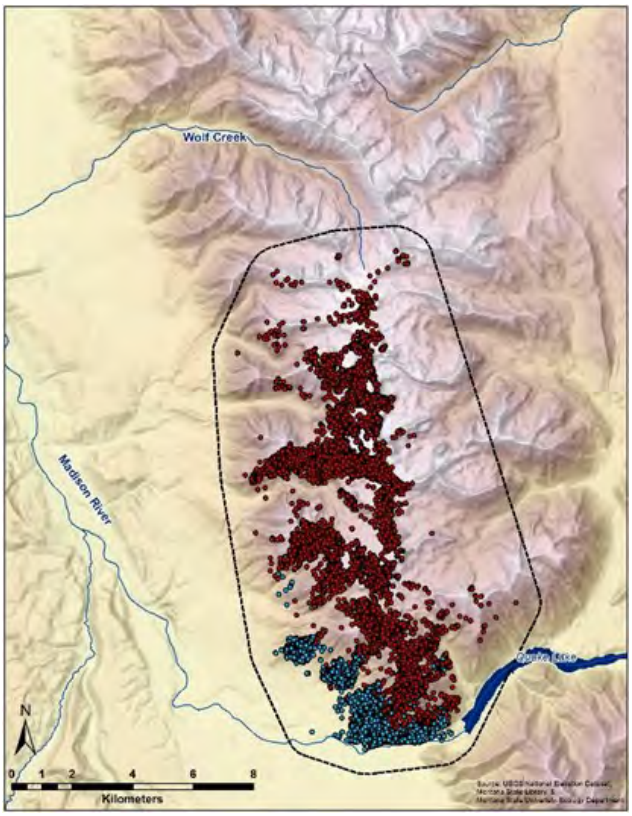


Figure 18. *The Taylor-Hilgard annual range (black line) delineated as our RSF extent of availability and encompassing summer (red) and winter (blue) GPS locations for bighorn sheep in the Taylor-Hilgard population 2013-2016*

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

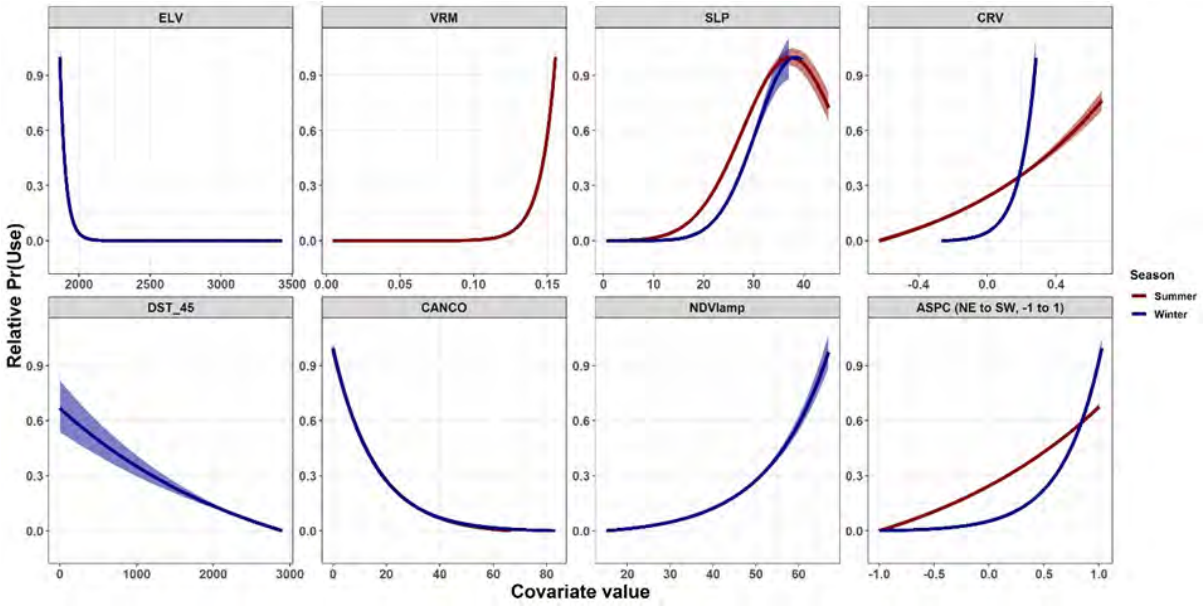


Figure 19. *Predictions of the relative probability of use for the top covariates in the winter and summer RS models. Predictions were generated across the observed covariate range with all other covariates held at their mean value.*

During the summer, bighorn sheep selected for rugged terrain (VRM), steep slopes, convex curvatures (i.e. ridgelines), decreased canopy cover and southwestern aspects (Figure 19). 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 19).

Predicted winter habitat largely occurred within the Madison Valley, along the low-elevation, southwest facing aspects associated with reduced snow cover (Figure 20). 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 three contiguous patches along high elevation ridgelines (Figure 20). We validated our results using *k*-fold validation (Boyce et al. 2002) and with additional GPS data from bighorns collared during the 2016 Taylor-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 Taylor-Hilgard annual range as well as in the Spanish Peaks (Figure 20).

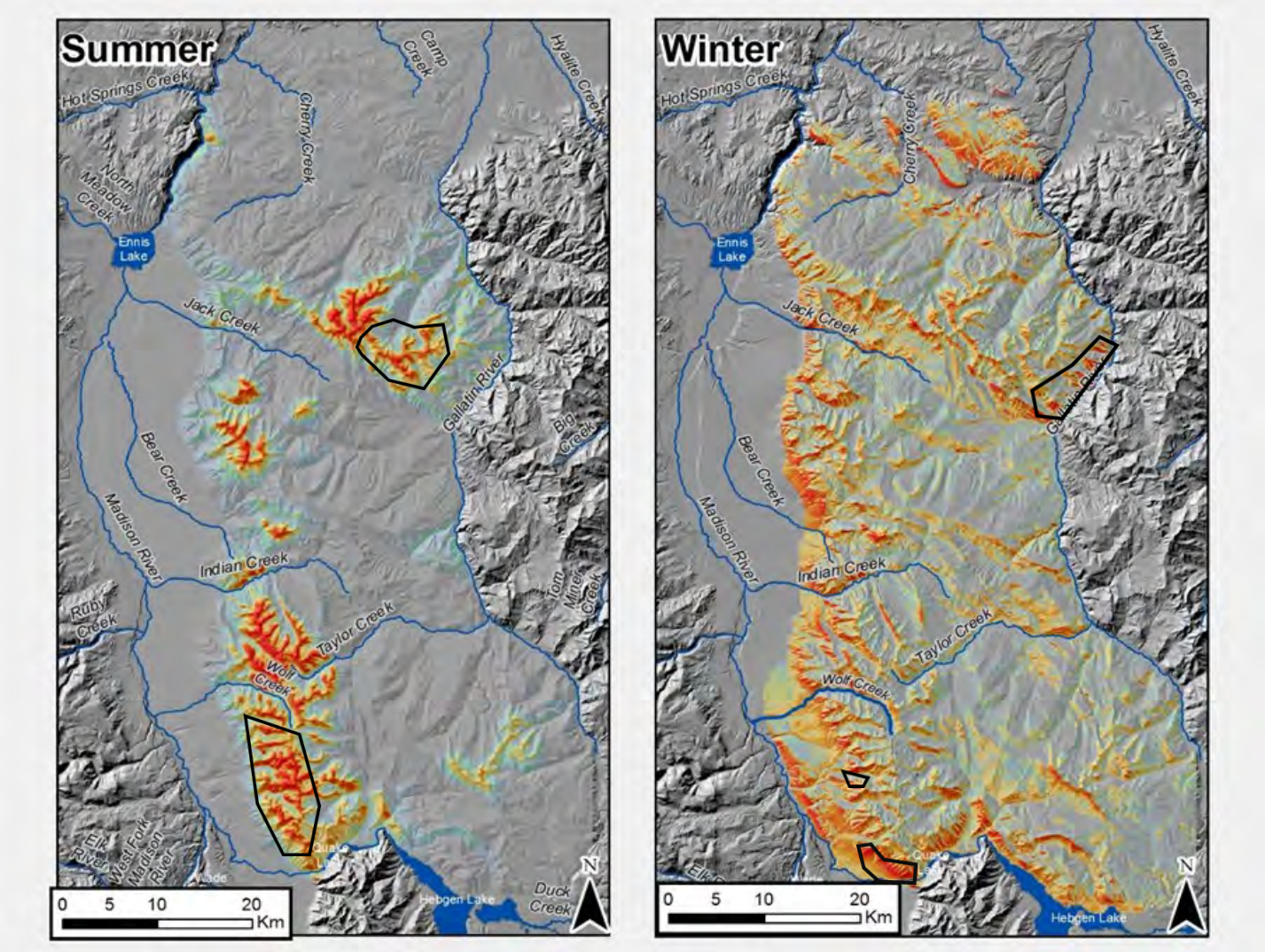


Figure 20 . *Seasonal RS model results extrapolated to the Madison Range study area where RSF scores were classified into 10 equal-area bins based on the seasonal predictions within the Taylor-Hilgard bighorn sheep population annual range. Cool colors represent low relative RSF probabilities while warm colors depict higher RSF values. Black polygons represent known distributions of extant bighorn sheep populations.*

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 Taylor-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 RS model to two measures of abundance within the Taylor-Hilgard population (Boyce and McDonald 1999, Boyce and Waller 2003). We utilized the Taylor-Hilgard population management objective (N_{min}=120) and 5 yr maximum observed population count (2013 – 2018, N_{max}= 255) to estimate densities of bighorn sheep on Taylor-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 Taylor-Hilgard population. Our results indicate that winter habitat within the Madison Range may be capable of supporting between 780 and 1,730 bighorn sheep; between two to four 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 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 GYE populations, consisting of localized wintering herds 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 herds and that historic extirpation resulted in an overall reduction of the broader geographic landscape known to the remaining herds. Once extirpated, wintering populations are unlikely to be naturally reestablished by neighboring herds 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 herds of bighorn sheep within the Madison Range (i.e. Taylor-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).

4.2.1 Management implications

Our results suggest significant potential for bighorn sheep restoration within the Madison Range, describing a population structure with the potential to dramatically increase abundances and distributions of bighorn sheep within the Range. Our model results provide managers with a useful tool for not only identifying future translocation sites that maximize the probability of herd establishment, but also provide 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 2017, Jesmer 2018), it may be useful to consider a progressive series of short-range translocations into adjacent winter habitat using animals from either the Taylor-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.

4.3 Evaluate migratory patterns of bighorn sheep populations

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 behaviors (i.e. life history traits) of anadromous fishes. 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, demographic) differences between resident and migratory components of partially migratory species (i.e., Hebblewhite and Merrill 2009, Middleton *et al.* 2013, Rolandsen et al. 2016) with will little focus on migratory diversity.

We used GPS location data collected from 209 female bighorn sheep (Ovis canadensis) 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 included seven study populations in Montana as well as seven populations in Wyoming, two populations in Idaho, and two populations in Colorado (Fig 21; Table 5). We characterized seasonal migrations between summer and winter core ranges, defined using the location data collected from 15-Jan to 28 -Feb and 15-Jul to 15-Aug for winter and summer, respectively. 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

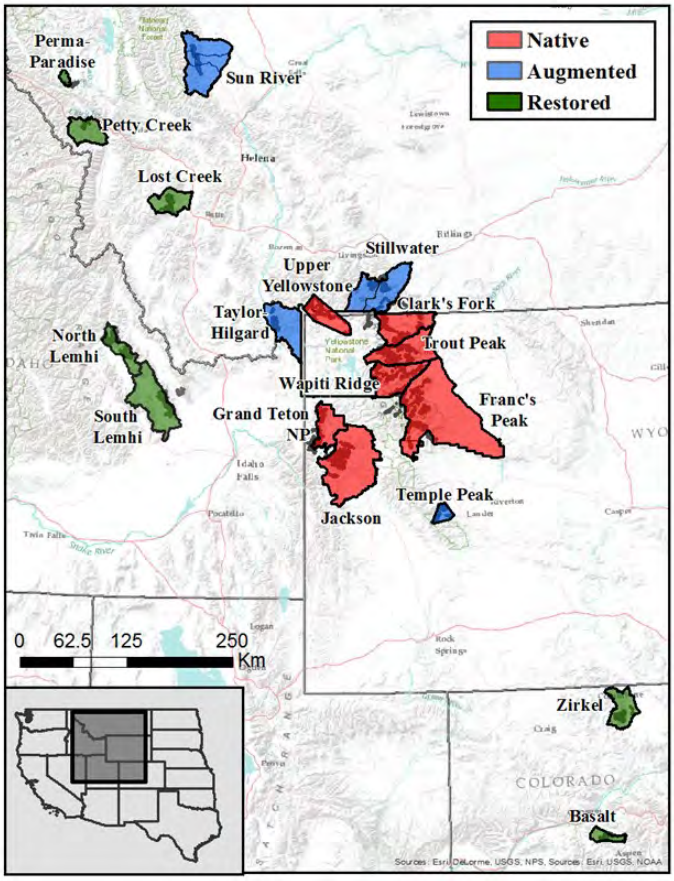


Fig 21. *Native (red; N = 7), augmented (blue; N = 4), and restored (green; N = 7) population units used to characterize female bighorn sheep migration patterns, Montana, Wyoming, Idaho, and Colorado, USA, 2008−2017.*

Table 5. *Summary information for the study populations, Montana, Wyoming, Idaho, and Colorado, USA, 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	Perma-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	Sun River	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	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 Appendix S1

[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

[4]Temple Peak is a non-hunted herd without a management unit.

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 three 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 (Fig 22). 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 (Fig 22).

