

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



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**Photo Credit: Jenny Jones,**

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## Project Background

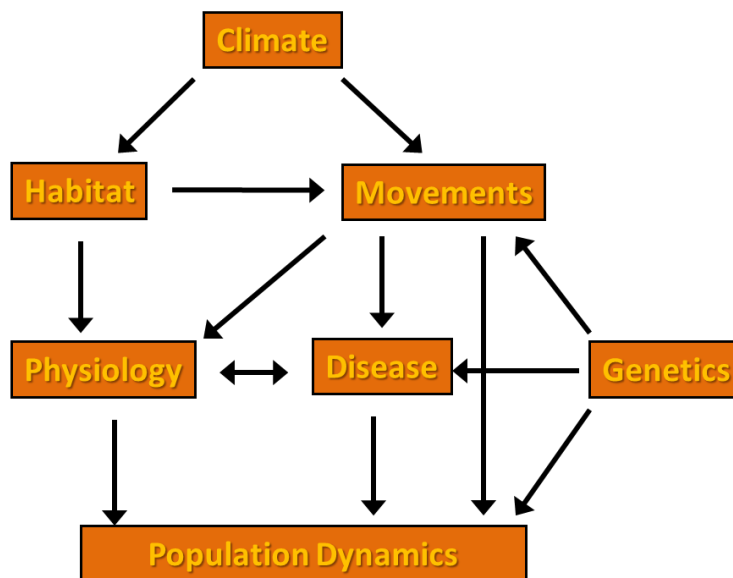
The history of bighorn sheep (*Ovis canadensis*) conservation shares many similarities with the conservation history of other North American ungulates, but is also quite distinctive. Similar to other ungulates, bighorn sheep existed in continuous and broadly distributed populations and likely numbered in the millions prior to colonization of western North America. Following settlement of western North America 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 20<sup>th</sup> century. This effort was successful in recovering most ungulate species back from the 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 and, 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 a substantial amount of 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 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.

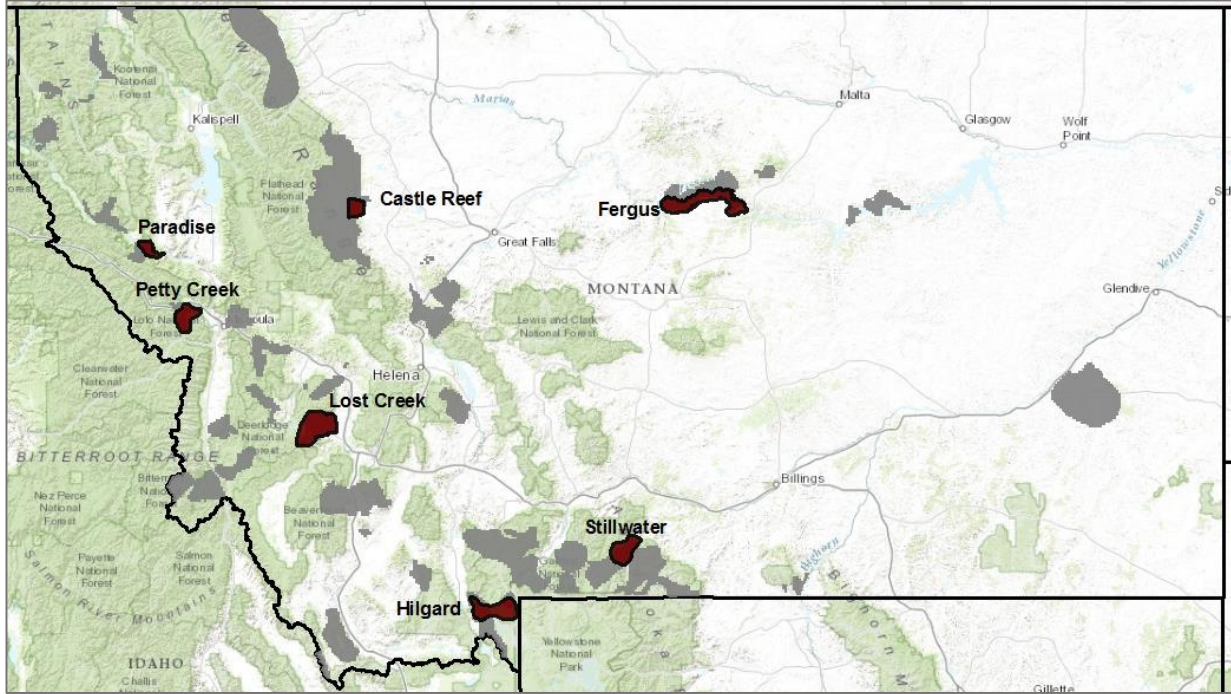
In 2013, MFWP and MSU initiated a collaborative six-year research program designed to assess factors driving bighorn sheep population dynamics across Montana. The integrated study design entails using standardized methods to investigate demographic rates, body condition and nutrition, respiratory pathogens, 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 second 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 used by this research program*

