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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 antelope. The most obvious factor hindering further bighorn sheep restoration is continued, widespread expression of respiratory disease. However, high predation rates, habitat loss, poor genetic diversity and "unique factors" are also cited as factors limiting bighorn sheep populations (Festa-Bianchet *et al.* 2006, Hogg *et al.* 2006, Johnson *et al* 2010). Given multiple potential limiting factors, managers often face difficult decisions regarding bighorn sheep conservation with insufficient information on the drivers of demographic processes. The small size of many populations makes management decisions even more challenging by heightening the consequences of these decisions. However, there still exist numerous populations that, for unknown but presumably tangible reasons, are well distributed, robust and require minimal management intervention. Thus, additional information regarding general bighorn sheep ecology would be useful for management agencies to have more confidence in predicting outcomes of different management actions.

As an initial start to establishing a statewide bighorn sheep research project, Montana Fish, Wildlife and Parks (MFWP) supported a six-month contract to Montana State University (MSU) during fiscal year 2012/2013 to consolidate all 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 lab 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 fourth produced by this research project. All findings reported herein should be considered preliminary, as data collection and analysis are ongoing.

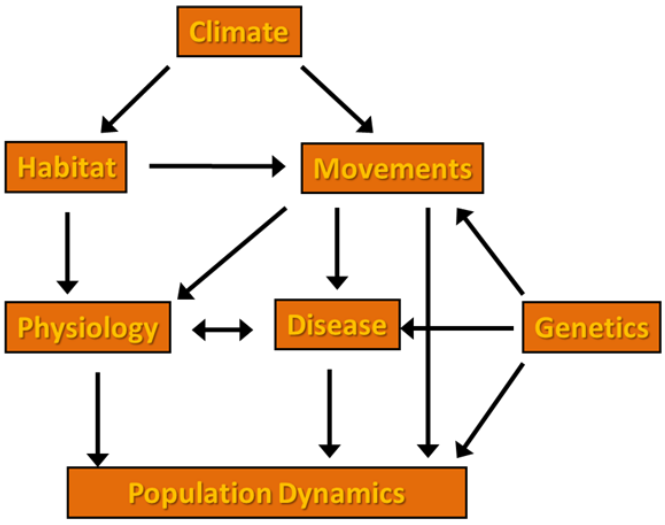


Figure 1. *Conceptual diagram of integrated study design of the Montana bighorn sheep research program as well as the Greater Yellowstone Area Mountain Ungulate Project which is also led by the same core research team. Where appropriate the data from the both research programs will be combined to provide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

Locations

Research conducted under this grant is 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), 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 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).

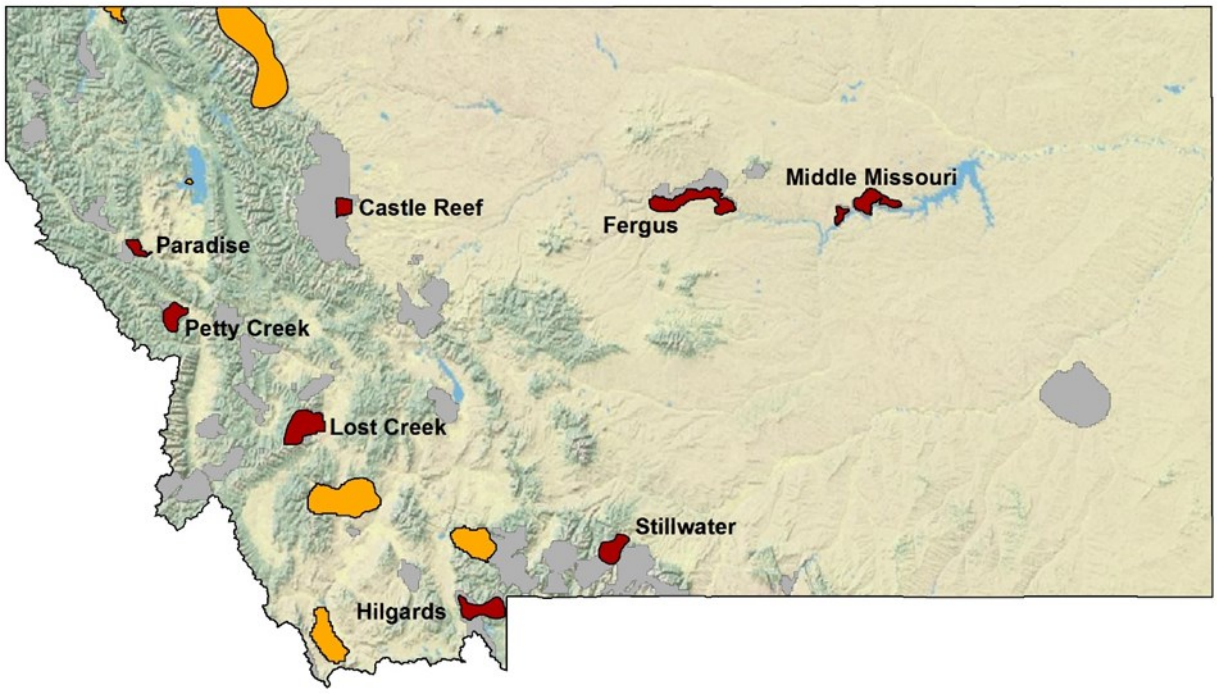


Figure 2. *Estimated distributions of bighorn sheep populations within Montana. Red polygons represent the eight Montana Bighorn Sheep Study populations. Polygons shaded in orange represent ancillary populations from which additional data are being incorporated into the statewide study to enhance biological 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 4 of 6-year study)

During the fourth 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) Explore advanced serum assay technologies to gain insight into the health and physiological status of bighorn sheep
4) Assess movement patterns of adult female bighorn sheep in sampled herds to determine seasonal patterns, migratory pathways, connectivity/isolation among herds, and habitat use
5) Collect data to estimate demographic rates of each herd included in the statewide study
6) Collect and provide samples for a bighorn sheep genetics study and complete preliminary genomic analyses

Objective # 1: *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 and MFWP staff transport a bighorn sheep captured during Hilgard drop net capture January 2018, graduate student Ethan Lula collaring a chemically immobilized ewe in the Stillwater population, and Quicksilver Air Inc. capturing bighorn sheep in the Spanish Peaks as part of collaborative MFWP sampling*

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.

The primary sampling objective in this fourth year of study was to resample 30 animals from the Petty Creek population and instrument 10 additional adult females with paired GPS/VHF (Models: TGW4400 [GPS] and MOD400 [VHF], Telonics Inc, Mesa, Arizona) collars. Once completed, this effectively met the broader study sampling objectives, and finalized bighorn sheep capture efforts for this research (Table 1).

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.

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.

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.

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.

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.

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.

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.

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 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.

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.

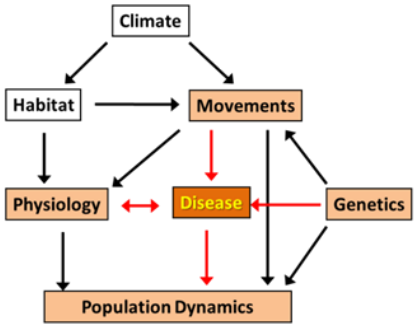
Table 1. *Sampling accomplishments to date in each of the eight study populations. Increased sampling in the Hilgard and Fergus populations resulted from coordination with MFWP during translocation captures. The increased number of radio-collars deployed in the Hilgard population also resulted from coordination 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	17
Petty Creek		0	17	0	21	**38**	24	22
Lost Creek		13	6	24	0	**43**	27	22
Hilgard *	29	50	35	31	32	**177**	32	23
Castle Reef		23	7	38	0	**68**	29	20
Fergus		60	0	30	0	**90**	40	33
Stillwater		16	3	11	1	**31**	21	16
Middle Missouri			19	20	0	**39**	20	14
TOTAL	**29**	**192**	**87**	**184**	**54**	**546**	**218**	**167**

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

Figure 4. *Bighorn sheep positioned 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 have been depopulated after many years of chronically poor performance following respiratory disease epizootics (Carlsen and Erickson, 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 very small enclosures. Commingling of free-roaming animals in rangeland and mountainous setting 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 commingling.

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 number of epizootics have certainly been caused by introduction of novel pathogens, 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; D.S. Miller et al., 2012, 2011, H. Edwards *unpublished data*, R. Garrott *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 (D. S. 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.

The respiratory pathogen aspect of this research effort aims to develop a framework to address these hypotheses and consists of two main initiatives. One initiative is focused on assessing detection probability for the different respiratory pathogens of interest in order to provide recommendations to management agencies for sampling intensity needed to reliably characterize pathogen communities given different sampling protocols. 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. The second initiative is an assessment of respiratory pathogen communities in numerous populations displaying a range of demographic performance to determine whether there are any associations between certain pathogen communities hosted by the population and poor demographic performance. Lack of associations would suggest that respiratory disease can be managed without the onerous and perhaps unattainable task of eradicating pathogens, and provide indirect evidence that disease expression can be caused by pathogens already present in a population.

2.1 Pathogen Sampling Methods

The Montana Bighorn Sheep Study adopted sampling methodologies that improve knowledge of both *Pasteurellaceae* and *M. ovipneumoniae* in study populations. Tonsil swabs were collected to assess presence of *Pasteurellaceae* organisms and the toxic agent they produce (leukotoxin) while nasal swabs were collected to assess presence of *M. ovipneumoniae* (Figure 5). In order to assess detection probability of the different pathogens, multiple tonsil and nasal swabs were collected from a subsample of captured animals. Further, multiple handling and testing protocols have been employed for both nasal and tonsil swabs to assess detection probability of the different protocols. Samples were collected using the same method as swabs collected from animals as part of the Greater Yellowstone Area Mountain Ungulate Project (MUP), allowing data collected by the two research programs to be pooled. Additionally, MtFWP collected samples using the same method and shared data from those samples in order to augment research sampling.