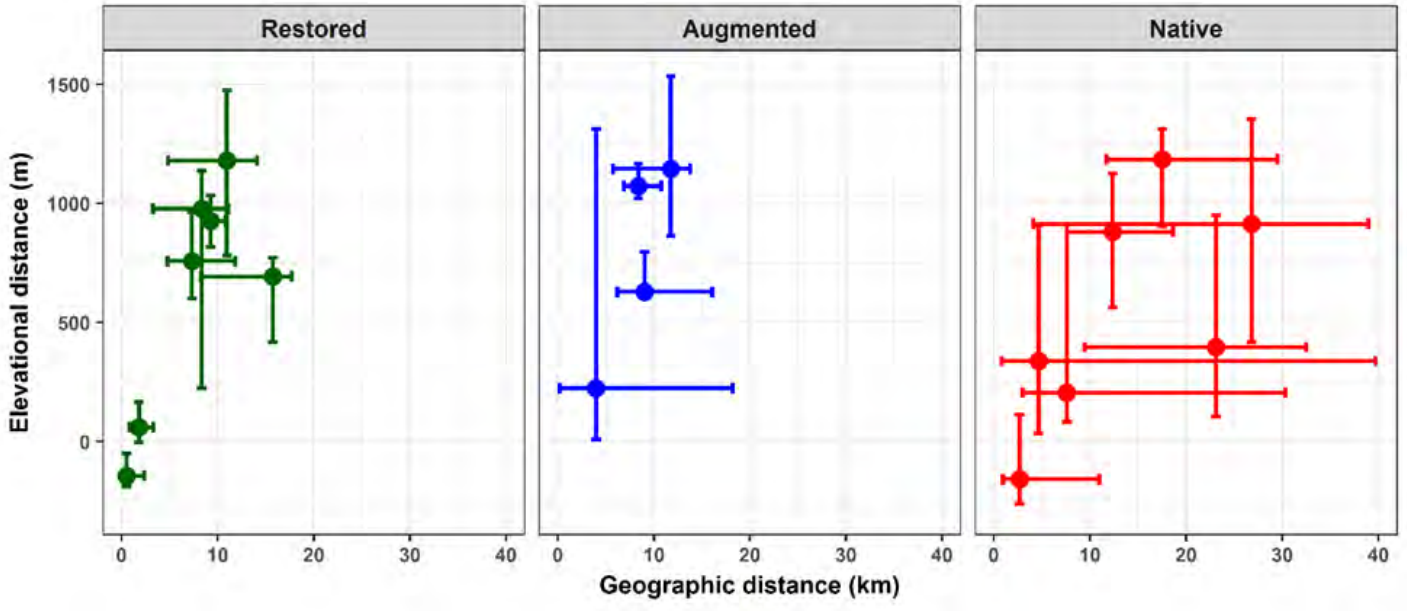
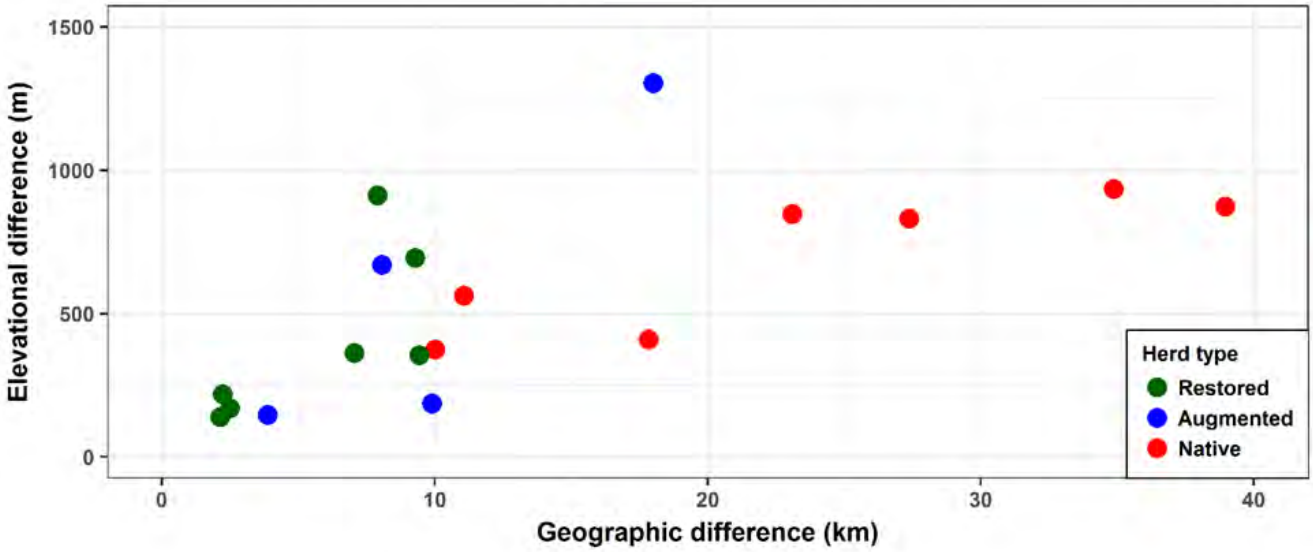


Fig 22. *Migration characterizations with respect to elevational and geographic distance between core seasonal ranges for restored (green), augmented (blue), and native (red) populations of female bighorn sheep, in Wyoming, Montana, Idaho, and Colorado, 2008−2017. Closed circles represent population-level 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.*

Fig 23. *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.*

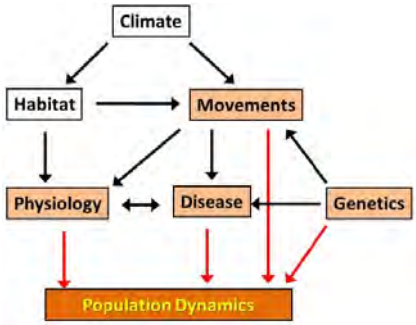


There were notable differences in individual variation within a population among the three management histories. As predicted, relative to native populations, restored and augmented populations had less variation among individuals with respect to elevational and geographic distance (Figs 22 and 23). 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 (Fig 23). Moreover, individual migrations in native populations spanned a continuum of elevation and geographic distances. In contrast, rather 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 (Fig 22).

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 all 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 four 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, Armstrong & Reed 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. A manuscript building from this work is currently in review in the Journal of Applied Ecology.

Objective # 4: *Collect data to estimate demographic rates of each herd included in the statewide study*



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 is to develop a simple cost effective monitoring procedure that wildlife managers could adopt as part of routine management activities, and use this procedure to estimate population size, adult female survival and pregnancy rates, and annual recruitment. Below are presentations of our initial efforts to use simple field protocols to collect data that can be used to estimate specific vital rates. As a final product of this research program we will provide a framework that integrates the individual data types and vital rate estimates into a comprehensive population monitoring plan that could potentially aid in gaining more ecological insight regarding processes that affect population dynamics of individual bighorn herds and assist in management decisions.

5.1 Adult Female Survival

Currently adult female mortality is being monitored in the eight study populations via VHF radio collars that began transmitting after the store-on-board GPS collars were released from the animals 1.5-2.0 years after they were instrumented. In a few herds, mortality is being monitored using satellite-linked GPS radio-collars equipped with mortality sensors. Both technologies allow for known fate survival estimation.

In addition, to the eight research herds that are part of the statewide bighorn sheep research project we also report on monitoring results for instrumented female bighorn sheep in the Upper Yellowstone herd complex and animals translocated from the Taylor-Hilgard population to the Wolf Creek area in the Madison Range during the statewide study. The Upper Yellowstone Complex was originally slated to be one of the core herds in the statewide study, but we failed to successfully instrument adequate numbers of animals to meet our research objectives and so an alternative herd was selected for incorporation into the statewide studies. The animals that were instrumented in the Upper Yellowstone Complex, however, were monitored and results are presented. In an effort to expand the distribution and abundance of bighorn sheep in the Madison Range in southwestern Montana, Julie Cunningham, the FWP's area biologist, partnered with the MSU research team to coordinate the capture and translocation of bighorn sheep from the Taylor-Hilgard winter range in the upper Madison Valley to the foothills of the Wolf Creek drainage approximately 25 km to the north. Three translocation events occurred during the winters of 2014-15, 2015-16, and 2017-18, moving a total

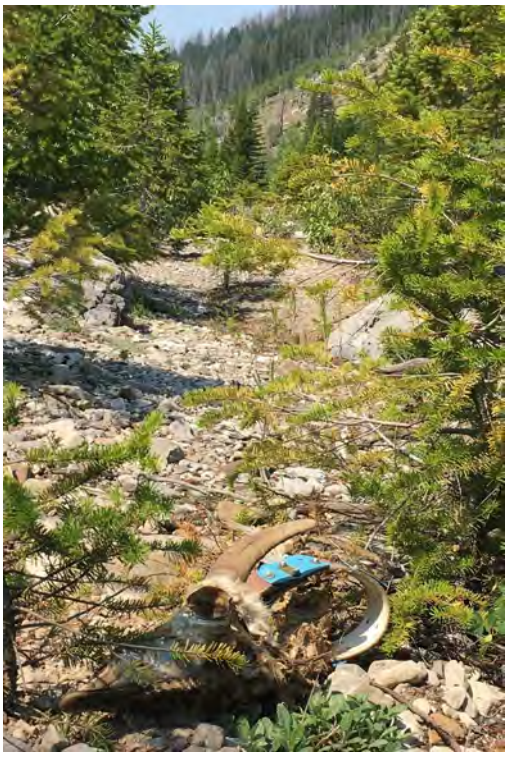


Figure 24. *A mortality in the Castle Reef population , summer 2018.*

of 97 animals to Wolf Creek. A modest proportion of adult ewes involved in each translocation were instrumented and monitored to determine basic movements and survival. Data from these animals were pooled to provide survival estimates that can be contrasted with those estimated for the Taylor-Hilgard herd over the same time period.

To date, 218 adult females from all eight study populations have been radio-collared and monitored for survival (Table 1), 69 of which have died. Causes of death have included hunter-harvest (n=16), cougar predation (n=6), trauma (n=2), vehicle/train collision (n=4), drowning (n=1) and disease (n=1), however; the cause of most mortalities (n=40) were undetermined (Table 6). In 2018, there were 23 mortalities. Herd specific summaries are presented below.

Table 6. *Cause of death for mortalities of adult female bighorn sheep in the seven study populations which have been monitored since winter 2014/2015.*

CAUSE OF DEATH	STUDY POPULATION								TOTAL
	Fergus	*Paradise*	*Hilgard*	*Stillwater*	*Castle Reef*	*Lost Creek*	*Petty Creek*	*Middle Missouri*	
Hunter Harvest	5	1	1	-	-	-	2	6	15
Disease	-	-	-	-	-	1	-	-	1
Trauma/Accident	-	-	1	-	-	1	-	-	2
Roadkill/Train	-	2	2	-	-	-	-	-	4
Drowning	-	-	-	-	1	-	-	-	1
Predation	-	1	1	-	3	1	-	-	6
Undetermined	3	8	5	6	9	3	2	4	40
TOTAL	**8**	**12**	**10**	**6**	**13**	**6**	**4**	**10**	**69**

Paradise:

A total of 25 bighorn sheep have been collared in this population, 12 of which have died. Of the 15 animals originally radio collared during the winter of 2014/2015, 7 (47%) are still alive. Four animals collared during the winter of 2016/2017 have also died, leaving a total of 13 animals alive and available for monitoring. During 2018, there were four mortalities. One was struck by a train sometime before May 29, and two others were detected in mid June but we could not determine cause of death. The most recent mortality was detected January 14, 2019 and has not been investigated.

Lost Creek:

A total of 27 bighorn sheep have been collared in this population, 6 of which have died. Twelve animals were collared during the winter of 2014/2015, 6 (50%) of which are still alive. No mortalities from the 2016/2017 winter capture have been detected leaving 21 animals available for monitoring. During 2018, there was only one recorded mortality, estimated as having occurred around June 22, evidence suggested this was not a predation but cause of death could not to determined with certainty.

Petty Creek:

A total of 24 bighorn sheep have been collared in this population, 4 of which have died. Of the 15 animals originally radio collared during the winter of 2016/2017, 12 (80%) are still alive. Nine additional collars were deployed during the winter of 2017/2018 and as of January 2018, 8 were still alive leaving a total of 20 animals available for monitoring. During 2018, two mortalities occurred October 20 and November 20. Both mortalities were hunter harvested.

Castle Reef:

A total of 29 bighorn sheep have been collared in this population, 13 of which have died. Of the 18 animals originally radio collared during the winter of 2015/2015, 8 (44%) are still alive and one collar has failed. Of the 11 animals collared during the 2016/2017 winter, 5 have died leaving a total of 16 animals alive and available for monitoring. Five mortalities occurred during 2018. Mortalities where cause of death could not be determined occurred on January 6, April 18, and June 20. The final two 2018 mortalities occurred Feb 4 and Feb 24 from predation and suspected drowning, respectively

Taylor-Hilgard:

A total of 32 female bighorn sheep have been collared in this population, 10 of which have died. Prior to this study, 5 adult female bighorn sheep were collared and incorporated into routine survival monitoring. Three of these animals have since died, and the remaining two collars are no longer transmitting. An additional 15 adult females were collared during the winter of 2013/2014, 12 (80%) of which are still alive. Four of the 11 animals collared during the winter of 2016/2017 have also died, with one of these collars redeployed winter 2017/2018, resulting in a total of 20 animals alive and available for monitoring. In addition, survival monitoring continues to be enhanced with the inclusion of animals collared as part of FWP intra-mountain range translocation efforts (n= 27). During 2018, two instrumented animals died, both as the result of vehicle collisions on March 27 and June 22.