### **Location**

Research conducted under this grant is focused within the range of seven distinct bighorn sheep populations across varying ecological settings in Montana. Bighorn sheep populations incorporated into this study occupy portions of Deer Lodge, Fergus, Lewis & Clark, Madison, Missoula, Sanders, Stillwater and Teton Counties, as well as the Flathead Indian Reservation. Populations included in the research program include Paradise, Lost Creek, Hilgard, Petty Creek (aka Grave Creek Range), Castle Reef, and Fergus.



**Figure 2.** Ranges of the seven study populations included in the Montana Bighorn Sheep Study. Polygons shaded in gray show ranges of the other bighorn sheep populations in Montana that are not part of this research effort.

**Study Objectives (Year 2 of 6-year study)**

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

- 1) Continue to capture, sample, and instrument animals in each study population in order to reach original capture and monitoring goals
- 2) Assess respiratory pathogen communities among sampled populations
- 3) Assess variation in body condition and physiological status among sampled populations
- 4) Monitor demographic rates in instrumented populations
- 5) Collect and provide samples for a pilot bighorn sheep genetics study

**Objective # 1:** *Capture, sample, and instrument animals in each study population*

**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. (30 mg Butorphanol/adult, 10 mg Azaparonone/adult, 12 mg Metatomidine/adult). Chemically immobilized animals were administered oxygen at two liters/minute and were also subcutaneously administered 5-7 mL Liquamycin (oxytetracycline antibiotic). Reversal entailed intramuscularly administering 200 mg Tolazaline followed by 31.25 mg Atipamezole. All capture and handling procedures followed

protocols approved by the Montana State University Institutional Animal Care and Use Committee (Permit # 2014-32).

### 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.



**Figure 3.** From left to right: Two ewes from Fergus population captured by Quicksilver Air Inc., a group of bighorn sheep from the Hilgard population under an 80' drop-net just prior to capture, and principle investigator Bob Garrott and technician Aaron McGuire processing a chemically immobilized ewe from the Stillwater population.

## **1.2 Study populations & Sampling Accomplishments**

The sampling objective was to capture and sample 30 animals in each of the seven study populations during the first year of the study, instrumenting 15 adult females with paired GPS and VHF (very high frequency) radio-collars equipped with mortality sensors (Models: TGW4400 [GPS] and MOD400 [VHF], Telonics Inc, Mesa, Arizona).

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 seven 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 300 animals, experiences moderate recruitment in most years, and is believed to be isolated from other bighorn populations. There is no known history of respiratory disease in this population.

Capture and sampling objectives were fully met at Paradise in December 2014. However, there are plans to chemically immobilize two additional adult females in winter 2015/2016 to redeploy radio-collars which were fit on animals that died since the original deployment.