Figure 5. *Field sampling techniques. A. Collecting nasal swab for M. ovipneumoniae detection. B. Collection of blood for detection of M. ovipneumoniae antibodies. C. Collecting tonsil swab for detecting Pasteurellaceae species. D. Plating tonsil swab onto Columbia Blood Agar plate at the animal.*

All *Pasteurellaceae* pathogens were detected using one set of five diagnostic protocols and *M. ovipneumoniae* was detected using a different set of three diagnostic protocols (Butler 2017). Diagnostic tests offered by a commercial fee-for-service (FFS) laboratory (Washington Animal Disease Diagnostic Laboratory-WADDL) were used to detect and identify respiratory pathogens for four protocols (FFS protocols). Diagnostic tests conducted at a non-commercial diagnostic laboratory (Wyoming Game and Department Wildlife Health Laboratory-WGFD) were used to detect and identify respiratory pathogens for three protocols (non-FFS protocols). A non-FFS diagnostic test also was conducted at WADDL as part of protocol development. For *Pasteurellaceae,* all FFS protocols detected pathogens by culture. The FFS *M. ovipneumoniae* protocol detected this pathogen by PCR. Non-FFS protocols used PCR (sometimes in conjunction with culture) to detect each pathogen, with the exception of *P. multocida,* which was only detected by culture. Exposure of study populations to *M. ovipneumoniae* was also assessed by sending serum from each animal to Washington Animal Disease Diagnostic Laboratory (WADDL) to detect antibodies against M. ovipneumoniae using an ELISA.

2.2 Assessing Pathogen Detection Probability-Results

One to four tonsil and nasal swabs were collected by trained personnel from bighorn sheep sampled in nine free-ranging populations in Montana, ten free-ranging populations in Wyoming, and one captive population in Wyoming. 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 completed and summarized in detail in the 2016 PR report and published in the peer-reviewed literature this year (Carson et al. 2017). An abbreviated summary of the results of this work follows.

Pathogen detection

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 diagnostics protocols for *Pasteurellaceae* available from the Wyoming Game and Fish Department Wildlife Health Laboratory uniformly detected pathogens at higher rates than the culture-based protocol with estimated detection probabilities for *Mannheimia sp., 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 for bighorn sheep.

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, ~39% would test positive once, and ~7% would not test positive either time.

4) Low detection probability of *Pasteurellaceae* pathogens using FFS 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 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

2.3 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 is 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 were hypothesized and, given the long history of domestic sheep grazing in the two states, it was hypothesized that the respiratory pathogens are resident in the majority of study populations.

2.4 Methods Used for Assessment of Respiratory Pathogen Communities and Demographic Attributes

Our survey included 20 bighorn sheep populations that occupy a wide range of habitat types across Montana and Wyoming and have varying disease and management histories. The areas inhabited by these populations represent much of the variation in habitat types that are realized across the species' range. Study populations represent native populations (n=12), restored populations (n=7), as well as native populations that have been augmented in efforts to increase population size (n=1). Connectivity levels varies among study populations, which include well-connected metapopulations (n=12), populations thought to have limited connectivity (n=3), and populations thought to be mostly isolated (n=5). Population structure, in terms of number of subpopulations, ranged from one sub-population to over ten sub-populations. Respiratory disease histories in the populations include no documented history of disease (n=10), a single all-age epizootic (n=5) and multiple all-age epizootics (n=5).

A total of 811 individual bighorn sheep from 20 populations in Montana and Wyoming were captured and sampled between December and March of each year from 2012-2017. Captured animals were live-sampled for presence of Mycoplasma ovipneumoniae, leukotoxigenic M. haemolytica or Mannhemia glucosida (combined as M. haemolytica as these two species are not reliably differentiated by available diagnostic tests), leukotoxigenic Mannheimia ruminalis or Mannheimia spp. (combined as Mannheimia spp. because the ability to identify Mannheimia ruminalis from other species was not available until the final year of data collection), leukotoxigenic B. trehalosi, and P. multocida. Demographic data used in this analysis were primarily collected by Montana Department of Fish Wildlife and Parks (FWP) or Wyoming Game and Fish Department (WGFD) personnel as part of regular bighorn sheep population surveys from 2006 to 2017. Demographic performance of study populations was characterized by their mean recruitment rates. Recruitment rates were indexed as the ratio of lambs to adult females (lamb: ewe ratio) that were counted in the classification surveys. Populations that experienced all-age epizootics within the range of years that recruitment data were collected were split into separate populations (before epizootic and after epizootic) for analyses if pathogen data were collected before and after epizootics; if pathogen data were not collected before epizootics, demographic data preceding the die-off were excluded from analysis.

2.5 Respiratory Pathogen Communities Resident in Sampled Bighorn Populations-Results

Five of the six respiratory pathogens were detected in over 70% of the study populations. M. ovipneumoniae was detected in 16 of 20 (80%) study populations and was not detected in the Galton, Perma-Paradise, Petty Creek, or Middle Missouri Breaks populations (Figure 5). Leukotoxigenic M. haemolytica was detected in 15 of 20 (75%) study populations (not detected in Galton, Lost Creek, Middle Missouri Breaks, Stillwater or Dubois Badlands populations) and leukotoxigenic Mannheimia spp. was detected in 17 of 20 (85%) study populations (not detected

in Galton, Highlands, or Upper Yellowstone populations). P. multocida was detected in 14 of 20 (70%) study populations (not detected in Galton, Petty Creek, Highlands, Choteau-Blaine, Middle Missouri Breaks, or Dubois Badlands populations) and leukotoxigenic B. trehalosi was detected in 10 of 20 (50%) study populations including all Wyoming study populations and two Montana study populations (Stillwater, Hilgard) that are adjacent to Wyoming. LktA was detected in all study populations. All populations that hosted M. ovipneumoniae also hosted leukoxigenic Pasteurellaceae, while leukotoxigenic Pasteurellaceae or lktA were detected in four populations (Galton, Perma-Paradise, Petty Creek, Middle Missouri Breaks) where M. ovipneumoniae was not detected (Figure 6).

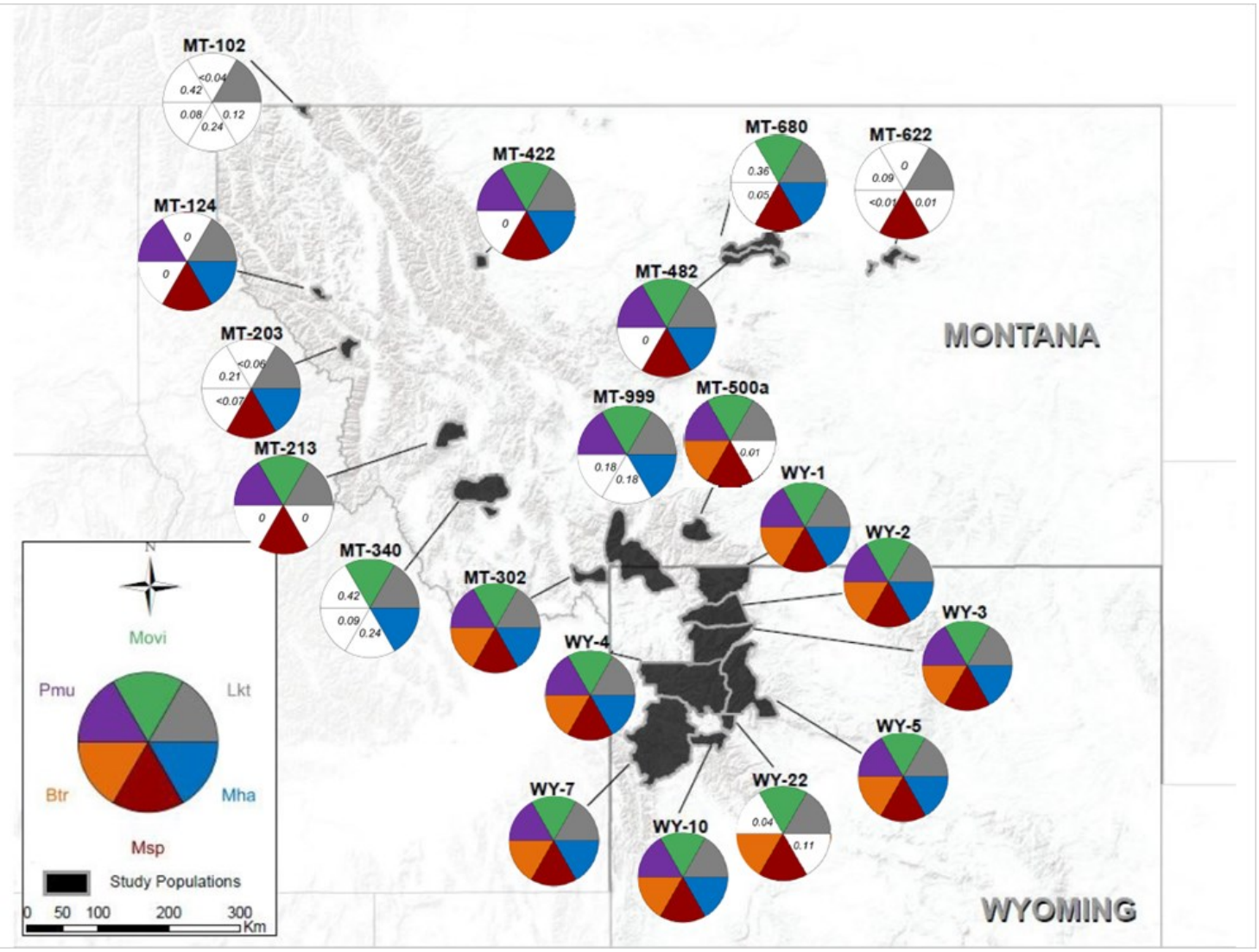


Figure 6. *Map of 20 bighorn sheep study populations and detected respiratory pathogen communities. All sections of the pie-charts are fixed to equal size and represent whether the respective pathogens were detected in the study population. The key for pathogen abbreviations are as follows: Movi= Mycoplasma 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 power of the employed sampling methodologies to detect the pathogen at 10% prevalence in the population.*

These results demonstrate the pervasiveness of the respiratory pathogens among the 20 bighorn sheep populations that were investigated. Intensive sampling found the two most cited agents responsible for respiratory disease, M. ovipneumoniae and leukotoxigenic Pasteurellaceae, were both present in at least 80% of the study populations within which nearly 7,630 bighorn sheep live. These pathogens are hosted in bighorn sheep populations that inhabit matrices of public and private land as well as those that inhabit the most remote areas of the continental United States that offer the most stringent protections against humans and livestock. These findings demonstrate that the combination of bighorn sheep ecology and anthropogenic use of the landscape results in a propensity for bighorn sheep across a variety of landscapes, including national parks and wilderness areas, to be exposed to these respiratory pathogens. It is not known how long the study 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, the fact that 80% percent of the study populations, including the most abundant populations, host both M. ovipneumoniae and leukotoxigenic Pasteurellaceae, highlights the substantial, landscape-level, challenges that wildlife agencies have faced and continue to face in preventing the spread of pathogens to bighorn sheep populations.