Fergus:

A total of 40 animals have been collared in this population, 8 of which have died. Of the 30 animals originally radio collared during the winter of 2014/2015, 23 (77%) are still alive and 1 collar has failed. Nine out of the ten animals collared during the winter of 2016/2017 are still alive, leaving a total of 31 animals available for monitoring. During 2018, a mortality occurred on approximately January 5, from unknown causes, another was harvested during the legal hunting season and a third unrecovered mortality was detected January 31, 2019.

Stillwater:

A total of 21 bighorn sheep have been collared in this population, 7 of which have died. Of the 15 animals originally radio collared during the winter of 2014/2015, 9 (60%) are still alive. All other animals collared during the winters of 2015/2016 (n=1) and 2016/2017 (n= 5) are alive, leaving 15 animals alive and available for monitoring. During 2018 two mortalities were detected in early January 2018, both from unknown causes though one was cached by a mountain lion. A third mortality was detected February 1, 2019 and has not yet been investigated.

Middle Missouri:

A total of 20 bighorn sheep were collared during the winter of 2016/2017. Of these animals, 10 (50%) are still alive and available for monitoring. During 2018, four mortalities were recorded. Two as the result of harvest during the legal hunting season and two were detected August 29, for which a cause of mortality was not able to be determined.

5.2 Adult Female Survival

Survival rates were estimated in Program MARK using a known-fate analysis (White and Burnham 1999) conducted via the nest-survival module (Dinsmore et al. 2002, Rotella et al. 2004), which is appropriate for telemetry data collected according to an irregular schedule and where an animal's fate is known but the exact dates for all mortality events are not known. This approach has been used in a variety of studies of survival of radio-marked individuals in recent years (e.g., Colwell et al. 2007, Mong and Sandercock 2007, Buckley et al. 2015). We estimated a unique survival rate for each herd and season using two different models. The first model

Table 7. *Known-fate seasonal and annual survival estimates and associated 95% confidence intervals for radio-collared adult ewes in each of the eight bighorn sheep herds in the state-wide study and a sample of animals from the upper Yellowstone complex. Also included are survival estimates from a sample of animals translocated from the Taylor-Hilgard herd in the Madison Range of southwestern Montana to the Wolf Creek area within the Madison Range north of the winter range of the Taylor-Hilgard herd.*

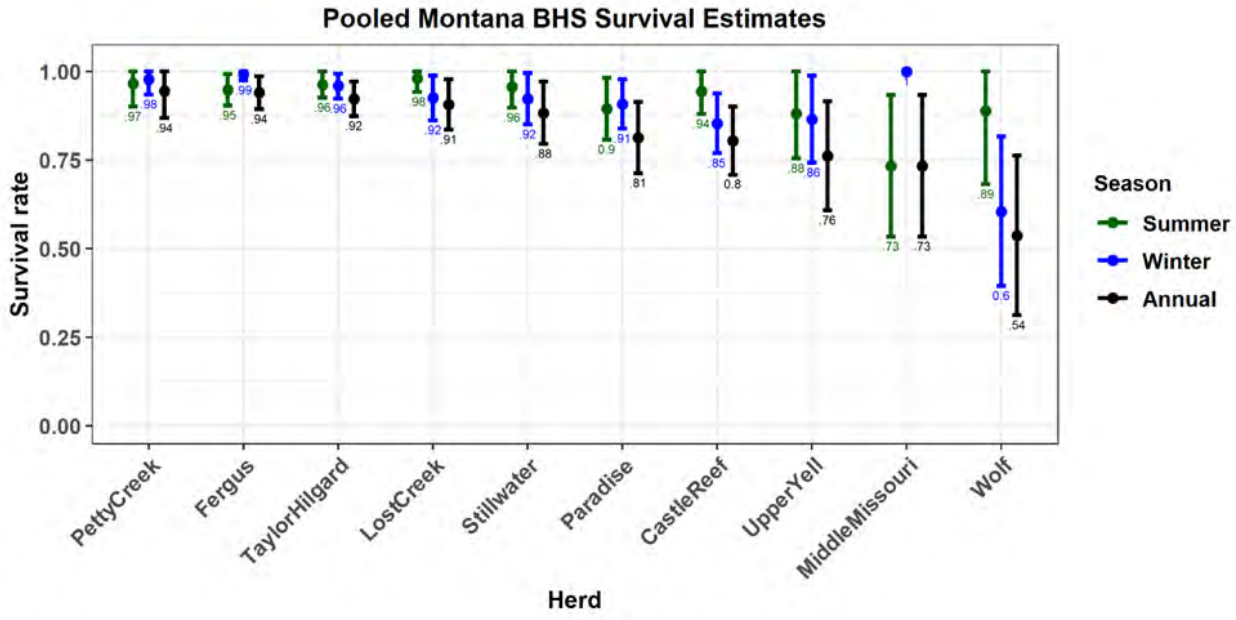
		Annual		Summer		Winter	
HERD	Year	Estimate	Conf. Inter.	Estimate	Conf. Inter.	Estimate	Conf. Inter.
Paradise	2014-15					0.93	0.80-1.00
	2015-16	0.86	0.68-1.00	0.93	0.80-1.00	0.93	0.78-1.00
	2016-17	0.78	0.59-0.97	0.92	0.77-1.00	0.85	0.69-1.00
	2017-18	0.89	0.75-1.00	0.95	0.84-1.00	0.94	0.83-1.00
	Pooled	0.81	0.71-0.91	0.90	0.81-0.98	0.91	0.84-0.98
PettyCreek	2015-16					1.00	1.00-1.00
	2016-17	0.93	0.80-1.00	1.00	1.00-1.00	0.93	0.80-1.00
	2017-18	0.93	0.80-1.00	0.93	0.80-1.00	1.00	1.00-1.00
	Pooled	0.95	0.87-1.00	0.97	0.90-1.00	0.98	0.94-1.00
LostCreek	2014-15					0.76	0.46-1.00
	2015-16	0.87	0.70-1.00	1.00	1.00-1.00	0.87	0.70-1.00
	2016-17	0.96	0.87-1.00	1.00	1.00-1.00	0.96	0.87-1.00
	2017-18	1.00	1.00-1.00	1.00	1.00-1.00	1.00	1.00-1.00
	2018-19			0.79	0.42-1.00		
	Pooled	0.91	0.84-0.98	0.98	0.94-1.00	0.93	0.86-0.99
TaylorHilgard	2011-12					1.00	1.00-1.00
	2012-13	1.00	1.00-1.00	1.00	1.00-1.00	1.00	1.00-1.00
	2013-14	0.95	0.85-1.00	1.00	1.00-1.00	0.95	0.85-1.00
	2014-15	0.95	0.86-1.00	1.00	1.00-1.00	0.95	0.86-1.00
	2015-16	0.92	0.81.1.00	0.92	0.81-1.00	1.00	1.00-1.00
	2016-17	0.93	0.84-1.00	1.00	1.00-1.00	0.93	0.84-1.00
	2017-18	0.88	0.76-1.00	0.92	0.82-1.00	0.96	0.87-1.00
	2018-19			1.00	1.00-1.00		
	Pooled	0.92	0.87-0.97	0.96	0.93-1.00	0.96	0.92-0.99
Wolf Creek	2014-15					0.82	0.50-1.00
	2015-16	0.17	0.00-0.48	0.47	0.00-1.00	0.35	0.00-0.77
	2016-17	1.00	1.00-1.00	1.00	1.00-1.00	1.00	1.00-1.00
	2017-18	0.51	0.17-0.85	1.00	1.00-1.00	0.51	0.17-0.85
	2018-19			1.00	1.00-1.00		
	Pooled	0.54	0.31-0.76	0.89	0.68-1.00	0.61	0.40-0.82
Upper Yellowstone	2011-12					1.00	1.00-1.00
	2012-13	1.00	1.00-1.00	1.00	1.00-1.00	1.00	1.00-1.00
	2013-14	0.91	0.75-1.00	1.00	1.00-1.00	0.91	0.75-1.00
	2014-15	0.79	0.53-1.00	0.90	0.72-1.00	0.78	0.65-1.00
	2015-16	0.11	0.00-0.39	0.60	0.18-1.00	0.19	0.00-0.62
	Pooled	0.76	0.61-0.92	0.88	0.75-1.00	0.87	0.74-0.99
CastleReef	2014-15					0.92	0.77-1.00
	2015-16	0.76	0.55-0.96	1.00	1.00-1.00	0.76	0.55-0.96
	2016-17	0.87	0.71-1.00	0.92	0.77-1.00	0.95	0.85-1.00
	2017-18	0.80	0.62-0.97	1.00	1.00-1.00	0.80	0.62-0.97
	2018-19			0.58	0.14-1.00		
	Pooled	0.80	0.71-0.90	0.94	0.88-1.00	0.85	0.77-0.94
Fergus	2014-15					1.00	1.00-1.00
	2015-16	0.93	0.85-1.00	0.93	0.85-1.00	1.00	1.00-1.00
	2016-17	1.00	1.00-1.00	1.00	1.00-1.00	1.00	1.00-1.00
	2017-18	0.88	0.78-0.99	0.92	0.83-1.00	0.96	0.89-1.00
	Pooled	0.94	0.89-0.99	0.95	0.90-0.99	0.99	0.98-1.00
Stillwater	2014-15					0.91	0.73-1.00
	2015-16	0.93	0.79-1.00	0.93	0.79-1.00	1.00	1.00-1.00
	2016-17	0.94	0.84.1.00	1.00	1.00-1.00	0.94	0.84-1.00
	2017-18	0.74	0.47-1.00	0.94	0.84-1.00	0.78	0.52-1.00
	Pooled	0.88	0.80-0.97	0.96	0.90-1.00	0.92	0.85-1.00
MiddleMissouri	2016-17					1.00	1.00-1.00
	2017-18	0.73	0.53-0.93	0.73	0.53-0.93	1.00	1.00-1.00
	Pooled	0.73	0.53-0.93	0.73	0.53-0.93	1.00	1.00-1.00

produced unique estimates for each herd in each season of each year (data modeled using an interaction of herd, season, and year). The second model produced a single estimate for each herd in each season across all years (data modeled using an interaction of herd and season but not year such that data were pooled across years for each herd). Seasons were defined as 1) winter (December through May) and 2) 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 = winter-DSR x 182.5; estimated survival rate for summer = summer-DSR x 182.5). The seasonal survival rates were then multiplied together to obtain estimates of annual survival rate. We used the delta method to derive measures of uncertainty (Seber 1982, Powell 2007) for seasonal and annual rates. We used program R (R Development Core Team 2017) to 1) implement the Program MARK analyses through the RMark package (Laake 2013) and 2) the delta method through the msm package (Jackson 2011).

5.2.1 Results

Survival rates were variable between seasons and among years and herds. 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 7). The exception to this pattern was in the Fergus, Middle Missouri and Petty Creek herds, where over half of documented mortalities to date have been the result of legal hunter harvest (Table 6). Hunter harvest occurs during the summer survival period (June-November) and is most noticeable for the Middle Missouri herd where of 4 instrumented ewes were legally harvested during the 2017 hunting season, resulting in a summer period survival estimate of 0.73. Since we incorporated the Middle Missouri herd into the statewide study in fall 2016 only four other mortalities have been recorded. Of these, one died shortly after capture suggesting high survival in the absence of human harvest. Caution should be exercised in interpretation of all single season and annual survival estimates as the modest number of instrumented animals present in each herd results in relatively wide confidence intervals on all estimates (Table 7). Among-herd comparisons are best made using survival estimates generated by pooling monitoring data across all years of monitoring. The pooled annual survival rates for the Petty Creek, Fergus, Taylor-Hilgard, and Lost Creek herds are relatively high, ranging from 0.91 to 0.94. Pooled survival estimates for Stillwater, Paradise, and Castle Reef herds, however, were notably lower, ranging between 0.80 to 0.88 (Figure 25).

Figure 25. *A comparison of the pooled seasonal and annual adult female survival estimates (filled circles) for the eight bighorn sheep herds in the state-wide study and a sample of animals from the upper Yellowstone complex. Also included are survival estimates from a sample of animals translocated from the Taylor-Hilgard herd in the Madison Range of southwestern Montana to the Wolf Creek area within the Madison Range north of the winter range of the Taylor-Hilgard herd. Estimates were calculated by combining all mortality data collected for instrumented animals in each herd over the entire monitoring period since animals were initially instrumented in each herd. Lines above and below the point estimates represent 95% confidence intervals for each point estimate.*



Large ungulate population growth rates are most sensitive to adult female survival rates and the lower survival estimates for these three herds suggests weaker overall demographic performance. While survival estimates for the upper Yellowstone complex 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. While the sample size of instrumented animals translocated from the Taylor-Hilgard winter range to Wolf Creek was also modest, with wide confidence intervals on the point estimates, the data clearly demonstrate that these animals had markedly lower survival than ewes in all other herds incorporated into the statewide study. These data suggest that more effort to formally monitor the behavior and fate of translocated animals would be worthwhile for if such low survival is typical, exploring potential modifications of current translocation procedures may be warranted in an effort to increase the success and efficacy of this important management and restoration tool.

5.3 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 herds 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 became pregnant after they were sampled, and were reported accordingly. Asymmetric binomial 90% confidence intervals were calculated for all point estimates for pregnancy rates.