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 160 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 chemical immobilization was planned for winter 2015/2016 in order to supplement drop-netting efforts. Seventeen adult females were captured and sampled using helicopter net-gunning on February 1<sup>st</sup> and 2<sup>nd</sup> 2016, and all 15 pairs of GPS/VHF collars were deployed. Additionally, there are plans to capture and sample additional animals through winter 2015/2016 using ground-based chemical immobilization to more closely reach original sampling goals.

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 ~60 animals due to the recent disease event and recruitment remains low.

In winter 2014/2015 seven animals (6 adult females and 1 adult male) were captured and sampled using a drop-net on January 3<sup>rd</sup>, 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. A total of 19 animals have been captured and sampled in this population. There are plans to continue chemical immobilization efforts through winter 2015/2016 to more closely reach original sampling goals.

### 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 populations and currently numbers at least 200 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. Additionally, a supplementary translocation of 25 animals is planned to occur February 20<sup>th</sup> 2016. The continued increased data and sample collection that has resulted from this collaboration will undoubtedly improve insights that will be obtained from the research program.

### 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 650-700 animals. Historically recruitment has been moderate to high, but since the most recent respiratory disease even, recruitment has been very low, although it may be closer to “normal” levels this year.

Twenty (20) animals were captured and sampled using a dropnet 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 there are plans to capture and sample up to four additional adult females in March 2016, redeploying two radio-collars which were deployed on animals which have died.

### 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 population in the state, numbering approximately 500 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.

**Table 1.** *Sampling accomplishments to date in each of the seven 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.*

	ANIMALS SAMPLED				RADIO-COLLARED EWES	
	<u>2013/ 2014</u>	<u>2014/ 2015</u>	<u>2015/ 2016</u>	<u>TOTAL</u>	<u>TOTAL COLLARED</u>	<u>CURRENTLY ON AIR</u>
Paradise	--	30	0	<b>30</b>	15	13
Petty Creek	--	0	17	<b>17</b>	15	15
Lost Creek	--	7	5	<b>12</b>	17	15
Hilgard	29	50	0	<b>79</b>	20	16
Castle Reef	--	23	3	<b>26</b>	16	14
Fergus	--	60	0	<b>60</b>	30	27
Stillwater	--	16	3	<b>19</b>	16	14
<b>TOTAL</b>	<b>29</b>	<b>177</b>	<b>28</b>	<b>234</b>	<b>129</b>	<b>114</b>

Stillwater:

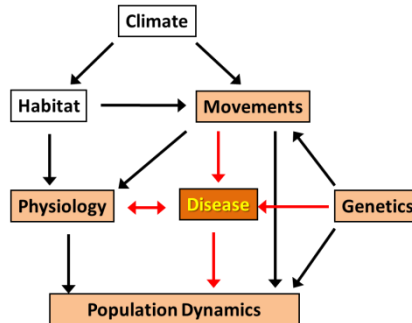
This native population is located in south-central Montana within the Southern Mountains ecoregion. The population is believed to be relatively isolated, is small (~60 animals) and has moderate recruitment. There are no known respiratory events in the population in recent times, but the population has 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.

**Objective # 2:** Assess variation in respiratory pathogen communities and exposure among sampled populations



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 (Besser et al., 2013). Bacterial organisms belonging to the family *Pasteurellaceae* have long been implicated as important agents for respiratory disease in bighorn sheep, and recent experimental inoculation studies have shown that it is likely leukotoxigenic (lktA) *Pasteurellaceae* organisms, including strains of *Mannheimia haemolytica* and *Bibersteinia trehalosi*, which cause respiratory disease in captive bighorn sheep, but not in domestic sheep (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 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 bighorns (Besser et al., 2014).

The very 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 (novel pathogen hypothesis) there are numerous examples of respiratory disease outbreaks in bighorn 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 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 respiratory disease and transmit those pathogens to bighorns in captive studies, these “resident pathogens” in bighorn populations likely originated from sympatric domestic sheep at some point since domestic sheep were introduced to western North America. 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, consistent with the principles behind the project’s design, 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 task of eradicating pathogens and provide indirect evidence that disease expression can be caused by pathogens already present in a population. The second 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.