2.6 Respiratory Pathogen Communities and Recruitment-Results

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 were *M. ovipneumoniae* and leukotoxigenic *Pasteurellaceae* were and were not detected are shown in Figure 7.

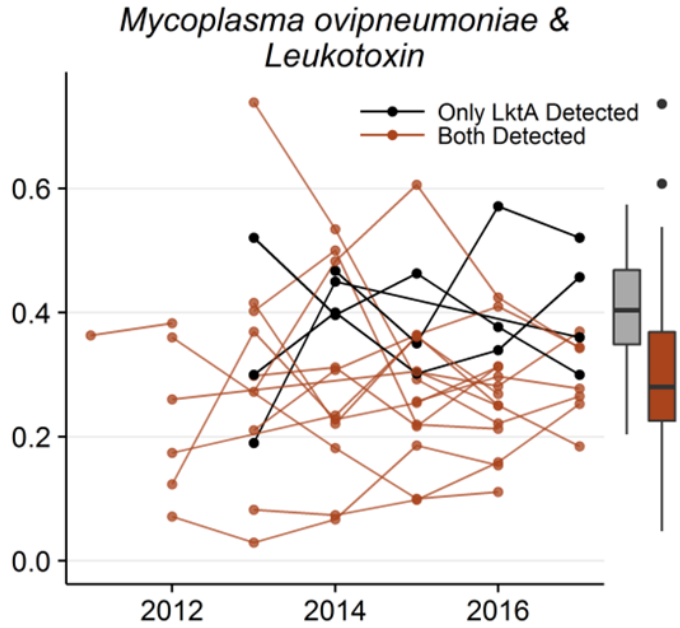


Figure 7. *Lamb:ewe ratios of 14 bighorn sheep populations in Montana and Wyoming where both M. ovipneumoniae and leukotoxigenic Pasteurellaceae were and were not detected.*

2.8 No Convincing Association Between Respiratory Pathogen Communities Hosted in Bighorn Sheep Populations and Demographic Performance

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 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 pathogen, the host, and the environment (the classic epidemiologic triad), which is the tradition model of infectious disease causation (Figure 8). This is likely the most challenging hypothesis to evaluate, but our integrated bighorn sheep research program is well positioned to begin addressing this hypothesis.

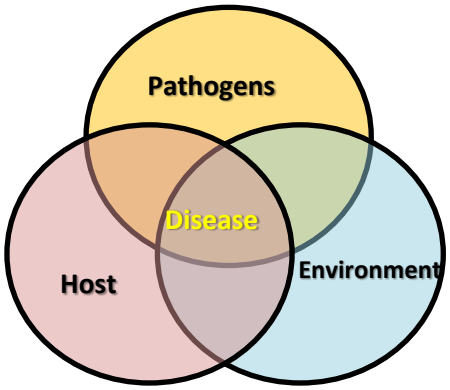
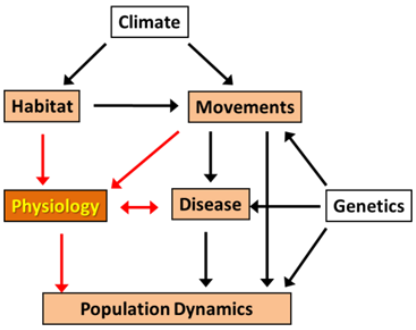


Figure 8. *The tradition model of infectious disease causation involving interactions between the host, the pathogen community, and the environment which may explain the variation in disease expression and demographic performance of bighorn populations that host the primary pathogens associated with respiratory disease in bighorn sheep.*

Objective # 3: *Explore advanced serum assay technologies to gain insight into the health and physiological status of bighorn sheep*



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

3.1 Metabolomics

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

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

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

3.2 Developing a Bighorn Sheep Metabolomics Data Set

Over the past 3 years we have assayed 949 serum samples collected during captures of 14 wild bighorn sheep herds in Montana and Wyoming in conjunction with the Montana Statewide Bighorn Sheep Research Project and the GYA Mountain Ungulate Research Project. Most of the Montana samples came from animals in the Castle Reef, Fergus, Lost Creek, Paradise, and Taylor-Hilgard herds, with most Wyoming samples originating from animals in the Absaroka, Dubois, and Jackson herds. In addition, we assayed serum samples from two captive bighorn sheep research facilities in Colorado and South Dakota and also included samples from a small experimental flock of domestic sheep (Rambouillet) used by Rashell Lambert for her thesis research. These samples represented animals suspected of experiencing a range of physiological conditions including gradients in dietary intake, degree and duration of starvation, and transitions from a healthy to a disease (pneumonia) state.

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

3.3 Evaluating Analytical Techniques for the Metabolomics Data Set

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

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

were captured under baited drop nets. The dietary intake from range forage for these animals was modestly augmented for appropriately 2 weeks prior to capture due to the daily baiting of the dropnet sites so we assume these animals had an overall higher daily dietary intake than the net-gunned bighorn sheep and, hence, these samples were labeled 'low'. The samples from each group were split evenly into a training dataset that was used with various analytical procedures to build models to discriminate among the 4 dietary intake groups, and a training dataset that was used to validate the models. We incorporated a total of 52 metabolites that were detected in all samples into this analysis. In addition, we included 5 specific metabolite ratios commonly considered in physiological assessments into the dataset resulting in a metabolic profile of 57 potential predictors of dietary intake.

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

Table 2. *A comparison of three analytical techniques (boosting, random forest, partial least squares-discriminant analysis) to discriminate among four categories of dietary intake (high, moderate, low, very low) based on the metabolic profiles of 518 captive bighorn and domestic sheep and wild bighorn sheep.*

BOOSTING	Reference			
Predication	High	Moderate	Low	Very Low
High	3	0	0	0
Moderate	0	13	0	0
Low	0	0	39	0
Very Low	5	0	14	184
92.3% correct classification				

RANDOM FOREST	Reference			
Predication	High	Moderate	Low	Very Low
High	4	0	0	0
Moderate	0	13	0	0
Low	1	0	35	0
Very Low	3	0	17	172
91.4% correct classification				

PLS-DA	Reference			
Predication	High	Moderate	Low	Very Low
High	4	0	0	1
Moderate	0	13	0	0
Low	0	0	37	5
Very Low	4	0	15	166
89.8% correct classification				

3.4. Identifying Informative Metabolites For Indexing Dietary Intake

The key outcome of this metabolomics analysis is the identification of the most important metabolites (biomarkers) for discriminating among the four categories of dietary intake. Figure 7 presents the relative influence of the 15 top-ranked metabolites and metabolite ratios from the boosting model that provided the best discrimination among the four categories of dietary intake. These results suggest that approximately 10 of the 57 potential predictors evaluated contribute most of the information that results in the strong discrimination among the categories of dietary intake. These biomarkers that provide good discrimination among the categories of dietary intake are associated with a variety of physiological processes which is only the initial step in the development of the envisioned 'health panel'. The ultimate goal of this work is to identify specific biomarkers for each physiological process of interest and interpret the quantitative values of the biomarkers with respect to that process. Figure 8 provides a visualization of the distribution of the quantitative values for a sample of the biomarkers identified as top predictors, demonstrating the potential of moving from qualitative to quantitative assessments.

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

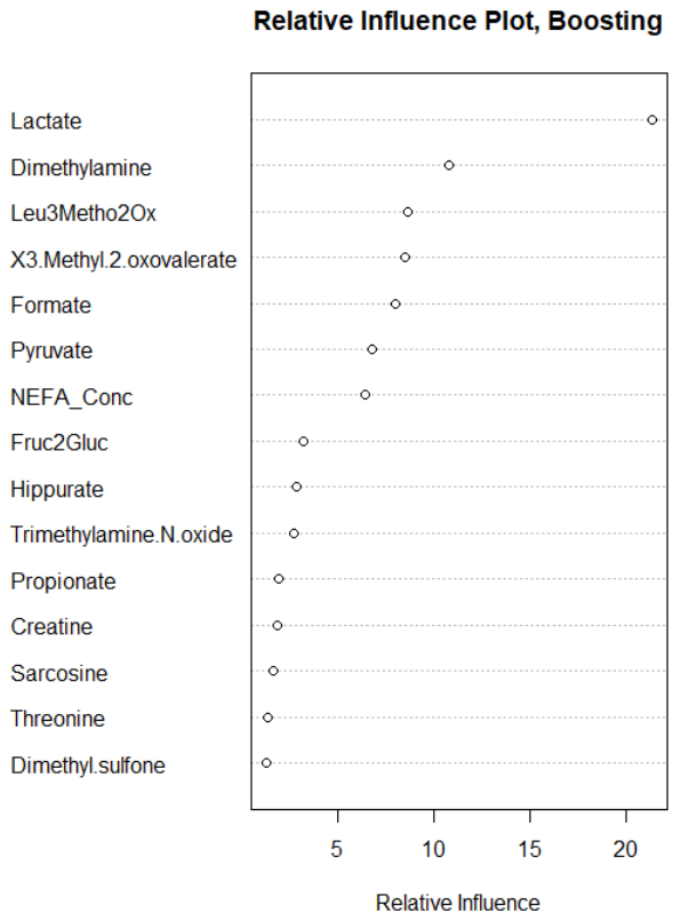


Figure 7. *The relative influence of the top 15 metabolites and metabolite ratios from the boosting model that provided the best discrimination among the four categories of dietary intake.*