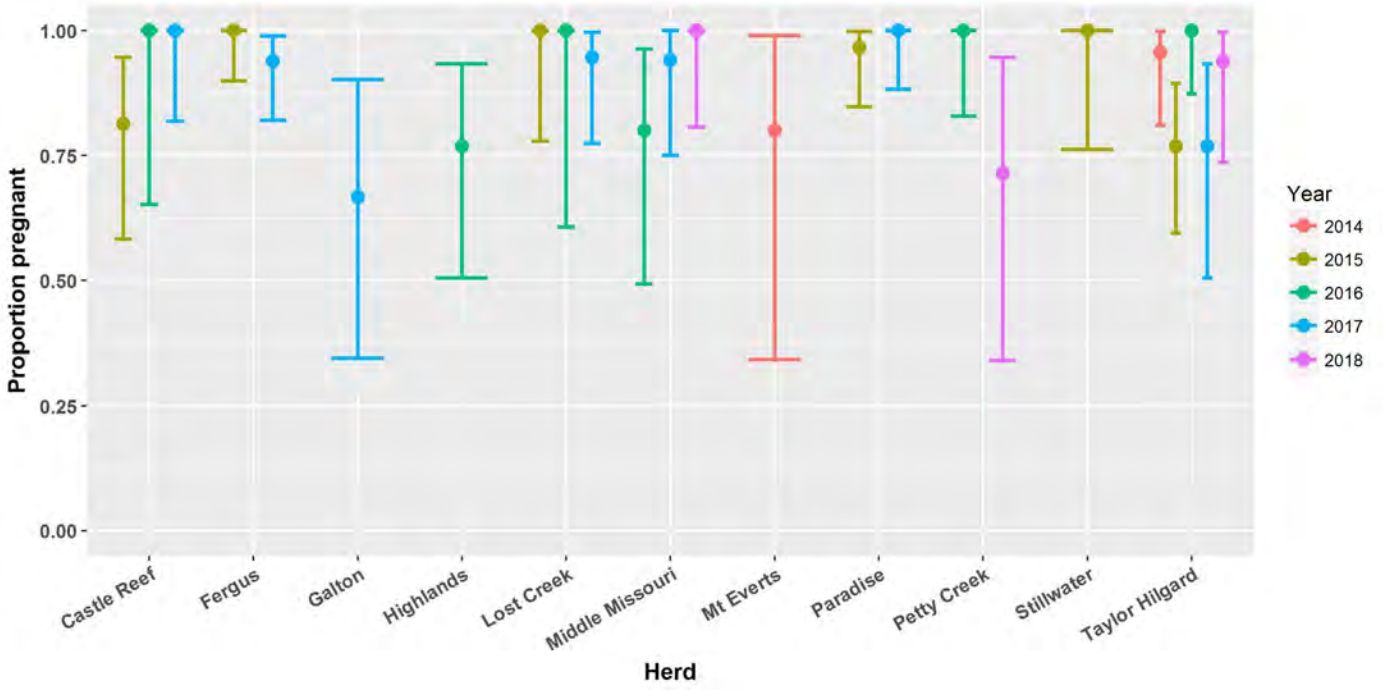


Figure 26. *Estimated pregnancy rates of the eight Montana bighorn populations captured and sampled as part of the statewide bighorn sheep research project, two herds (Galton, Highland) were sampled as part of Montana FWP's herd health program, and a herd located in the upper Yellowstone River drainage within Yellowstone National Park that was sampled as part of the GYA Mountain Ungulate Research Program.*

5.3.1 Results

Estimated pregnancy rates for most herds were very high, generally >0.90 (Figure 26). 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.* 2000*b*, Cassirer and Sinclair 2007, Stephenson *et al.* 2012). Despite the evidence for overall high pregnancy rates, our sampling has produced some results that indicate potentially lower pregnancy rates occur in some herds and in some years that could have the potential to dampen demographic performance of herds. For example, pregnancy rate estimates for the Galton and Highland populations, two herds sampled as part of Montana FWP's herd health program, were 0.67 and 0.77, respectively. The Galton herd 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 herd has experienced very poor demographic performance which 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 herd. We also found some evidence for significant annual variation in pregnancy rates for two of the three herds (Castle Reef, Taylor-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 herds should also be interpreted with caution as there is considerable uncertainty in these estimates, as reflected in the wide confidence intervals (Figure 15), 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.

5.5 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 first year of life to become recruited into the adult population. Herds where sex-age classification surveys are routinely conducted at the optimal time to index recruitment (April to early-May) include Castle Reef, Taylor-Hilgard, Lost Creek, Paradise, and Petty Ck. Classification surveys for the two prairie bighorn herds in the statewide studies (Fergus, Middle Missouri), as well as the Stillwater herd 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 significant 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. Since 2015, we have been able to coordinate with the area biologist managing the Fergus herd to conduct spring age-sex classification surveys in addition to her normal mid-winter surveys in order to obtain lamb:ewe ratios more comparable to most of the other herds in the statewide studies.

5.5.1 Results

As is typical for large ungulate populations, the age-sex classification surveys documented substantial annual variation in recruitment rates for all herds included in the statewide research project over the past decade (Figure 27). The three herds with the most pronounced annual variation are the Taylor-Hilard, Castle Reef, and Lost Creek herds. The latter two herds experienced a pneumonia epizootic during the winter of 2010. Subsequent to the disease-related die-offs in these herds lamb:ewe ratios were depressed (0.03-0.12) for four to five years, which is a pattern routinely observed in bighorn sheep herds after pneumonia events (Cassirer et al. 2017). However, lamb:ewe ratios in both of these herds have improved in the last two years (0.15-0.25), suggesting recruitment in both herds may be returning to more typical rates experience in the herds 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. As we move into more concentrated research on the demographic attributes of each research herd in the last year of this project we anticipate working with the area biologist managing the Taylor-Hilgard herd to produce annual estimates from only late winter to early spring surveys to better estimate annual variation in recruitment.

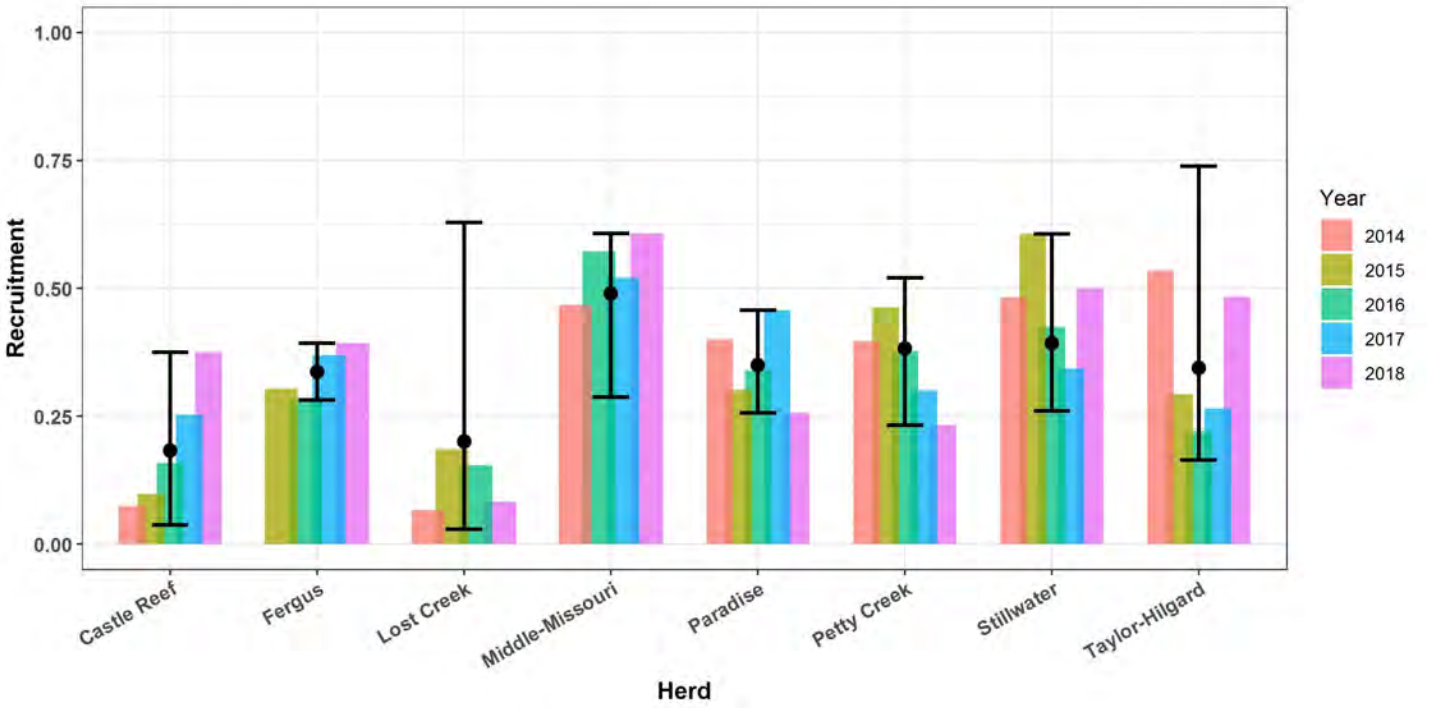
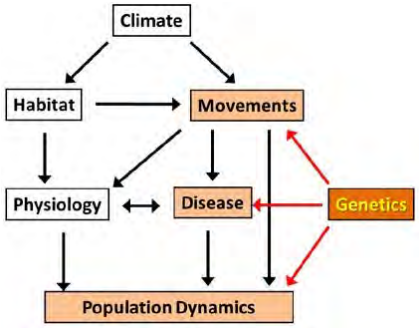


Figure 27. *Annual variation in lamb:ewe ratios determined from routine population monitoring surveys conducted by area biologists responsible for managing each of the research herds in the statewide bighorn sheep studies. For most herds the surveys were conducted in April-May and can be considered reasonable 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.*

Objective #5: *Collect and provide samples for a bighorn sheep genetics study and complete preliminary genomic analyses.*



Genetics

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 can influence translocation success. Comparing genetics of different bighorn sheep herds could potentially provide information to describe genetic connectivity and diversity of examined herds, as well as discover links between herd 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.

5.1 Generating high-quality genotype data

We have over 500 high-quality bighorn sheep genotypes from different populations across Montana, Wyoming, Colorado, and California available for genomic analysis (Table 8). 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, Dinosaur 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 four 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 and whole blood. Biopsy punches were obtained from ear cartilage during ear tagging and stored frozen in diluted ethanol.

Table 8. 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, and California. Herd units not managed by Montana FWP are shaded in gray.*

Herd	Management Agency	Samples currently assayed
Castle Reef[rh]	Montana FWP	25[c]
Fergus[rh]	Montana FWP	30[c]
Grave Creek[rh] (Petty Creek)	Montana FWP	25[c]
Lost Creek[rh]	Montana FWP	25[c]
Middle Missouri Breaks[rh]	Montana FWP	25[c]
Paradise[rh]	Montana FWP	25[c]
Stillwater[rh]	Montana FWP	24[a,c]
Taylor/Hilgards[rh]	Montana FWP	30[c]
Galton	Montana FWP	5[c]
Highlands	Montana FWP	17[c]
Spanish Peaks	Montana FWP	20[c]
Tendoys	Montana FWP	25[a,c]
Wild Horse Island	Montana FWP	25[a,c]
Glacier National Park	NPS	95[b]
Beartooth-Absaroka Metapopulation	Wyoming F&G and NPS	90[a]
Dinosaur National Monument	NPS	20[d]
Sierra Nevada	CA Dept. of Fish and Wildlife	5[c]
Total		511

[rh] Herds in Montana state-wide 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.

[d] Analysis of these samples was funded by Dinosaur National Monument.

5.2 Extraction of genetic samples and 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.

Figure 28. *PhD candidate Elizabeth Flesch with a bighorn captured during the 2018 Taylor-Hilgard drop-net capture*

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 for a limited number of captures 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.

5.3 Preliminary genomic analysis results

5.3.1 Evaluating sample size to estimate genomic relatedness

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 herd attributes to investigate genomic relatedness within and between herds and estimate an optimal sample size per population for evaluating genetic diversity and distance (Flesch et al. 2018). We also presented results of the sample size study at the Montana Chapter of the Wildlife Society annual conference in February 2018 and the Northern Wild Sheep and Goat Council annual conference in May 2018. The literature provides little insight into this issue and while we had a target of 15 animals per herd 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 2008). Thus, we determined the optimal number of animals to sample from each herd for genetic analyses. Information regarding optimal sample size would serve 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 four 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 four populations provided samples across a spectrum of these herd attributes and included Fergus, Taylor-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 herd unit to evaluate the effect of sample size on estimated variance and relative bias. We evaluated mean kinship (Manichaikul et al. 2010) within each herd to determine how related individuals were on average in the same area. 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 herds. Thus, we sought to evaluate the following hypothesis:

Hypothesis: Genetic metrics (heterozygosity, uniqueness, and genetic distance) for each herd will be highly variable for smaller sample sizes. As sample size increases, variability in genetic metrics will decrease and stabilize at a higher sample size that is adequate to characterize that herd.

Characteristics of Herds in the Sample Size Study

We examined population attributes that may impact genetics of each examined herd to predict genomic results in order to predict what differences among herds would be detected for within herd relatedness results (Figure 29). First, we expected native and reintroduced herds to have differing genetics, because initial genetic composition and diversity of founders in a newly established herd can have a strong impact on the population genetics. This "founder effect" can result in low genetic diversity and subsequent genetic drift, because the herd was founded by a small number of individuals (Fitzsimmons et al. 1997, Hedrick et al. 2001, Olson et al. 2013). In contrast, native herds are more likely to contain more genetic diversity and adaptations to their local environment (Nachman et al. 2003, Reed and Frankham 2003). Secondly, we expected population size to impact herd genetics. Small population size can result in lower likelihood of herd persistence, limited adaptive potential, and increased susceptibility to inbreeding, which can impact overall herd recruitment (Berger 1990, Willi et al. 2006, Frankham 2007). We categorized herds into three different population sizes: "small" (on average less than 100 individuals), "medium" (100-200 individuals), and "large" (greater than 200 bighorn sheep).