## **2.1 Disease 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*. 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 previously collected from animals as part of the Greater Yellowstone Area Mountain Ungulate Project (MUP), allowing data collected by the two efforts to be directly comparable. Additionally, MFWP collects

samples using the same method and has agreed to share data from those samples in order to augment research sampling.

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*. To assess presence of *M. ovipneumoniae* in study populations, nasal swabs were collected from each animal and sent frozen either in tryptic soy broth with glycerol (TSB) or in an empty cryovial to WADDL for PCR detection. Additionally, Dr. Tom Besser at WADDL is conducting genetic strain typing of *M. ovipneumoniae* detected in PCR tests, allowing exploration of whether different strains of this species show different pathogenicity and potentially to gain inferences on pathogen transmission across the landscape.



**Figure 4** 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.

To detect *Pasteurellaceae* pathogens, three sampling strategies have been used to allow for future comparison of respiratory pathogen communities in bighorn sheep populations that are outside of this research effort. Tonsil swabs were either immediately frozen in TSB transport media, stored cool in BBL Port-A-Cul™ tubes and shipped to the lab within 24 hours, or used to inoculate a culture plate at the animal. Swabs placed in TSB or Port-A-Cul™ tubes were sent to WADDL for *Pasteurellaceae* culture, with a subset also tested for the leukotoxin gene using PCR. Inoculated culture plates were incubated in a custom built incubator in order to facilitate growth of more *Pasteurellaceae* organisms and increase detection rate of individual species. This

more rigorous sampling protocol was developed under guidance from Hank Edwards and the Wyoming Game and Fish (WGF) Wildlife Disease Lab protocol and also allows the project to cryogenically preserve bacterial cells and DNA, resulting in a collection of samples for future disease testing. For most animals that were sampled in each population, a swab of bacterial growth from the culture plate was also shipped to WADDL for *Pasteurellaceae* culture.