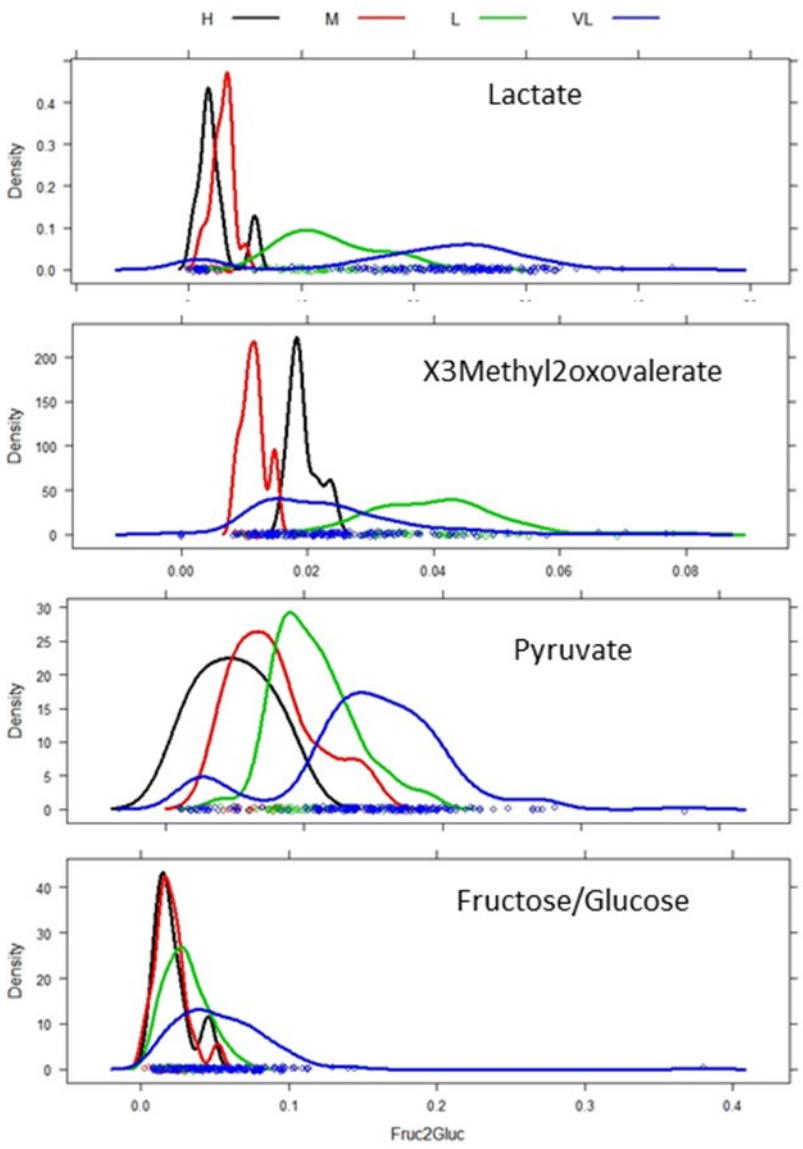
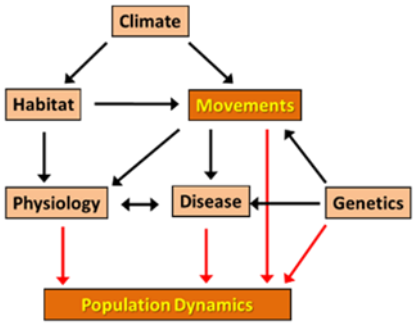


Figure 8. *The distribution of values for four of the metabolites and metabolite ratios identified by the boosting model as biomarkers for discriminating among the four categories of dietary intake.*

Objective #4:

Assess movement patterns of adult female bighorn in sampled herds to determine seasonal patterns, migratory pathways, connectivity/isolation among herds, and habitat use.



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 would begin transmitting, so as to continue survival monitoring for an additional 3-5 years. Field crews, using telemetry would navigate to the dropped GPS collar and retrieve the stored data for analysis.

Figure A. *Graduate student Ethan Lula using radio-telemetry to recover store-on-board GPS collar in the Lost Creek population.*

A total of 197 adult female bighorn sheep have been instrumented with GPS radio collars (Table 3). Of these, data has been retrieved from 116, and 4 have either failed or are considered unrecoverable. Fifty-five store-on-board GPS collars remain on animals and are scheduled for recovery during the spring of 2018 and summer of 2019. Twenty-two animals in the Castle Reef and Hilgard populations were instrumented with Iridium satellite linked radio collars and their location and survival are being monitored in real time.

Table 3. *The number and current status of GPS collars deployed among study populations since 2014.*

Study Population	Total	Recovered	Failed/ Unrecoverd	Iridium	Remaining
Castle Reef	28	16	1	10	1
Fergus	25	15			10
Lost Creek	27	18			9
Middle Missouri	20	6			14
Paradise	25	17	1		7
Petty Creek	24	14	1		9
Hilgard	27	15		12	0
Stillwater	21	15	1		5
TOTAL	**197**	**116**	**4**	**22**	**55**

Among all study populations, a total of 425,355 GPS locations have been collected, averaging about 59,000 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 4). All study populations experienced good overall GPS precision, specified as being ≥75% successful fixes. The Stillwater population experienced the highest proportion of censored locations (13.5%). After censoring, a total of 402,740 locations are available for spatial analysis. GPS data from the 55 unrecovered collars will be incorporated in future analyses, and used to validate initial results.

Table 4. *Summary of collected bighorn sheep GPS locations for the eight study populations. Note: The sample size for Middle Missouri population will be comparable after May 2018 when the remaining collars are scheduled to release from the animals.*

Study Population	Number of Individuals	GPS Location Data			GPS Fix Success
		Good	Censored	Total	
Castle Reef	16	55,097	3,492	58,589	93.7
Fergus	15	62,173	2,041	64,214	96.7
Lost Creek	18	57,083	1,524	58,607	97.3
Middle Missouri	5	10,807	270	11,077	97.5
Paradise	17	60,091	2,510	62,601	95.8
Petty Creek	14	48,316	3,289	51,605	93.2
Hilgard	15	58,019	2,576	60,595	95.6
Stillwater	15	51,154	6,913	58,067	86.5
TOTAL	**115**	**402,740**	**22,615**	**425,355**	

4.2 Initial comparison of population movement strategies

We averaged the daily elevation of individuals within each study population to determine the population's migration strategy (Figure 9**).** Initial results clearly show that four study populations of bighorn sheep (Castle Reef, Stillwater, Hilgard, and Lost Creek) are seasonal migrants, wintering at a low elevations, conducting a spring migration to high elevation summer range and returning to winter range during the fall. The remaining four populations (Paradise, Petty Creek, Fergus and Petty Creek) can be classified as non-migratory, occupying the same general area year long. There may be some short seasonal use of relatively close geographic features (Figure 10) within Petty Creek and Middle Missouri populations, but further analysis is needed. Furthermore, among migratory populations, while there are distinguishable seasonal ranges, there are also periodic movements to lower elevation during the summer (Figure 11). Exploration of the GPS data reveal that many of these movements are by individuals briefly returning to winter range. Further movement analysis will continue through 2018.

Figure 9. *Mean daily elevation (color) plotted over individual daily elevation (grey) for each study population.*

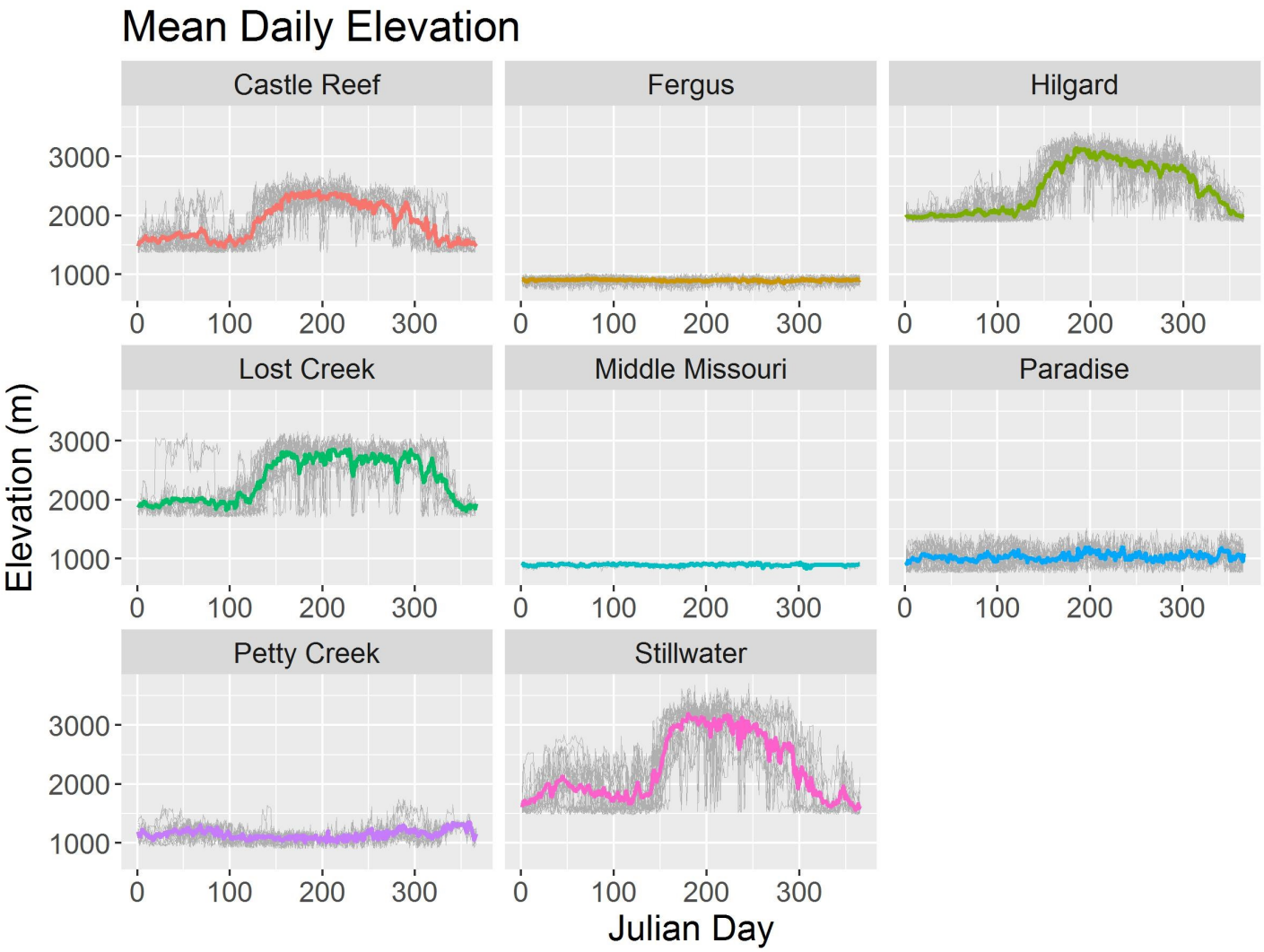


Figure 10. *GPS locations displayed for the four non- migratory study populations.*