Thirdly, we expected that past bottlenecks (a severe reduction in population size at a point in time) in herd history could impact population genetics. Bottlenecks can result in a decrease in genetic variation, an increase in inbreeding, and greater frequency of detrimental alleles, which can all negatively impact probability of herd persistence (Lande 1988, Ralls et al. 1988, Hedrick and Miller 1992, Brakefield and Saccheri 1994, Jiménez et al. 1994, Lande 1994, Mills and Smouse 1994, Frankham 1995). We classified three categories of potential bottlenecks, including "mild" (large populations with no record of past bottlenecks), "moderate" (possible past bottlenecks), and "strong" (known past bottlenecks). Finally, connectivity with other bighorn sheep herds can impact population genetics, as isolation and consequent lack of gene flow can cause a decline in genetic diversity (Epps et al. 2005). Lack of gene flow in isolated herds has been cited to promote strategic genetic augmentation of bighorn sheep (Hogg et al. 2006). We classified herd connectivity as "high" when a herd was a part of a known, large metapopulation of bighorn sheep, "some" when limited connectivity with other herds was suspected, and "isolated" when no known connectivity (other than augmentation) occurred.

Bighorn sheep populations located in Glacier National Park, Montana, and across the Beartooth Absaroka Mountains in Wyoming served as baseline examples of large, native herds with high anticipated connectivity and genetic diversity. The selected samples from Glacier National Park spanned the eastern front of the park, with approximately 16 from the northern and 14 from the southern areas of the park. The samples from the Beartooth Absaroka metapopulation spanned the eastern front of the Greater Yellowstone Area, across Wyoming hunt units 1, 2, 3, 5, and 22. The Fergus and Taylor-Hilgard herds served as examples of herds with more complex management histories. The Fergus herd is a large population that was reintroduced (43 bighorn sheep reintroduced from 1958 to 1961), experienced a population bottleneck of a limited number of individuals, and was supplemented with additional augmentations. Thus, this population is representative of a herd with a successful reintroduction and a current population size of greater than 200 individuals, as well as a past bottlenecks and augmentations. The Taylor Hilgard herd represents a native population that experienced multiple augmentations and catastrophic die-offs that reduced the population to several 10s of animals, but has recovered to a moderate size between 100 and 200 individuals. In addition, this herd has been impacted by respiratory disease, which is a major limiting factor to bighorn sheep conservation and management throughout the western

U.S. (Monello et al. 2001, Cassirer and Sinclair 2007, Besser et al. 2008, Miller 2008, Besser et al. 2012, Cassirer et al. 2013). Based on a synthesis of these herd history characteristics, we expected inbreeding and relatedness to be lower within the Beartooth Absaroka and Glacier National Park herds, in comparison to the Fergus and Taylor-Hilgard herds.

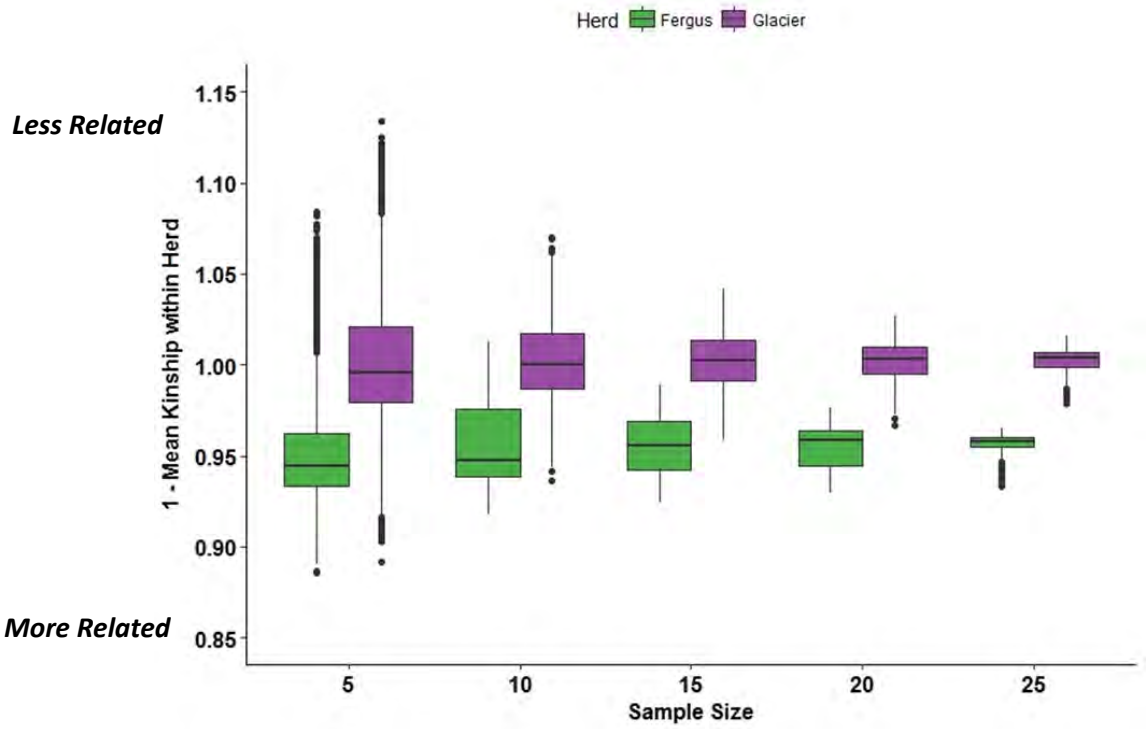
Herd Attribute	Beartooth Absaroka	Glacier National Park	Fergus	Taylor-Hilgard
Native or Reintroduced	Native	Native	Introduced	Native
Population Size	Large	Large	Large	Medium
Potential Bottlenecks	Mild	Mild	Strong	Strong
Connectivity	High	High	High	Some

Figure 29. *Herd attributes of four bighorn sheep herds analyzed in the sample size study. There was a range of attributes among herds that were predicted to cause different herd genetics.*

Sample Size Study Results

By evaluating our simulation results, we concluded that a sample size of 20-25 is adequate for assessing intra- and inter-population relatedness. In regard to within herd relatedness, 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 Taylor-Hilgard herds, which had more complex herd histories. A comparison of a native metapopulation (Glacier) and a reintroduced herd (Fergus) using the mean kinship metric is in Figure 30 Relatedness within a herd decreases as (1 - Mean Kinship with Herd) increases. Figure 30 also demonstrates that estimates regarding within herd relatedness differences between the two different herds 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 herd. 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 herds. In regard to relatedness between herds, differences in relatedness among herd comparisons were also more clearly differentiated at a sample size of 25 (Figure 31). Thus, we decided to use 20-25 samples per herd to evaluate population genomics of additional herds that will be assessed through the statewide study (Table 8).

Figure 30. *Boxplots of intrapopulation relatedness estimates based on 10,000 replicate simulations using empirical SNP genotypes from populations of bighorn sheep, including one minus mean kinship by increasing sample size. Center lines 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, points represent outliers. Different populations are indicated by color, including Fergus (green) and Glacier (purple).*



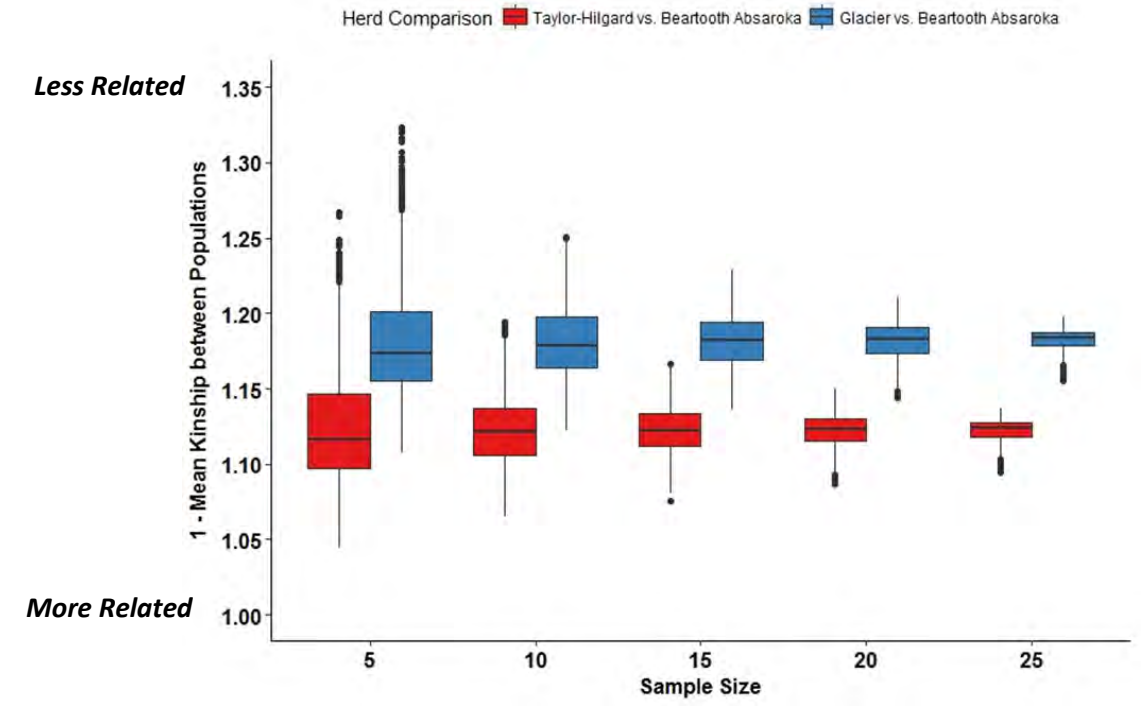


Figure 31. *Boxplots of interpopulation relatedness estimates based on 10,000 replicate simulations using empirical SNP genotypes from populations of bighorn sheep, including one minus mean kinship by increasing sample size per individual population included. Center lines represent the median, box limits represent the 25th and 75th percentiles, whiskers indicate 1.5 multiplied by the interquartile range from the 25th and 75th percentiles, points represent outliers. Different population comparisons are indicated by color.*

5.3.2 Ancient DNA

Anthropologists working in the high alpine environments in the Greater Yellowstone Area have recovered bighorn sheep skull caps, horn cores, and bones that have been dated to pre-European settlement from receding ice patches. We developed a partnership with an anthropologist that has a number of bighorn sheep specimens that were collected on the Beartooth Plateau along the Montana-Wyoming border in the northeast GYA that have been radiocarbon dated. The six different samples have been dated to about 879, 2210, 3296, 3346, 3665, and 3885 years before present. To take advantage of the potential of these samples to better understand the genetics of contemporary bighorn sheep populations in the region, we are collaborating with Dr. Beth Shapiro (Univ. Calif. Santa Cruz) and her team, who are experts in the extraction and analyses of ancient DNA. Dr. Shaprio's team successfully recovered adequate DNA from five of the ice patch specimens. Due to DNA degradation of these ancient samples, we did not use the Ovine HD SNP genotyping array for ancient DNA analysis, but instead implemented mitochondrial DNA analysis for comparison with the extant bighorn sheep population occupying the Beartooth-Absaroka ranges of the GYA. We plan to compare 26 contemporary bighorn sheep mitochondrial DNA genomes from the Beartooth-Absaroka complex with the five ancient samples (Figure 5). In 2017 Dr. Shapiro's team successfully generated mitochondrial genomes for five ancient samples and 26 contemporary samples. Graduate student Elizabeth Flesch visited Dr. Shapiro's lab to discuss the project in March 2018. Based on their discussions, Dr. Shapiro's team is currently adding additional samples to this dataset and working on building a phylogenetic tree.

Figure 32. *An example of an ancient bighorn sheep specimen radiocarbon dated to pre-European settlement that was recovered from a receding high-elevation ice patch located on the Beartooth Plateau in the northeast GYA near the Montana-Wyoming border*

This represents an exciting opportunity to compare the genome of the bighorn populations that existed in the GYA prior to contact with domestic sheep and their associated respiratory pathogens that were introduced to the region at the time of European settlement. We can expect the genome of the pre-settlement bighorn sheep to represent the historic condition of native bighorn sheep when their populations were both numerous and robust. The introduction of exotic respiratory pathogens into the naive GYA bighorn populations when domestic sheep were initially introduced to the region undoubtedly resulted in catastrophic mortalities and strong selection for bighorn that could mount a successful immunological defense against the pathogens. Recent sampling of bighorn sheep populations in the region indicate that these exotic pathogens are present in nearly all population segments, suggesting that the current bighorn populations have likely been under continuous selection pressure for resilience against the exotic pathogens since they were introduced approximately 150 years ago. Current and historical population sizes, as well as past bottlenecks can be successfully detected by comparing mitochondrial DNA genomes (Avise et al. 1988). Thus, we expect significant differences in the genetic characteristics of pre-settlement bighorn populations of the eastern GYA and the populations that occupy the region today that should provide significant biological insight for the conservation and management of bighorn sheep.

5.3.3 Upcoming Research Efforts

In addition to our research, we have coordinated with the WAFWA Wild Sheep Working Group to interact with the broader community of researchers generally working on bighorn sheep genetic studies. In regard to future research, we have the following projects planned for the upcoming year:

1) Assess population genetics of herds

We will assess genetic relatedness within and between Montana herds, as well as relate genomic results to herd history to help inform future management. We will assess herd attributes that may impact herd genetics and produce a summary table, similar to Figure 2, to predict general herd genetic characteristics, including genetic differences and diversity, as well as evaluate likely genetic impacts of past augmentation efforts. Using the information provided by the sample size study, we genotyped 20-25 samples per herd to evaluate mean kinship (Table 1). To determine genetic relatedness within and between herds, we will apply the same methods used in the sample size study across all herds of interest. To determine genetic differences among herds, we will calculate genetic distances among individuals and herds, as well as conduct a principle component analysis and multidimensional scaling plot. To evaluate contributions of past translocations, we will generate STRUCTURE plots and a herd-based phylogenetic tree that can detect past translocations (Pickrell and Pritchard 2012, Raj et al. 2014). This effort can be helpful for evaluation of connectivity among herds and translocation planning, as we could use genetic markers to determine genetic contribution of past augmentations and interrelatedness among individuals and herds. Balancing the importance of both genetic variation and uniqueness can be important in determining translocation strategies, and comparing SNP genotypes can be useful in statewide planning to both conserve existing genetic sources and maximize heterozygosity.

2) Compare herd disease presence with genomics

Disease is an important factor that can impact herd population dynamics, and immune response to outbreaks may be at least partially determined by genetics. Since some bighorn sheep typically survive in herds that experience catastrophic die-offs associated with disease events, it is reasonable to expect that there has been strong selective pressure on bighorn sheep to survive outbreaks since the pathogens were introduced into native populations over a century ago. Genetic diversity has been linked with disease susceptibility in some species, and thus, we will assess genetic diversity and prevalence of disease in herds. The Ovine array also provides SNP coverage of genomic regions associated with immune response that are informative for bighorn sheep, including known locations of 136 out of 149 known MHC genes. These informative SNPs may allow for identification of variation related to respiratory disease susceptibility. Thus, we can use cross-species alignment of the Ovine array to look for important SNPs involved in disease resistance. We can look for genetic signatures of adaptation to pathogen presence by comparing herds that have hypothesized local adaptation to outbreaks and those that do not, to identify candidate genes important to the disease process in bighorn sheep. Information regarding the genetic basis of resistance can help inform selection of translocation source and recipient herds to potentially reduce probability of die-off events due to disease outbreaks. In addition, managers could use this research to assess genetic impacts of other actions intended to address disease. Therefore, the Ovine array provides a powerful tool that we can relate to disease information already collected through the statewide project and GYA Mountain Ungulate Project to potentially derive insight with significant implications for disease management.

3) Compare movement and habitat selection with genomics

In some cases, dispersal distance of wildlife has been linked to genetic heterozygosity, and researchers hypothesize that genotypes associated with low fitness, which can be caused by low heterozygosity or inbreeding, may disperse to increase genetic diversity and thus fitness of their offspring (Gueijman et al.

2013). This topic has been examined for mountain goats, and individuals that dispersed more widely had lower heterozygosity (Shafer et al. 2011). We can assess this possibility for bighorn sheep in Montana by relating genotypes to movement data. In addition, we will examine if SNP markers detected by the Ovine array are correlated with movement patterns in bighorn sheep, such as particular movement strategies, rates, or distances. GPS telemetry collected as part of the GYA Mountain Ungulate Project and the Montana Statewide Bighorn Sheep Study suggests that bighorn sheep display diverse seasonal movement strategies, including high elevation non-migrants, low elevation non-migrants, short-distance seasonal migrants (within a local mountain complex), and long-distance seasonal migrants (across multiple drainage systems). In some populations we document multiple movement strategies among animals within the same herd. Variation in movement strategy has also been observed in insects and birds, and research suggests that genetics may be correlated with migratory activity. Relating genetics to movement would be possible in Glacier National Park, the Beartooth-Absaroka complex, and in Montana bighorn sheep herds included in the statewide study with GPS data available.

Figure 33. *Bighorn sheep on Castle Reef summer range.*

Deliverables

Annual Reports

R.A. Garrott, K.M. Proffitt, J.J. Rotella, C.J. Butler. 2014, 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.

R. Garrott, 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.

R. Garrott, 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.

Thesis

C.J. Butler. 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.

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

Peer-reviewed Publications

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

E. Flesch, J. Rotella, J. Thompson, T. Graves, and R. Garrott. 2018. Evaluating sample size to estimate relatedness in the genomics era. Molecular Ecology Resources, DOI: 10.1111/1755-0998.12898.

B.J. Lowrey, C.J. Butler, R.A. Garrott, S.R. Dewey, W.H. Edwards, G.L. Fralick, J. Jennings-Gaines, H. Killion, D.E. McWhirter, H. Miyasaki, S.T. Stewart, K.S. White, P.J. White, and M.E. Wood. 2018. A survey of bacterial respiratory pathogens in native and introduced mountain goats. Journal of Wildlife Diseases 54:852-858.

C.J. Butler, W.H. Edwards, J.T. Paterson, K.M. Proffitt, J. Jennings-Gaines, H.J. Killion, M.E. Wood, J.M. Ramsey, E.S. Almberg, S.R. Dewey, D.E. McWhirter, A.B. Courtemanch, P.J. White, J.J. Rotella, and R.A. 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.

Lowrey B., R.A. Garrott, D.E. McWhirter, P.J. White, N.J. DeCesare, and S.T. Stewart 2018. Niche similarities among introduced and native mountain ungulates. Ecological Applications 28:1131-1142.

B. Lowrey, K. Proffitt, D. McWhirter, P.J. White, A. Courtemanch, S. Dewey, H. Miyasaki, K. Monteith, J. Mao, J. Grigg, C. Butler, E. Lula, and R. Garrott. In review. Contrasting seasonal movements in native and restored populations; a case for conserving migratory portfolios. Journal of Applied Ecology.

Professional Presentations

C.J. Butler, R.A. Garrott, J.J. Rotella. 2014. Correlates of recruitment in Montana bighorn sheep populations. Montana Chapter of the Wildlife Society 52nd Annual Conference, Bozeman, MT.

R.A. Garrott, J.J. Rotella, K.M. Proffitt, J. Ramsey, C.J. Butler. 2014. Montana's new statewide bighorn sheep research initiative. Montana Chapter of the Wildlife Society 52nd Annual Conference, Bozeman, MT.

R.A. Garrott, J.J. Rotella, K.M. Proffitt, J. Ramsey, C.J. Butler. 2014. Montana's new statewide bighorn sheep research initiative. 19th Biennial Northern Wild Sheep and Goat Council Symposium, Fort Collins, CO.

C.J. Butler, R.A. Garrott, H. Edwards, J. Ramsey, D. McWhirter, N. Anderson. 2014. A collaborative regional initiative to correlate respiratory pathogens demographic attributes of bighorn populations. 19th Biennial Northern Wild Sheep and Goat Council Symposium, Fort Collins, CO.

C.J. Butler, R.A. Garrott, K.M. Proffitt, J.J. Rotella. 2015. One year progress report for the Montana Statewide Bighorn Sheep Research Project. Montana Chapter of the Wildlife Society 53rd Annual Conference, Helena, MT.

R.A. Garrott, C.J. Butler, J. Ramsey, K.M. Proffitt. 2015. Approaches initiated to gain insight into respiratory disease in Montana's bighorn sheep herds. Montana Chapter of the Wildlife Society 53rd Annual Conference, Helena, MT.

C.J. Butler, R.A. Garrott, J.J. Rotella, D. McWhirter, H. Edwards, P.J. White, E. Almberg, J. Ramsey, K.M. Proffitt. 2015. Northern Rockies collaborative bighorn sheep research initiative. West-wide, Adaptive Disease Management Venture Oversight Committee Meeting, Salt Lake City, UT.

C.J. Butler, and R.A. Garrott. 2016. What does it all mean? Interpreting respiratory pathogen survey results for bighorn sheep management. Montana Chapter of the Wildlife Society 54nd Annual Conference, Missoula, MT.

E.P. Flesch, J.M. Thomson, R.A. Garrott, and T.A. Graves. 2016. An initial assessment of the potential of genomic analysis to help inform bighorn sheep management. Montana Chapter of the Wildlife Society 54nd Annual Conference, Missoula, MT.

M. R. Herrygers, J.R. White, J.M. Thomson, C.J. Butler, D.E. McWhirter, W.H. Edwards, K. Monteith, R.A. Garrott, and J.G. Berardinelli. 2016. Pregnancy rates, metabolites and metabolic hormones in bighorn sheep during and after the breeding season. Montana Chapter of the Wildlife Society 54nd Annual Conference, Missoula, MT.

J.R. White, M.R. Herrygers, J.M. Thomson, V. Copie, B. Tripet, C.J. Butler, D.E. McWhirter, K. Monteith, R.A. Garrott, and J.G. Berardinelli. 2016. Developing physiological profiles using nuclear magnetic resonance spectroscopy to inform bighorn sheep (*Ovis canadensis*) management. 2016. Montana Chapter of the Wildlife Society 54nd Annual Conference, Missoula, MT.

M.R. Herrygers, J.G. Berardinelli, J.R. White, V. Copie, B. Tripet, C.J. Butler, D.E. McWhirter, W.H. Edwards, K. Monteith, and R.A. Garrott. 2016. Pregnancy rates, metabolites, metabolic hormones, and application of nuclear magnetic resonance spectroscopy of metabolic profiles for assessing physiological status in bighorn sheep (*Ovis canadensis*). 20th Biennial Northern Wild Sheep and Goat Council Symposium, Moscow, ID.

R.A. Garrott, P.J. White, D.E. McWhirter, W.H. Edwards, K. Proffitt, J. Ramsey, M. Wood, E. Almberg, and J.J. Rotella. 2016. The Montana-Wyoming collaborative bighorn sheep research program. 20th Biennial Northern Wild Sheep and Goat Council Symposium, Moscow, ID.

E.P. Flesch, J.M. Thomson, R.A. Garrott, and T.A. Graves. 2016. An initial assessment of the potential of genomic analysis to help inform bighorn sheep management. 20th Biennial Northern Wild Sheep and Goat Council Symposium, Moscow, ID.

C.J. Butler, and R.A. Garrott. 2016. What Does It All Mean? Interpreting respiratory pathogen survey results for bighorn sheep management. 20th Biennial Northern Wild Sheep and Goat Council Symposium, Moscow, ID.

C.J. Butler , W.H. Edwards, J. Jennings-Gaines, H.J. Killion, M.E. Wood, J.T. Paterson, K.M. Proffitt, E.S. Almberg, P.J. White, D.E. McWhirter, J.J. Rotella, and R.A. Garrott. 2017. Imperfect Tests, Pervasive Pathogens, and Variable Demographic Performance: Thoughts on Managing Bighorn Sheep and Respiratory Disease after Five Years of Research. Montana Chapter of the Wildlife Society 55nd Annual Conference, Helena, MT.

C.J. Butler, K. Proffitt, W.H. Edwards, and R. Garrott. 2017. Addressing respiratory disease and bighorn sheep management through an integrated science program. Sheep in Montana - Domestic and Wild: The State of Things and What We Know About Disease

Feb. 2017, Helena, Montana.

C.J. Butler, R. Garrott, T. Paterson, J.J. Rotella, W.H. Edwards, J. Jennings-Gaines, H.J. Killion, D.E. McWhirter, M.E. Wood, K. Proffitt, E.S. Almberg, and P.J. White. 2017. Imperfect tests, pervasive pathogens and variable demographic performance: thoughts on managing bighorn sheep pneumonia. Wyoming TWS conference, December 2017, Jackson, Wyoming.

C.J. Butler, W.H. Edwards, J.T. Patterson, K.M. Proffitt, J. Jennings-Gaines, H.J. Killon, M.E. Wood, J.M. Ramsey, E.S. Almberg, S.R. Dewey, D.E. McWhirter, A.B. Courtemanch, P.J. White, J.J. Rotella, and R.A. Garrott. 2018. Detection error and demographic variability amid pervasive pneumonia pathogens in bighorn sheep. 21th Biennial Northern Wild Sheep and Goat Council Symposium, Whitefish, MT.

J. Cunningham, H. Burt, R. Garrott, K. Proffitt, C. Butler, E. Lula, J. Ramsey, and K. Carson. 2018. Evaluating success for an intramountain range transplant of bighorn sheep in southwestern Montana. 21th Biennial Northern Wild Sheep and Goat Council Symposium, Whitefish, MT.

E.P. Flesch, J.J. Rotella, J.M. Thomson, T.A. Graves, and R.A. Garrott. 2018. Evaluating sample size to estimate genomic relatedness in bighorn sheep populations. 21th Biennial Northern Wild Sheep and Goat Council Symposium, Whitefish, MT.

R.A. Garrott, R. Lambert, J. Berardinelli, J. Weeding, and K.M. Proffitt. 2018. An exploration of metabolomics to assess physiological states in bighorn sheep. 21th Biennial Northern Wild Sheep and Goat Council Symposium, Whitefish, MT.

B. Lowrey, R.A. Garrott, D.E. McWhirter, P.J. White, N.J. DeCesare, and S.T. Stewart. 2018. Niche similarities among introduced and native mountain ungulates. 21th Biennial Northern Wild Sheep and Goat Council Symposium, Whitefish, MT.

B. Lowery, R.A. Garrott, P.J. White, K.M. Proffitt, D.E. McWhirter, K.L. Monteith, H. Miyasaki, E.S. Lula, J. Grigg, A.B. Courtemanch, and C.J. Buter. 2018. Characterizing the seasonal movements of native and restored bighorn sheep: a case for conserving migratory portfolios. 21th Biennial Northern Wild Sheep and Goat Council Symposium, Whitefish, MT.

J.T. Patterson, C.J. Butler, J.J. Rotella, and R.A. Garrott. 2018. The implications of imperfect detection for establishing the presence/absence of pathogens: a web-based resource for managers. 21th Biennial Northern Wild Sheep and Goat Council Symposium, Whitefish, MT.

E.S. Lula, J.A. Cunningham, K.M. Proffitt, A.R. Litt, and R.A. Garrott. 2018. Is habitat constraining bighorn sheep (*Ovis canadensis*) distribution and restoration? A case study in the Greater Yellowstone Ecosystem. Montana Chapter of the Wildlife Society 54nd Annual Conference, Butte, MT.