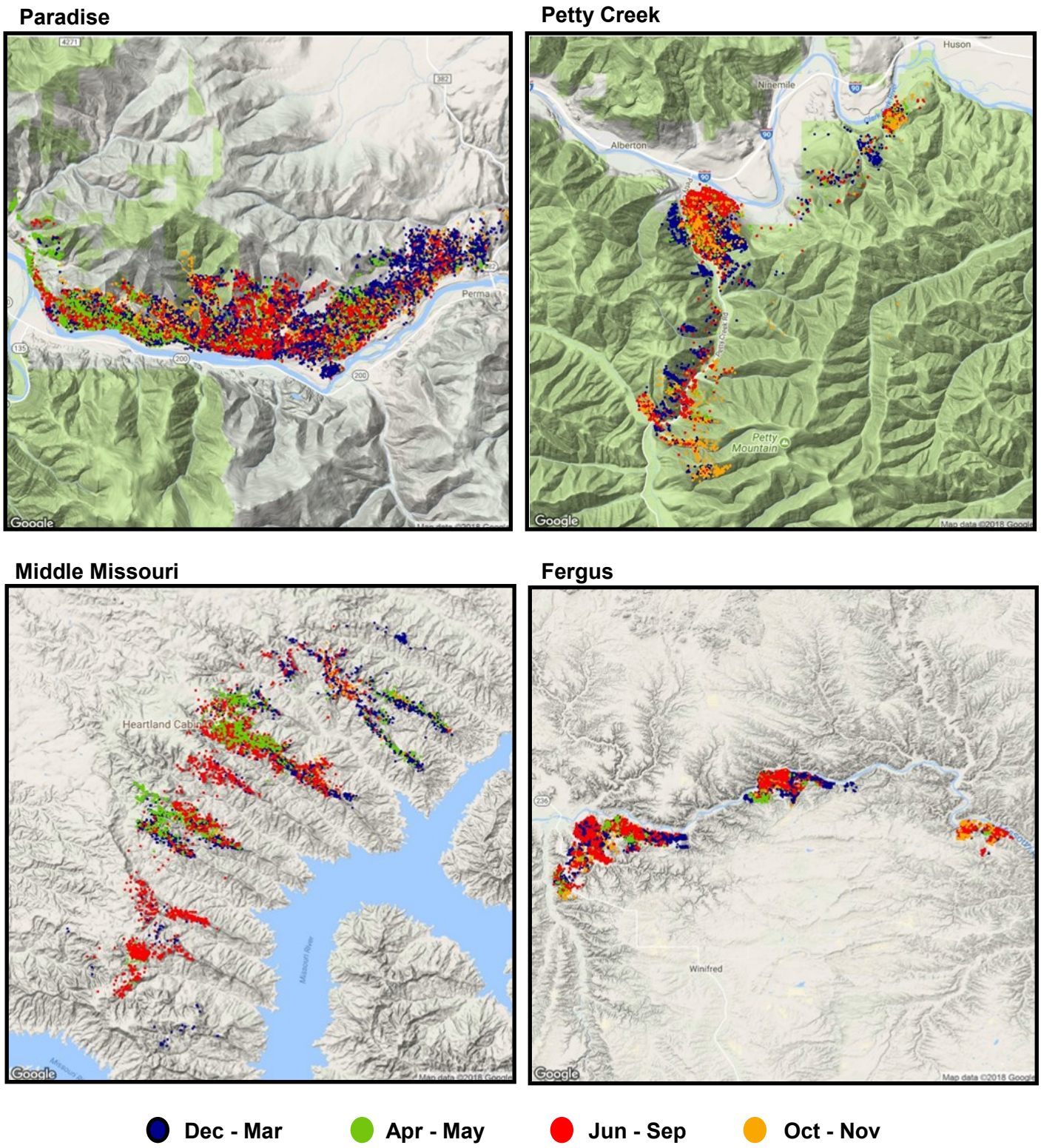
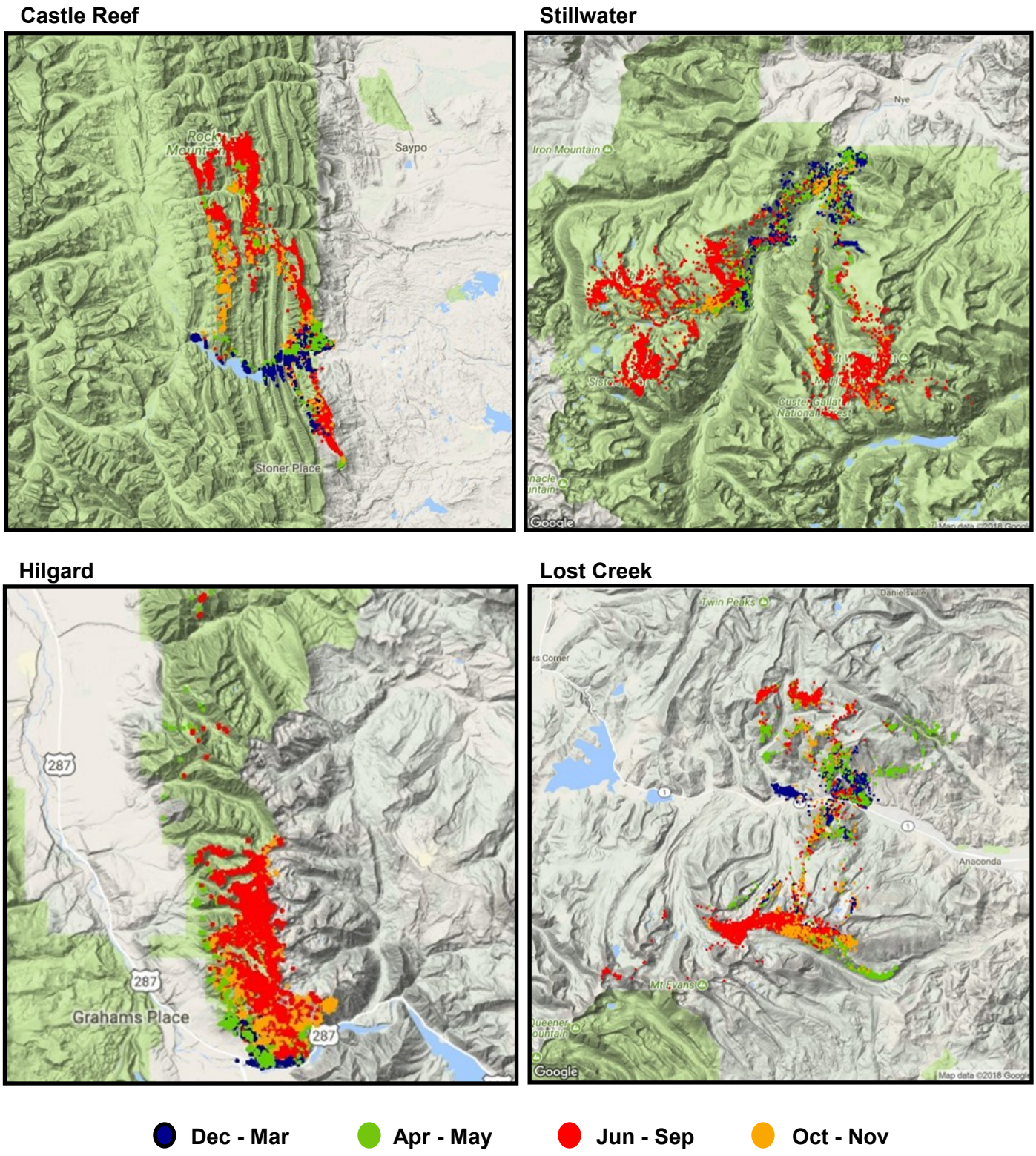


Figure 11. *GPS locations displayed for the four migratory study populations.*



4.3 Current and Planned Research Efforts

1.) Inform restoration efforts in the Madison Mountain Range.

Bighorn Sheep are believed to have historically existed within geographically distinct areas (e.g., mountain ranges) as naturally structured metapopulations, and efforts focused on restoring metapopulations may provide currently unrealized restoration opportunities. By rebuilding metapopulations, managers may not only increase bighorn sheep abundance and distribution, but may also promote natural recolonization, improve genetic heterozygosity and improve population resiliency to stochastic disease events. The Madison Mountain Range, located on the western edge of the Greater Yellowstone Ecosystem (GYE), is a good example of a mountain complex with apparent unrealized potential for metapopulation restoration; containing two distinct populations of bighorn sheep isolated from each other on opposite ends of the range (Figure 12), and apparently abundant yet unoccupied perceived habitat. In 2014, Montana Fish, Wildlife and Parks began translocating bighorn sheep from the Hilgard population to nearby historic winter range in Wolf Creek, thereby reducing local herd densities in the Hilgard population and attempting to restore a bighorn sheep metapopulation throughout the Madison Range. While restored populations may fail or be constrained by a number of factors (e.g. predation), we hypothesized that the Madison Range is capable of supporting a metapopulation of bighorn sheep, and that current distributions are not primarily limited by habitat availability.

To test this hypothesis, we are using GPS data from the Hilgard population and a suite of environmental variables associated with bighorn sheep habitat selection to generate seasonal resource selection function (RSF) models for the Hilgard population. These models will be validated, in part, with GPS data from translocated animals, and then extrapolated to generate predictive seasonal models to identify areas of unoccupied habitat that may be considered for future translocation efforts. Analyses are expected to be completed by May 2018, and results will be presented at the 2018 Northern Wild Sheep and Goat Council (NWSGC) Symposium.

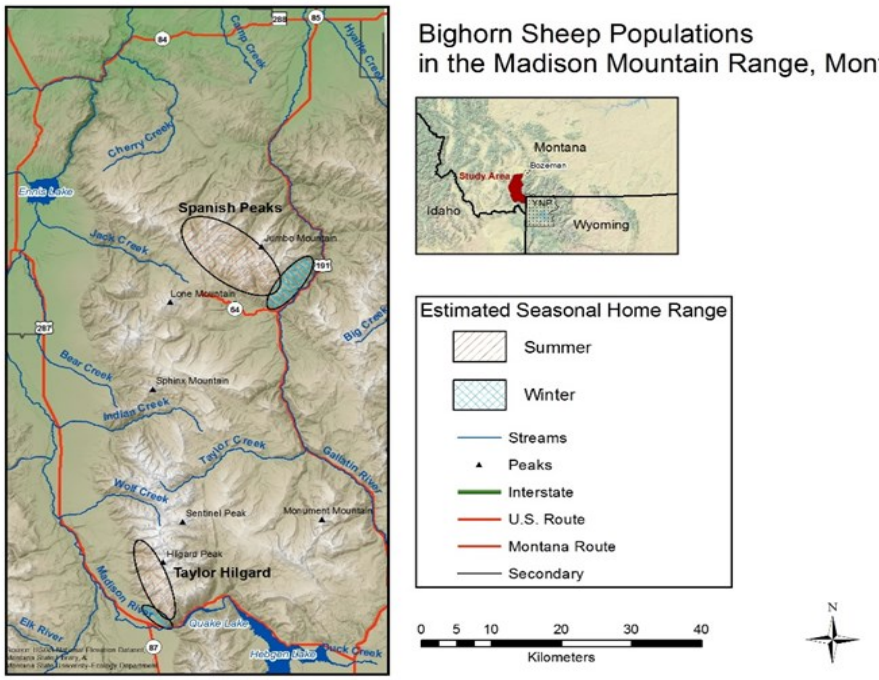
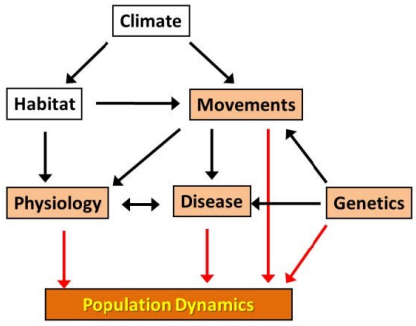


Figure 12. *Map displaying estimated seasonal distributions of the two primary bighorn sheep populations in the Madison Range.*

2.) Compare resource selection among study populations and generate predictive statewide models.

Planned future research will utilize GPS data from all eight study populations and, as with the Madison Range research, we plan to generate seasonal RSF models for each study population. From this analysis, we hope to gain new insights into how bighorn sheep, demonstrating varying movement strategies across a diverse landscape, utilize the available components of their habitat.

Objective # 5: *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 inference of 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 implementing both effective research and management programs of wildlife populations. An important objective of the Montana Bighorn Sheep Study is to develop a simple, cost effective monitoring program that wildlife managers will be able to adopt as part of routine management activities, and use this program to estimate population size, adult female survival and pregnancy rates, and annual recruitment.

5.1 Adult Female Survival

Adult female survival is being monitored in the eight study populations by use of VHF and store-on-board GPS radio-collars equipped with mortality sensors which allow for known fate survival estimation. Survival of radio-collared animals is generally monitored at least once every three months, though the instrumented animals are often checked more frequently. The wide survival monitoring intervals often precluded determining cause of death, however; date of death can frequently be inferred from GPS collar data.

Figure 13. *A mortality in the Lost Creek population detected during May survival monitoring. Cause of death was likely due to a broken femur.*

To date, 218 adult females from all eight study populations have been radio-collared and monitored for survival (Table 1), 48 of which have died. Animals instrumented with radio-collars in winter 2017/2018 (n=10) are not included in the following discussion, however all were found to be alive during follow up survival checks in January 2018. Causes of death have included hunter-harvest (n=10), cougar predation (n=5), trauma (n=2), vehicle collision (n=1), and disease (n=1), however; the cause of most mortalities (n=29) were undetermined (Table 5). In 2017, there were 21 mortalities, 4 of which occurred in January-February, 3 in March-April, 5 in May-June, 5 in Sept-Oct, and 4 in November. An additional four animals died early January 2018. The percentage of instrumented adult females captured during or prior to winter 2014/2015 that entered 2017 alive and survived to present, ranged from approximately 53% at Castle Reef to approximately 80% at Fergus. Herd specific summaries are presented below.

Table 5. *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	4	1	1	-	-	-	-	4	10
Disease	-	-	-	-	-	1	-	-	1
Trauma/Accident	-	-	1	-	-	1	-	-	2
Roadkill	-	1	-	-	-	-	-	-	1
Predation	-	1	1	-	2	1	-	-	5
Undetermined	2	5	5	5	6	2	2	2	29
TOTAL	**6**	**8**	**8**	**5**	**8**	**5**	**2**	**6**	**48**

Paradise:

A total of 25 bighorn sheep have been collared in this population, 8 of which have died. Of the 15 animals originally radio collared during the winter of 2014/2015, 10 (66%) are still alive. Two animals collared during the winter of 2016/2017 have also died, leaving a total of 17 animals alive and available for monitoring. During 2017, there were four mortalities, occurring January 9, March 12, March 30, and November 25. With the exception of the November mortality, which was a hunter harvest, all 2017 mortalities were indeterminable.

Lost Creek:

A total of 27 bighorn sheep have been collared in this population, 5 of which have died. Twelve animals were collared during the winter of 2014/2015, 7 (58%) of which are still alive. Animals captured during the winter of 2015/2016 (n=6) and in 2016/2017 (n=9) are still alive, leaving a total of 25 available for monitoring. During 2017, there was only one mortality, estimated as having occurred around June 1, as a result of a broken leg.

Petty Creek:

A total of 24 bighorn sheep have been collared in this population, 2 of which have died. Of the 15 animals originally radio collared during the winter of 2016/2017, 13 (87%) are still alive. Nine additional collars were deployed during the winter of 2017/2018 and as of January 2018, all were still alive leaving a total of 22 animals available for monitoring. During 2017, two mortalities occurred January 10 and November 5. The cause of both mortalities was undetermined.

Castle Reef:

A total of 29 bighorn sheep have been collared in this population, 8 of which have died. Of the 15 animals originally radio collared during the winter of 2015/2015, 8 (53%) are still alive and one collar has failed. One animal collared during the 2016/2017 winter also died, leaving a total of 20 animals alive and available for monitoring. During 2017, one mortality occurred January 3 due to unknown causes and another occurred January 6, 2018 from currently unknown causes.

Hilgard:

A total of 32 female bighorn sheep have been collared in this population, 8 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, 13 (86%) of which are still alive. Three 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 23 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 2017, there were four mortalities, occurring January 31, May 2, June 14, and September 29. The cause of all mortalities was undetermined, though predation was suspected for those occurring in January and September, and trauma/fall was suspected for the mortality in June.

Fergus:

A total of 40 animals have been collared in this population, 6 of which have died. Of the 30 animals originally radio collared during the winter of 2014/2015, 24 (80%) are still alive and 1 collar has failed. All animals collared during the winter of 2016/2017 (n=10) are still alive, leaving a total of 33 animals available for monitoring. During 2017, one instrumented animal was harvested November 18, and another died from unknown causes on approximately June 26. Another mortality occurred on approximately January 5, 2018, from unknown causes.

Stillwater:

A total of 21 bighorn sheep have been collared in this population, 5 of which have died. Of the 15 animals originally radio collared during the winter of 2014/2015, 10 (66%) are still alive. All other animals collared during the winters of 2015/2016 (n=1) and 2016/2017 (n= 5) are alive, leaving 16 animals alive and available for monitoring. During 2017, one mortality occurred April 1, from unknown causes, and another two were detected in early January 2018. One of the January detections in currently uninvestigated, and the other was discovered cached by a mountain lion, though actual cause of death was indeterminable.

Middle Missouri:

A total of 20 bighorn sheep were collared during the winter of 2016/2017. Of these animals, 14 (70%) are still alive and available for monitoring. Of the six mortalities, one occurred shortly after capture on December 17, 2016 from undetermined causes; though is suspected to be capture related. Another mortality occurred June 25, 2017 from undetermined causes, and the remaining four occurred as hunter harvest, September 9, October 8, October 26 and November 13.

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). The model estimated a unique survival rate for each herd, and season. 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-DSR182.5; estimated survival rate for summer = summer-DSR182.5). 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 6). The exception to this pattern was in the Fergus herd were four of the five documented mortalities to date have been the result of legal hunter harvest (Table 5). Variability in annual survival rate estimates was most notable for the Castel Reef, Hilgard, Lost Creek, and Petty Creek herds were mortalities in a single year of monitoring of instrumented animals resulted in an exceptionally low survival estimate of 0.76. Caution should be exercised in interpretation of all single season or year survival estimates, however, as the modest number of instrumented animals present in each herd results in relatively wide confidence intervals on all estimates (Table 6). Among-herd comparisons are best made using survival estimates generated by pooling monitoring data across all years of the study. The pooled annual survival rates for the Petty Creek, Fergus, and Stillwater herds were relatively strong, ranging from 0.92 to 0.94. Pooled survival estimates for Castle Reef, Hilgard, Lost Creek, and Paradise herds, however, were notably lower, ranging between 0.83 to 0.87 (Figure 14). Large ungulate population growth rates are most sensitive to adult female survival rates and the low survival estimates for this four herds suggests weaker overall demographic performance.