R.A. Garrott, J.J. Rotella, K. Proffitt, C. Butler, E. Lula, E.P. Flesch, and B. Lowrey. 2018. Montana statewide bighorn sheep research project: a progress report. Montana Chapter of the Wildlife Society 54nd Annual Conference, Butte, MT.

E.P. Flesch, J.J. Rotella, J.M. Thomson, T.A. Graves, and R.A. Garrott. 2018. Evaluating sample size to estimate genomic relatedness in bighorn sheep populations. Montana Chapter of the Wildlife Society 54nd Annual Conference, Butte, MT.

B. Lowrey, R.A. Garrott, D.E. McWhirter, P.J. White, N.J. DeCesare, and S.T. Stewart. 2018. Niche similarities among introduced and native mountain ungulates. Montana Chapter of the Wildlife Society 54nd Annual Conference, Butte, MT.

C.J. Butler, W.H. Edwards, J.T. Patterson, K.M. Proffitt, J. Jennings-Gaines, H.J. Killon, M.E. Wood, J.M. Ramsey, E.S. Almberg, S.R. Dewey, D.E. McWhirter, A.B. Courtemanch, P.J. White, J.J. Rotella, and R.A. Garrott. 2017. Addressing respiratory disease to inform bighorn sheep management: a regional collaborative science program. Wyoming Chapter of The Wildlife Society annual meeting, Cody, WY.

R.A. Garrott, J.J. Rotella, K. Proffitt, C.J. Butler, E. Lula, E. Flesch, and B. Lowrey. 2018. The Montana-Wyoming bighorn sheep project: Integrated research to inform management. Wyoming Chapter of The Wildlife Sheep Foundation, Casper, WY.

C.J. Butler, W.H. Edwards, J.T. Patterson, K.M. Proffitt, J. Jennings-Gaines, H.J. Killon, M.E. Wood, J.M. Ramsey, E.S. Almberg, S.R. Dewey, D.E. McWhirter, A.B. Courtemanch, P.J. White, J.J. Rotella, and R.A. Garrott. 2018. Addressing respiratory disease to inform bighorn sheep management: a regional collaborative science program. The Wildlife Sheep Foundation Affiliate Chapter Meetings, Jackson, WY.

R.A. Garrott, J.J. Rotella, K. Proffitt, C.J. Butler, E. Lula, E. Flesch, and B. Lowrey. 2018. The Montana-Wyoming bighorn sheep project: Integrated research to inform management. The Wildlife Sheep Foundation Affiliate Chapter Meetings, Jackson, WY.

R.A. Garrott. 2018. Bighorn sheep and mountain goat conservation and ecology in the northern Rockies. Public lecture, Madison Valley, Aug 2018.

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Literature Cited

Avise, J. C., R. M. Ball, and J. Arnold. 1988. Current versus historical population sizes in vertebrate species with high gene flow: a comparison based on mitochondrial DNA lineages and inbreeding theory for neutral mutations. Molecular Biology and Evolution 5:331–344.

Bavananthasivam, J., R.P. Dassanayake, A. Kugadas,S Shanthalingam, D.R. Call, D.P. Knowles, and S. Srikumaran. (2012). Proximity-dependent inhibition of growth of Mannheimia haemolytica by Pasteurella multocida. Applied and Environmental Microbiology 78: 6683–6688.

Berger, J. 1990. Persistence of different-sized populations: an empirical assessment of rapid extinctions in bighorn sheep. Conservation Biology 4**:**91-98.

Besser, T.E., E.F. Cassirer, K.A. Potter, J. VanderSchalie, A. Fischer, D.P. Knowles, C. Herndon, F.R. Rurangirwa, G.C. Weiser, and S. Srikumaran. 2008. Association of *Mycoplasma ovipneumoniae* infection with population limiting respiratory disease in free-ranging Rock Mountain bighorn sheep (*Ovis canadensis canadensis)*. Journal of Clinical Microbiology *46:423-430.*

Besser, T.E., E.F. Cassirer, C. Yamada, K.A. Potter, C. Herndon, W.J. Foreyt, D.P. Knowles, and S. Srikumaran. 2012a. Survival of bighorn sheep (*Ovis canadensis*) commingled with domestic sheep (*Ovis aries*) in the absence of *Mycoplasma ovipneumoniae*. Journal of Wildlife Diseases *48:168-172.*

Besser, T.E., M.A. Highland, K. Baker, E.F. Cassirer, N.J. Anderson, J.M. Ramsey, K. Mansfield, D.L. Bruning, P. Wolff, J.P. Smith, and J.A. Jenks. 2012b. Causes of pneumonia epizootics among bighorn sheep, western United States, 2008-2010. Emerging Infectious Diseases 18:406-415.

Besser, T.E., E.F. Cassirer, M.A. Highland, P. Wolff, A. Justice-Allen, K. Mansfield, M.A. Davis, and W. Foreyt. 2013. Bighorn sheep pneumonia: sorting out the cause of a polymicrobial disease. Preventive Veterinary Medicine 108:85-93.

Besser, T. E., E. F. Cassirer, K. A. Potter, K. Lahmers, J. L. Oaks, S. Shanthalingam, S. Srikumaran, and W. J. J. P. O. Foreyt. 2014. Epizootic pneumonia of bighorn sheep following experimental exposure to Mycoplasma ovipneumoniae**.** 9:e110039.

Bleich, V. C., J. D. Wehausen, R. R. Ramey, and R. J. L. 1996. Metapopulation theory and mountain sheep; implication for conservation. Pages 353-373 *in* D. R. McCullough, editor. Metapopulations and Wildlife Conservation. Island Press, Washington, D.C., USA

Bolger, D.T., Newmark, W.D., Morrison, T.A. & Doak, D.F. 2008. The need for integrative approaches to understand and conserve migratory ungulates. Ecology Letters 11, 63–77.

Boyce, M. S., and L. L. McDonald. 1999. Relating populations to habitats using resource selection functions. Trends in Ecology & Evolution 14:268-272.

Boyce, M. S., P. R. Vernier, S. E. Nielsen, and F. K. A. Schmiegelow. 2002. Evaluating resource selection functions. Ecological Modelling 157:281-300.

Boyce, M. S., and J. S. Waller. 2003. Grizzly Bears for the Bitterroot: Predicting Potential Abundance and Distribution. Wildlife Society Bulletin 31:670-683.

Brakefield, P. M., and I. J. Saccheri. 1994. Guidelines in conservation genetics and the use of the population cage experiments with butterflies to investigate the effects of genetic drift and inbreeding. Pages 165–179 in. Conservation genetics. Springer. <http://link.springer.com/chapter/10.1007/978-3-0348-8510-2_14>. Accessed 19 May 2016.

Buckley, B. R., A. K. Andes, B. A. Grisham, and C. Brad Dabbert. 2015. Effects of broadcasting supplemental feed into roadside vegetation on home range and survival of female northern bobwhite. Wildlife Society Bulletin, 39:301-309.

Buechner, H.K. 1960. The bighorn sheep in the United States, its past, present, and future. Wildlife Monographs 4:3-174.

Butler, C.J., R.A. Garrott, and J.J. Rotella. 2013. Correlates of recruitment in Montana bighorn

sheep populations. Montana Department of Fish, Wildlife and Parks, Helena, USA.

Butler, C.J. 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.

Butler, C. J., W. H. Edwards, J. T. Paterson, K. M. Proffitt, J. E. Jennings-Gaines, H. J. Killion, M. E. Wood, J. M. Ramsey, E. S. Almberg, and S. R. Dewey. 2018. Respiratory pathogens and their association with population performance in Montana and Wyoming bighorn sheep populations. PloS one 13:e0207780.

Cassirer, E.F. and A.F.E. Sinclair. 2007. Dynamics of pneumonia in a bighorn sheep metapopulation. Journal of Wildlife Management 71:1080-1088.

Cassirer, E. F., R. K. Plowright, K. R. Manlove, P. C. Cross, A. P. Dobson, K. A. Potter, and P. J. Hudson. 2013. Spatio-temporal dynamics of pneumonia in bighorn sheep. Journal of Animal Ecology 82:518–528.

Cassirer, E.F., K.R. Manlove, E.S. Almberg, P.L. Kamath, M. Cox, P. Wolff, A. Roug, J. Shannon, R. Robinson, R.B. Harris, B.J. Gonzales, R.K. Plowright, P.J. Hudson, P.C. Cross, A. Dobson, and T.E. Besser. 2017. Pneumonia in Bighorn Sheep: Risk and Resilience. Journal of Wildlife Management. DOI: 10.1002/jwmg.21309.

Colwell, M. A., S. J. Hurley, J. N. Hall, and S. J. Dinsmore. 2007. Age related survival and behavior of snowy plover chicks. The Condor 109:638–647.

Cook, R.C., J.G. Cook, D.J. Vales, B.K. Johnson, S.M. Mccorquodale, L.A. Shipley, R.A. Riggs, L.L. Irwin, S.L. Murphie, B.L. Murphie, K.A. Schoenecker, F. Geyer, P.B. Hall, R.D. Spencer, D.A. Immell, D.H. Jackson, B.L. Tiller, P.J. Miller, L. Schmidtz. 2013. Regional and seasonal patterns of nutritional condition and reproduction in elk. Wildlife Monographs 184:1-44.

Dassanayake, R.P., S. Shanthalingam, C.N. Herndon, P.K. Lawrence, E.F. Cassirer, K.A. Potter, W.J. Foreyt, K.D. Clinkenbeard, and S. Srikumaran. 2009. *Mannheimia haemolytica* serotype A1 exhibits differential pathogenicity in two related species, *Ovis canadensis* and *Ovis aries*. Veterinary Microbiology *133:366-371.*

Dassanayake, R.P., S. Shanthalingam, C.N. Herndon, R. Subramaniam, P.K. Lawrence, J. Bavananthasivam, E.F. Cassirer, G.J. Haldorson, W.J Foreyt, F.R. Rurangirwa, D.P. Knowles, T.E. Besser, and S. Srikumaran. 2010. *Mycoplasma ovipneumoniae* can predispose bighorn sheep to fatal *Mannheimia haemolytica* pneumonia. Veterinary Microbiology 145:354-359.

Dassanayake, R.P., S. Shanthalingam, R. Subramaniam, C.N. Herndon, J. Bavananthasivam, G.J. Haldorson, W.J. Foreyt, J.F. Evermann, L.M. Herrmann-Hoesing. D.P. Knowles, and S. BavanSrikumaran. 2013. Role of *Biberstenia trehalosi*, respiratory syncytial virus, and parainfluenza-3 virus in bighorn sheep pneumonia. Veterinary Microbiology 162:166-172.

DeCesare, N.J., M. Hebblewhite, M. Bradley, K.G. Smith, D. Hervieux, and L. Neufeld. 2012. Estimating ungulate recruitment and growth rates using age ratios. Journal of Wildlife Management 76:144-153.

Demarchi, R. A., C. L. Hartwig, and D. A. Demarchi. 2000. Status of the California Bighorn Sheep in British Colombia. Wildlife Bulletin No. B-98.

Dinsmore, S. J., G. C. White, and F. L. Knopf. 2002. Advanced techniques for modeling avian nest survival. Ecology 83:3476–3488.

Eberhardt, L.L. 2002. A paradigm for population analysis of long-lived vertebrates. Ecology 83:2841-2854.

Edwards, V.L., J. Ramsey, C. Jourdonnais, R. Vinkey, M. Thompson, N. Anderson, T. Carlsen, and C. Anderson. 2010. Situational Agency Response to Four Bighorn Sheep Die-offs in Western Montana. Proceedings of the 17th Biennial Symposium Northern Wild Sheep and Goat Council 17:29-50.

Enk, T. A., H. D. Picton, and J. H. Williams. 2001. Factors limiting a bighorn sheep population in Montana following a dieoff. Northwest Sciences 75:280-291.

Epps, C. W., P. J. Palsbøll, J. D. Wehausen, G. K. Roderick, R. R. Ramey, and D. R. McCullough. 2005. Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep: Highways reduce genetic diversity. Ecology Letters 8:1029–1038.

Festa-Bianchet, M. 1986. Seasonal Dispersion of Overlapping Mountain Sheep Ewe Groups. The Journal of Wildlife Management 50:325-330.

Festa-Bianchet, M. 1988*a*. A Pneumonia epizootic in bighorn sheep, with comments on preventative management, in: Proceedings Bienniel Symposium of the Northern Wild Sheep and Goat Council. Northern Wild Sheep and Goat Coucil, pp. 66–76.

Festa-Bianchet, M. 1988*b*. Seasonal range selection in bighorn sheep: conflicts between forage quality, forage quantity, and predator avoidance. Oecologia 75:580-586.

Festa-Bianchet, M., T. Coulson, J.M. Gaillard, J.T. Hogg, and F. Pelletier. 2006. Stochastic predation events and population persistence in bighorn sheep. Proceedings of the Royal Society of B-Biological Sciences 45**:**1537-1543.

Fitzsimmons, N. N., S. W. Buskirk, and M. H. Smith. 1997. Genetic Changes in Reintroduced Rocky Mountain Bighorn Sheep Populations. The Journal of Wildlife Management 61:863–872.

Flesch, E. P., J. J. Rotella, J. M. Thomson, T. A. Graves, and R. A. Garrott. 2018. Evaluating sample size to estimate genetic management metrics in the genomics era. Molecular Ecology Resources.

Frankham, R. 1995. Inbreeding and extinction: a threshold effect. Conservation biology 9:792–799.

Frankham, R. 2007. Effective population size/adult population size ratios in wildlife: a review. Genetics Research 89:491–503.