Table 6. *Known-fate seasonal and annual survival estimates for radio-collars adult ewes in each of seven bighorn sheep research herds. Estimates for the Middle Missouri herd are not shown as adequate data were not yet available when estimates were generated.*

		Annual		Summer		Winter	
HERD	Year	Estimate	CI	Estimate	CI	Estimate	CI
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	0.76	0.54-0.97	0.91	0.74-1.00	0.83	0.66-1.00
	2017-18			1.00	1.00-1.00		
	Pooled	0.83	0.71-0.95	0.93	0.83-1.00	0.89	0.80-0.98
Petty Creek	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			0.92	0.77-1.00		
	Pooled	0.92	0.82-1.00	0.96	0.89-1.00	0.96	0.88-1.00
Lost Creek	2014-15	0.76	0.46-1.00	1.00	1.00-1.00	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	0.94	0.81-1.00	1.00	1.00-1.00	0.94	0.81-1.00
	Pooled	0.87	0.77-.098	1.00	1.00-1.00	0.87	0.77-0.98
Hilgard	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	0.91	0.80-1.00	1.00	1.00-1.00	0.91	0.80-1.00
	2015-16	0.76	0.59-0.93	0.87	0.73-1.00	0.87	0.73-1.00
	2016-17	0.93	0.85-1.00	1.00	1.00-1.00	0.93	0.85-1.00
	2017-18			0.85	0.65-1.00		
	Pooled	0.87	0.80-0.94	0.94	0.89-0.99	0.92	0.87-0.97
Castle Reef	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	0.81	0.56-1.00	0.88	0.66-1.00	0.92	0.76-1.00
	2017-18			1.00	1.00-1.00		
	Pooled	0.83	0.71-0.94	0.97	0.90-1.00	0.85	0.74-0.96
Fergus	2014-15					1.00	1.00-1.00
	2015-16	0.93	0.84-1.00	0.93	0.84-1.00	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			0.80	0.54-1.00		
	Pooled	0.94	0.88-1.00	0.94	0.88-1.00	1	1.00-1.00
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			1.00	1.00-1.00		
	Pooled	0.93	0.85-1.00	0.98	0.93-1.00	0.95	0.89-1.00

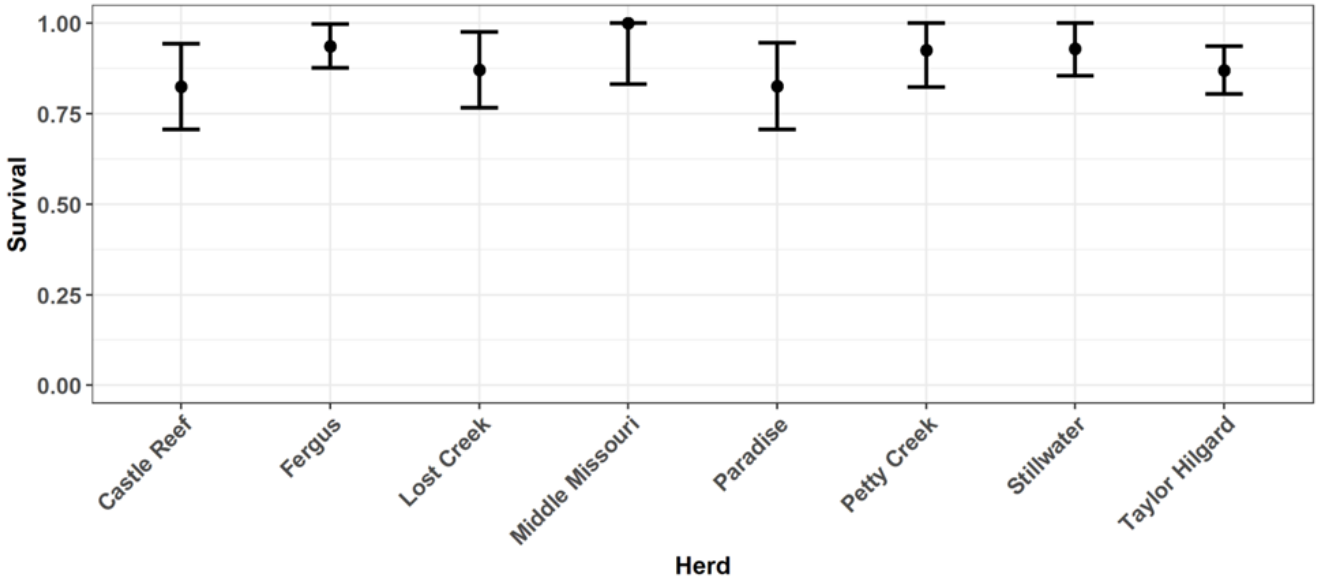


Figure 14. *Annual survival estimates for the eight bighorn sheep herds in the state-wide study calculated by pooling all mortality data collected for instrumented animals in each herd.*

5.3 Pregnancy

Pregnancy rates of adult female animals (≥ 1.5 years old) in 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 indicate cycling is, or will become pregnant, and report these as pregnant.

5.3.1 Results

Estimated pregnancy rates for most herds were very high, generally >0.90 (Figure 15). This pattern of high pregnancy rate 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, 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 3-4 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 Middle Missouri herd we suspect is a reflection of the fact that this herd was sampled in December and we have only completed the PSPB assays. Once results of progesterone assays (P4) are completed we expect the pregnancy estimate for this herd to increase substantially. The low pregnancy rate for the Mt. Evert herd should also be interpreted with caution as there is considerable uncertainty in this estimate, as reflected in the wide confidence interval (Figure 15), due to the small number of animals sampled.

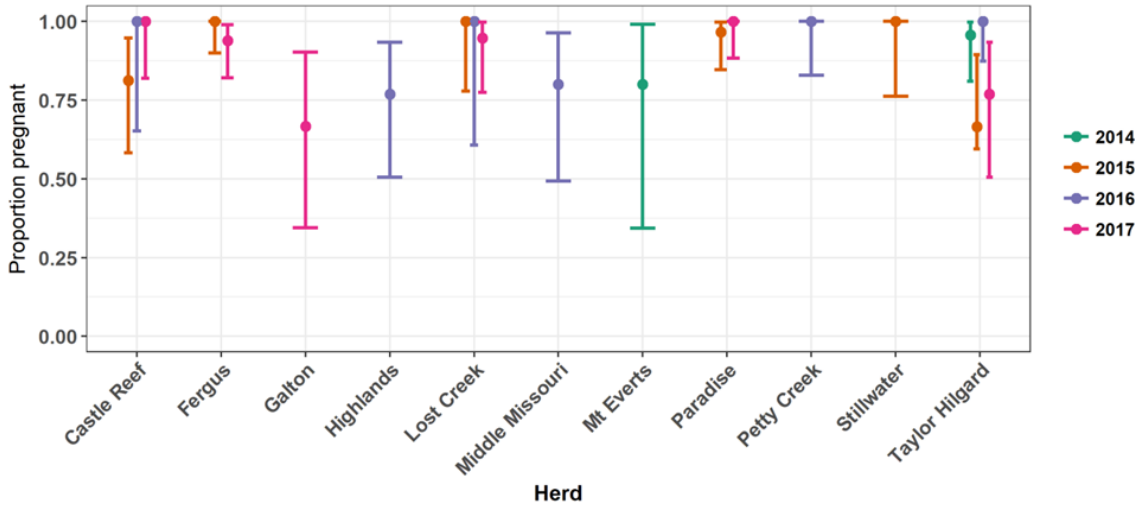


Figure 15. *Estimated pregnancy rates of the eight Montana bighorn populations captured and sampled as part of the statewide bighorn sheep research project, two herds (Galton, Highland) sampled as part of Montana FWP's herd health program, and a herd located in the upper Yellowstone River drainage within Yellowstone National Park that was sampled as part of the GYA Mountain Ungulate Research Program.*

5.5 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. We have been able to coordinate with the area biologist managing the Fergus herd to conduct spring age-sex classification surveys for the past 3 years 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 16). 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 two years 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 reflect estimate annual variation in recruitment.

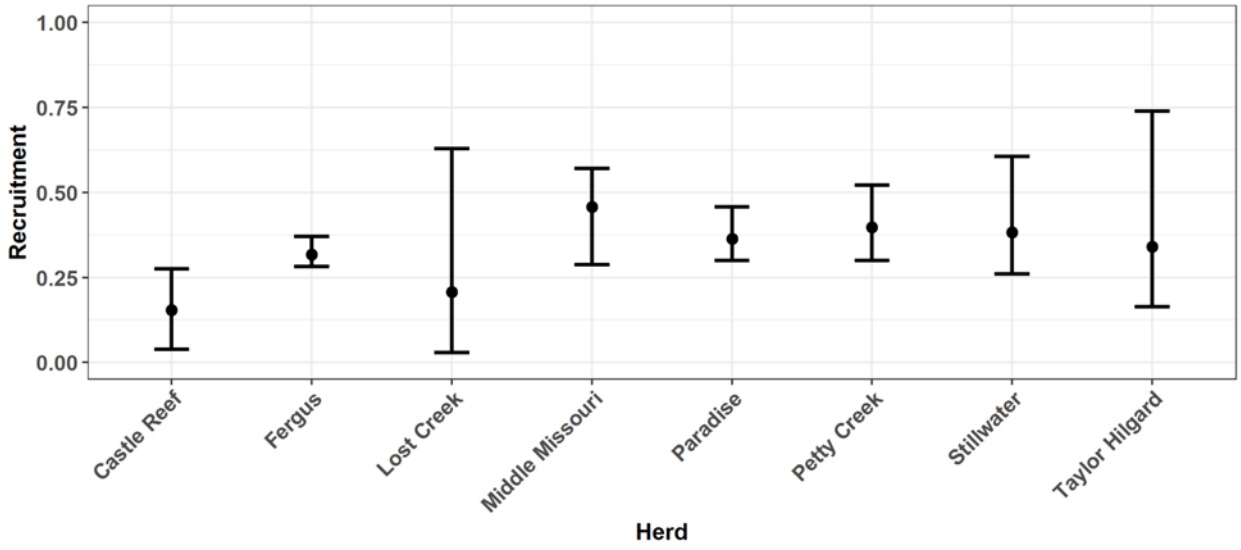
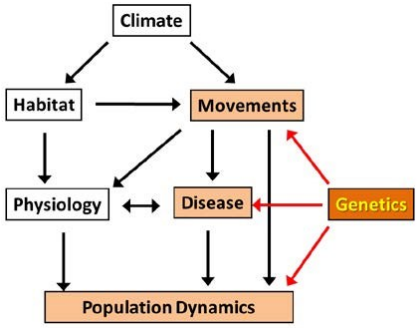


Figure 16. *Annual variation in lamb:ewe ratios determined from routine population monitoring surveys conducted by area biologists responsible for managing each of the research herds in the statewide bighorn sheep studies. For most herds the surveys were conducted in April-May and can be considered reasonable 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. The data presented for the Fergus herd, however, only represents spring ratios recorded for the most recent 3 years as ratios in prior years are only available from mid-winter surveys.*

Objective #6: *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 Collection of genetic samples

We have over 900 high-quality bighorn sheep genetic samples from different populations across Montana and Wyoming available for genomic analysis (Table 7). Samples are 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, and USGS. During capture efforts by MSU and Montana Fish, Wildlife and Parks from March 2017 to January 2018, we collected genetic samples from 45 different bighorn sheep. These genetic samples were obtained from the Petty Creek, Taylor-Hilgard, and Stillwater herds. 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. 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. In addition to the samples collected this year, Montana Fish Wildlife and Parks has been collecting DNA using gene cards since 2004.

Table 7. *High quality genetic samples (gene cards, ear biopsy punches, tissue, nasal swabs, and/or DNA extractions) from different animals currently available for bighorn sheep genomic analyses from Montana and Wyoming. Herd units not managed by Montana FWP are shaded in gray.*

Herd	Management Agency	Samples currently available	Samples currently assayed	Minimum no. samples to be assayed
Castle Reef[rh]	Montana FWP	56	25[c]	0
Fergus[rh]	Montana FWP	59	30[c]	0
Grave Creek[rh] (Petty Creek)	Montana FWP	36	16[c]	9
Lost Creek[rh]	Montana FWP	38	25[c]	0
Middle Missouri Breaks[rh]	Montana FWP	35	25[c]	0
Paradise[rh]	Montana FWP	44	25[c]	0
Stillwater[rh]	Montana FWP	25	24[a,c]	1
Taylor/Hilgards[rh]	Montana FWP	124	30[c]	0
Clark Fork Cut-off	Montana FWP	6	0	0
Galton	Montana FWP	32	0	25
Highlands	Montana FWP	20	18[c]	2
North Clark Fork	Montana FWP	1	0	0
Spanish Peaks	Montana FWP	4	0	25[d]
Tendoys	Montana FWP	25	25[a,c]	0
Wild Horse Island	Montana FWP	27	25[a]	0
Glacier National Park	NPS	98	51[b]	47[e]
Mount Everts-Yellowstone NP	NPS	5	0	0
Rocky Boy Reservation	Tribal	22	0	0
Absaroka Metapopulation	Wyoming F&G	224	87[a]	0
National Bison Range	USFWS	21	0	0
Sierra Nevada	CA Dept. of Fish and Wildlife	15	0	5
Totals		**917**	**406**	**114**

[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 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] Additional samples expected to be captured in February 2018.

[e] Analysis of these samples will be funded by the Glacier National Park Conservancy and the National Science Foundation Graduate Internship Program.

5.2 Extraction of genetic samples and assessment of DNA quality

Figure 17. *Graduate student Elizabeth Flesch collecting an ear punch for genetic sampling with MSU students at Taylor-Hilgard drop net capture in January 2018.*

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

Thus, during capture efforts from March 2017 to February 2018, 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 2017 we conducted an empirical simulation study that 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. We currently have a manuscript in preparation concerning this work and plan to submit it for peer review in spring 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 will aid 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 estimate 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 18). 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 18. *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 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 19. Relatedness within a herd decreases as (1 - Mean Kinship with Herd) increases. Figure 19 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 20). Thus, we decided to use 25 samples per herd to evaluate population genomics of additional herds that will be assessed through the statewide study (Table 7).

Figure 19. *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).*

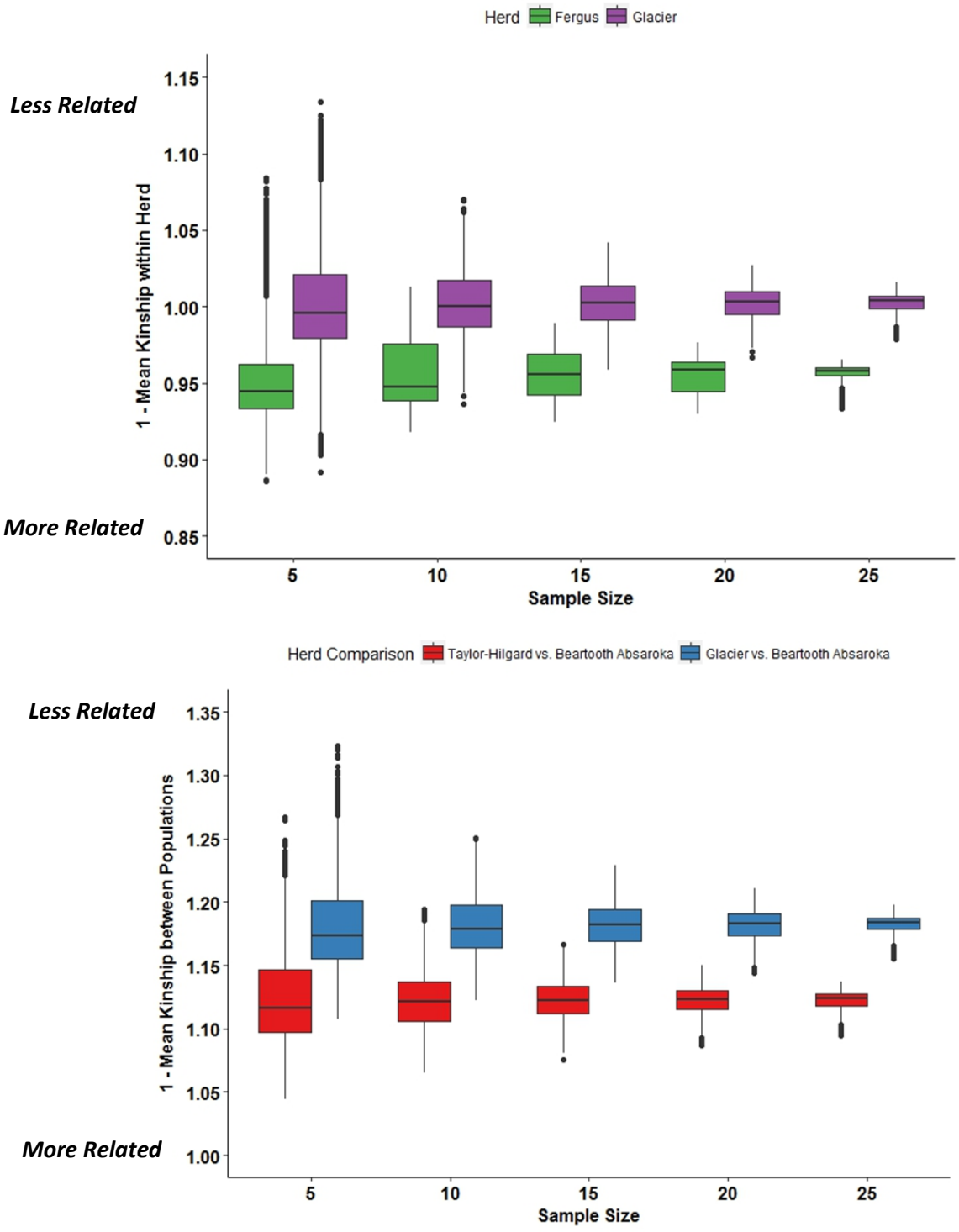


Figure 20. *Boxplots of interpopulation relatedness estimates based on 10,000 replicate simulations using empirical SNP genotypes from populations of bighorn sheep, including one minus mean kinship by increasing sample size per individual population included. Center lines represent the median, box limits 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 do not plan to use the Ovine HD SNP genotyping array for ancient DNA analysis, but instead will implement 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 21). In 2017 Dr. Shapiro's team successfully generated mitochondrial genomes for five ancient samples and 26 contemporary samples and is currently working on building a phylogenetic tree. Graduate student Elizabeth Flesch plans to visit Dr. Shapiro's lab to discuss the project in March 2018.

Figure 21. *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

We plan to present results of the sample size study at the Montana Chapter of the Wildlife Society annual conference in February 2018. We also plan to submit an abstract for the Northern Wild Sheep and Goat Council annual conference in May 2018. A manuscript regarding the sample size simulations will be submitted to the peer-reviewed journal Molecular Biology no later than April 2018. In addition, 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 additional research, we have the following projects planned for the upcoming two years:

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 management. Using the information provided by the sample size study, we plan to genotype 25 samples per herd to evaluate population genomics, and most of the samples have already been assayed (Table 7). 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. 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. In general, SNP genotyping relatedness estimates are highly correlated to those calculated from a known pedigree (Li et al. 2011, Zhang et al. 2015). Therefore, it is a useful approach to estimate herd genetic diversity for wild populations without pedigree information. 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 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. Graduate student Elizabeth Flesch will work with Dr. Tabitha Graves at the USGS Glacier Field Station in summer 2018 to compare genotypes of bighorn sheep in Glacier National Park with movement patterns. In addition, Elizabeth will work with graduate student Blake Lowrey for GYA herds located in Wyoming and with graduate student Ethan Lula for Montana herds where we have both GPS data and genetic samples from an adequate number of individuals.

3) Compare herd disease presence and demographic performance with genomics

We can compare population genetic results with herd demography to identify potential associations between demographic performance of herds and their genetic attributes. The effects of inbreeding can depend on sex, interactions with the environment, and genetics of the species/population. Our use of the genome-wide Ovine array can help determine the trait impacts of inbreeding by identifying specific functional genes that are homozygous, as the genetic causation of negative inbreeding effects can differ by population (Kristensen et al. 2010). By studying the impacts of low heterozygosity, we can help identify herds with low heterozygosity that may benefit from genetic augmentation. Disease is also 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.

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.

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

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.

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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.

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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.

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