Franklin, A. B., D. R. Anderson, R. J. Gutiérrez, and K. P. Burnham. 2000. Climate, habitat quality and fitness in northern spotted owl populations in Northwestern California. 70:539-590.

Garel, M., J.M. Gaillard, T. Chevrier, J. Michallet, D. Delorme, and G.V. Laere. 2010. Testing reliability of body size measurements using hind foot length in roe deer. Journal of Wildlife Management 74:1382-1386.

Geist, V. 1971. Mountain sheep; a study in behavior and evolution. University of Chicago Press, Chicago, Illinois USA.

Gilroy, J.J., Gill, J.A., Butchart, S.H.M., Jones, V.R., and Franco, A.M.A. (2016) Migratory diversity predicts population declines in birds. Ecology Letters, 19, 308–317.

Griffiths, J.R., Schindler, D.E., Armstrong, J.B., Scheuerell, M.D., Whited, D.C., Clark, R.A., Hilborn, R., Holt, C.A., Lindley, S.T., Stanford, J.A. & Volk, E.C. (2014) Performance of salmon fishery portfolios across western North America. Journal of Applied Ecology, 51, 1554–1563.

Gueijman, A., A. Ayali, Y. Ram, and L. Hadany. 2013. Dispersing away from bad genotypes: the evolution of Fitness-Associated Dispersal (FAD) in homogeneous environments. BMC Evolutionary Biology 13:125

Hebblewhite, M. & Merrill, E.H. (2009) Trade-offs between predation risk and forage differ between migrant strategies in a migratory ungulate. Ecology, 90, 3445–3454.

Hedrick, P. W., G. A. Gutierrez-Espeleta, and R. N. Lee. 2001. Founder effect in an island population of bighorn sheep. Molecular Ecology 10:851–857.

Hedrick, P. W., and P. S. Miller. 1992. Conservation genetics: techniques and fundamentals. Ecological Applications 30–46.

Hemming, J.E. 1969. Cemental deposition, tooth succession, and horn development as criteria of age in Dall sheep. The Journal of Wildlife Management 33:552-558.

Hogg, J.T., S.H.,Forbes, B.M. Steele, G. and Luikart. 2006. Genetic rescue of an insular population of large mammals. Proceedings of the Royal Society B: Biological Sciences 273: 1491–1499.

Jackson, C.H. 2011. Multi-state models for panel data: The msm Package for R. Journal of Statistical Software, 38(8), 1-29. URL http://www.jstatsoft.org/v38/i08/.

Jesmer, B. R., J. A. Merkle, J. R. Goheen, E. O. Aikens, J. L. Beck, A. B. Courtemanch, M. A. Hurley, D. E. McWhirter, H. M. Miyasaki, and K. L. J. S. Monteith. 2018. Is ungulate migration culturally transmitted? Evidence of social learning from translocated animals**.** 361:1023-1025.

Jiménez, J. A., K. A. Hughes, G. Alaks, L. Graham, and R. C. Lacy. 1994. An experimental study of inbreeding depression in a natural habitat. Science 266:271–273.

Johnson, H. E., L. S. Mills, T. R. Stephenson, and J. D. Wehausen. 2010. Population-specific vital rate contributions influence management of an endangered ungulate. 20:1753-1765.

Laake, J. L. 2013. RMark: an R interface for analysis of capture-recapture data with MARK. AFSC processed report 2013-01. Alaska Fisheries Science Center, NOAA, National Marine Fisheries Service, Seattle, Washington, USA.

Laforge, M. P., E. Vander Wal, R. K. Brook, E. M. Bayne, and P. D. McLoughlin. 2015. Process-focussed, multi-grain resource selection functions. Ecological Modelling 305:10-21.

Lande, R. 1988. Genetics and demography in biological conservation. Science 241:1455–1460.

Lande, R. 1994. Risk of population extinction from fixation of new deleterious mutations. Evolution 1460–1469.

Lawrence, P.K., S. Shanthalingam, R.P. Dassanayake, R. Subramaniam, C.N. Herndon, D.P. Knowles, F.R. Rurangirwa, W.J. Foreyt, G. Wayman, A.M. Marciel, S.K. Highlander, and S. Srikumaran. 2010. Transmission of *Mannheimia haemolytica* from domestic sheep (*Ovies aries)* to bighorn sheep (*Ovies canadensis)*: unequivocal demonstration with green fluorescent protein-tagged organisms. Journal of Wildlife Diseases 46:706-717.

Manichaikul, A., J. C. Mychaleckyj, S. S. Rich, K. Daly, M. Sale, and W.-M. Chen. 2010. Robust relationship inference in genome-wide association studies. Bioinformatics 26:2867–2873.

Manly, B. F., L. L. McDonald, D. Thomas, T. L. McDonald, and W. P. Erickson. 2002. Resource selection by animals: statistical design and analysis for field studies. 2nd edition. Klwer Academic Berlin, Germany.

Meyer, C. B., and W. Thuiller. 2006. Accuracy of resource selection functions across spatial scales. Diversity and Distributions 12:288-297.

Middleton, A.D., Kauffman, M.J., McWhirter, D.E., Cook, J.G., Cook, R.C., Nelson, A.A., Jimenez, M.D. & Klaver, R.W. (2013) Animal migration amid shifting patterns of phenology and predation: lessons from a Yellowstone elk population. Ecology, 94, 1245–1256.

Miller, D.A.W., B.L. Talley, K.R. Lips, E.H. Campbell Grant. 2012. Estimating patterns and drivers of infection prevalence and intensity when detection is imperfect and sampling error occurs: Detection in epidemiological studies. Methods in Ecology and Evolution 3: 850–859.

Miller, D.S., G.C. Weiser, K. Aune, B. Roeder, M. Atkinson, N. Anderson, T.J. Roffe, K.A. Keating, P.L. Chapman, C. Kimberling, J. Rhyan, P.R. Clarke. 2011. Shared Bacterial and Viral Respiratory Agents in Bighorn Sheep (*Ovis canadensis*), Domestic Sheep (*Ovis aries*), and Goats (*Capra hircus*) in Montana. Veterinary Medicine International 2011: 1–12.

Miller, D.S., E. Hoberg, G.C. Weiser, K. Aune, M. Atkinson, and C. Kimberling. 2012a. A review of hypothesized determinants associated with bighorn sheep (*Ovis canadensis*) die-offs. Veterinary Medicine International 2012:1-19.

Miller, J. M., Moore, S. S., Stothard, P., Liao, X., and Coltman, D. W. 2015. Harnessing cross-species alignment to discover SNPs and generate a draft genome sequence of a bighorn sheep (Ovis canadensis). BMC Genomics 16:397.

Mills, L. S., and P. E. Smouse. 1994. Demographic consequences of inbreeding in remnant populations. American Naturalist 412–431.

Milner-Gulland, E.J., Fryxell, J.M. & Sinclair, A.R.E. (2011) Animal Migration: A Synthesis. Oxford, NY: Oxford University Press.

Monello, R. J., D. L. Murray, and E. F. Cassirer. 2001. Ecological correlates of pneumonia epizootics in bighorn sheep herds. Canadian Journal of Zoology 79:1423–1432.

Mong, T. W., and B. K. Sandercock. 2007. Optimizing radio retention and minimizing radio impacts in a field study of upland sandpipers. Journal of Wildlife Management 71:971–980.

Nachman, M. W., H. E. Hoekstra, and S. L. D'Agostino. 2003. The genetic basis of adaptive melanism in pocket mice. Proceedings of the National Academy of Sciences 100:5268–5273.

Montana Department of Fish, Wildlife, and Parks . 2010. Montana bighorn sheep conservation strategy. Montana Department of Fish, Wildlife, and Parks, Helena, USA.

Olson, Z. H., D. G. Whittaker, and O. E. Rhodes. 2013. Translocation history and genetic diversity in reintroduced bighorn sheep. The Journal of Wildlife Management 77:1553–1563.

Parker, K.L., P.S. Barboza, M.P. Gillingham. 2009. Nutrition integrates environmental responses of ungulates. Functional Ecology 23:57-69.

Picton, H.D. and T.N. Lonner. 2008. Montana's wildlife legacy: decimation to restoration. Media Works Publishing, Bozeman, USA.

Plowright, R. K., K. Manlove, E. F. Cassirer, P. C. Cross, T. E. Besser, and P. J. J. P. O. Hudson. 2013. Use of exposure history to identify patterns of immunity to pneumonia in bighorn sheep (Ovis canadensis). 8:e61919.

Powell, L. 2007. Approximating variance of demographic parameters using the delta method: a reference for avian biologists. Condor 109:949-954.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ralls, K., J. D. Ballou, and A. Templeton. 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. Conservation biology 2:185–193.

Reed, D. H., and R. Frankham. 2003. Correlation between Fitness and Genetic Diversity. Conservation Biology 17:230–237.

Rolandsen, C.M., Solberg, E.J., Sæther, B.-E., Moorter, B.V., Herfindal, I. & Bjørneraas, K. (2016) On fitness and partial migration in a large herbivore – migratory moose have higher reproductive performance than residents. Oikos, 126, 547–555.

Rotella, J.J., S. J. Dinsmore, and T.L. Shaffer. 2004. Modeling nest-survival data: a comparison of recently developed methods that can be implemented in MARK and SAS. Animal Biodiversity and Conservation 27:187-204.

Ryder, T.J., E.S. Williams, K.W. Mills, K.H. Bowles, E. Thorne. 1992. Effect of pneumonia on population size and lamb recruitment in Whiskey Mountain bighorn sheep, in: Proceedings Bienniel Symposium of the Northern Wild Sheep and Goat Council. pp. 136–146.

Safaee, S., G.C. Weiser, E.F. Cassirer, R.R. Ramey, and S.T. Kelley. 2006. Microbial diversity in bighorn sheep revealed by culture-independent methods. Journal of Wildlife Diseases 42:545–555.

Sasaki, T., and D. Biro. 2017. Cumulative culture can emerge from collective intelligence in animal groups. Nature Communications 8:15049.

Schindler, D.E., Armstrong, J. B., & Reed, T. E. (2015). The portfolio concept in ecology and evolution. Frontiers in Ecology and the Environment, 13, 257–263.

Schindler, D.E., Hilborn, R., Chasco, B., Boatright, C.P., Quinn, T.P., Rogers, L.A. & Webster, M.S. (2010) Population diversity and the portfolio effect in an exploited species. Nature, 465, 609–612.

Schwartz, M. K., and K. S. McKelvey. 2008. Why sampling scheme matters: the effect of sampling scheme on landscape genetic results. Conservation Genetics 10:441–452.

Shafer, A. B. A., J. Poissant, S. D. Côté, and D. W. Coltman. 2011. Does reduced heterozygosity influence dispersal? A test using spatially structured populations in an alpine ungulate. Biology Letters rsbl20101119.

Seber, G. A. F. 1982. The estimation of animal abundance and related parameters. 2nd ed. Chapman, London and Macmillan, New York.

Shackleton, D. M., C. C. Shank, and B. M. Wikeem. 1999. Natural history of Rocky Mountain and California bighorn sheep. Pages 78-138 *in* R. Valdez and P. R. Krausman, editors. Mountain sheep of North America. University of Arizona Press, Tucson, Arizona, USA.

Shanthalingam, S.A. Goldy, J. Bavananthasivam, R. Subramaniam, S.A. Batra, A. Kugadas, et al. 2014. PCR assay detects *Mannheimia haemolytica* in culture-negative pneumonic lung tissues of bighorn sheep (*Ovis canadensis*) from outbreaks in the western USA, 2009-2010. Journal of Wildlife Diseases 50: 1–10.

Singer, F. J., V. C. Bleich, and M. A. Gudorf. 2000*a*. Restoration of bighorn sheep metapopulations in and near western national parks. Restoration Ecology 8:14-24.

Singer, F. J., E. Williams, M.W. Miller, and L.C. Zeigenfuss. 2000*b*. Population Growth, Fecundity, and Survivorship in Recovering Populations of Bighorn Sheep. Restoration Ecology 8: 75–84.

Stephenson, T.R., J.D. Wehausen, A.P. Few, D.W. German, D.F. Jensen, D. Spitz, K. Knox, B.M. Pierce, J.L. Davis, J. Ostergard, and J. Fusaro. 2012. 2010-2011 annual report of the Sierra Nevada Bighorn Sheep Recovery Program: a decade in review. California Department of Fish and Game.

Toweill, D.E., and V. Geist. 1999. Return of Royalty: Wild sheep of North America. Boone and

Crockett Club and Foundation for North American Wild Sheep, Missoula, MT.

Webster, M.S., Marra, P.P., Haig, S.M., Bensch, S., and Holmes, R.T. (2002) Links between worlds: Unraveling migratory connectivity. Trends in Ecology & Evolution, 17, 76–83.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-Statistics for the Analysis of Population Structure. Evolution 38:1358 –1370.

White, G. C. and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46 Supplement:120–138.

Wilcove, D.S. & Wikelski, M. (2008) Going, going, gone: Is animal migration disappearing? PLoS Biol, 6, e188

Wild, M.A., and M.W. Miller 1994. Effects of modified cairy and blair medium on recovery on nonhemolytic *Pasteurella haemolytica* from Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) pharyngeal swabs. Journal of Wildlife Diseases 30:16–19.

Willi, Y., J. van Buskirk, and A. A. Hoffmann. 2006. Limits to the Adaptive Potential of Small Populations. Annual Review of Ecology, Evolution, and Systematics 37:433–458.

Zannése, A., A. Baïsse, J.M. Gaillard, A.J.M. Hewison. K. Saint-Hilaire, C. Toïgo, G.V. Laere, and N. Morellet. 2006. Hind foot length: an indicator for monitoring roe deer populations at a landscape scale. Wildlife Society Bulletin. 34:351-358.