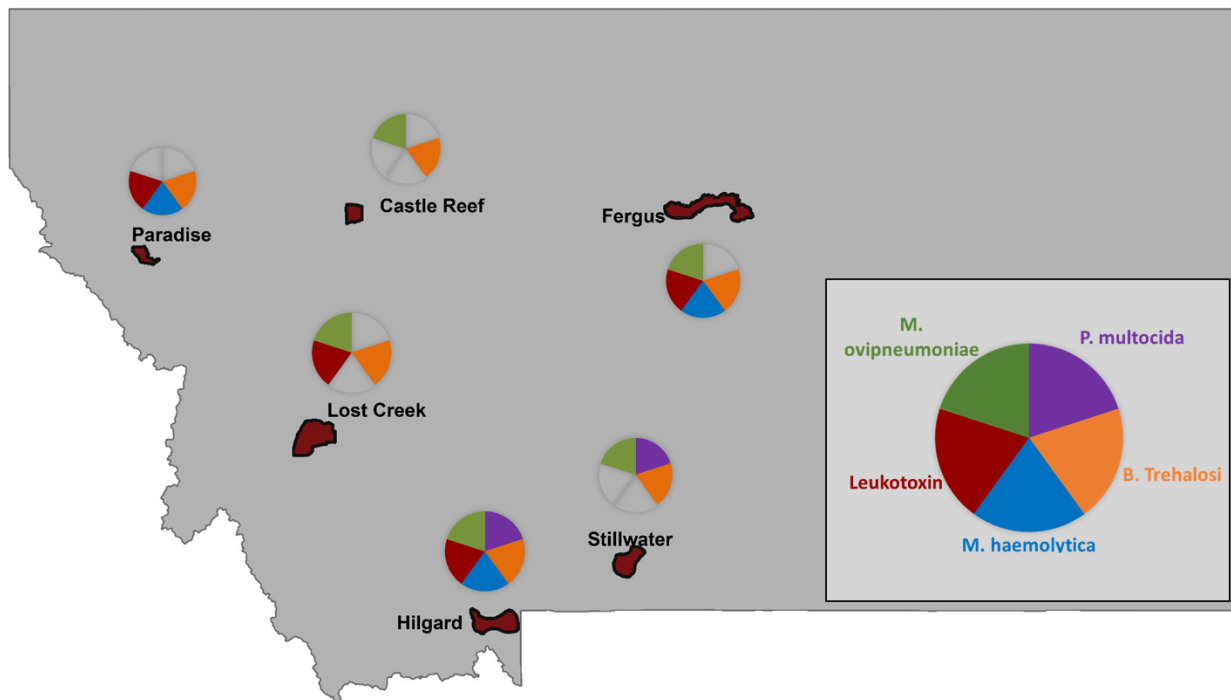
## 2.2 Disease Sampling Results

Serum, nasal, and tonsil swabs, have been collected from 234 animals in all seven study populations (n = 29 in 2013/2014, n = 177 in 2014/2015, n = 28 in 2015/2016 Table 1). Thirty-five sets of samples have been collected in winter 2015/2016 from three “non-study” populations and up to 40 sets of samples from the Hilgard population which will be sampled in late February as part of management activities by MFWP. Samples collected during winter 2014/2015 or earlier were sent to WADDL for diagnostic testing and results have been received, however results for samples collected from animals in the Petty Creek population as well as other animals sampled in winter 2015/2016 are not yet available.

Diagnostic tests detected seven types of potential respiratory pathogens. These pathogens include *M. ovipneumoniae* (detected in 68 animals in five study populations), *M. haemolytica* (detected in 23 animals in 3 study populations), *B. trehalosi* (detected in 182 animals in six study populations), *P. multocida* (detected in 17 animals in two study populations), unidentified *Mannheimia species* (detected in 22 animals in five study populations), unidentified *Pasteurella species* (detected in 27 animals in five study populations), and *Trueperella pyogenes* (detected in 36 animals in six study populations). Additionally, the leukotoxin gene (specific to any *Pasteurellaceae* species) was detected in 14 animals in 4 study populations; however only a subset of samples was tested for the leukotoxin gene, based on culture test result, making comparison of findings related to leukotoxin with other test results difficult. Of these eight groups of pathogenic agents, those currently of most interest are *M. ovipneumoniae*, *M. haemolytica*, *B. trehalosi*, *P. multocida*, as well as leukotoxin and the following summaries will be restricted to these groups (Figure 5).

*B. trehalosi* was detected in every sampled population at high prevalence, with minimum prevalence ranging from 59% to 100% of animals sampled. However, none of the *B. trehalosi* isolates displayed beta-hemolysis, which is thought to be indicative of leukotoxin production (Fisher 1999) and, accordingly, virulence. The only population without evidence for presence of or exposure to *M. ovipneumoniae* was Paradise. Minimum prevalence of *M. ovipneumoniae* in the other populations (based on PCR testing) varied widely, ranging from 5% of animals sampled (Fergus) to 86% of animals sampled (Hilgard 2013). Minimum prevalence of animals with serum antibodies against this pathogen also varied widely, ranging from 18% of animals sampled (Hilgard 2013) to 77% of animals sampled (Castle Reef & Hilgard 2015). *M. haemolytica* was detected at low rates in the Paradise (10% of animals sampled), Fergus (17% of animals sampled), and Hilgard populations (24% animals sampled 2013 and 6% animals sampled 2014) populations. *P. multocida* was detected in a single animal from the Stillwater population (6% of animals sampled) and in 33% of animals sampled from the Hilgard population in 2015. The leukotoxin gene was detected in samples collected from animals at Paradise, Hilgard (test was only available for 2015 sampling), Fergus, and Lost Creek. It should be noted that the number of animals sampled in several populations (eg. Lost Creek and Stillwater) is

rather modest and also that the true prevalence of these pathogens is higher than the detection rate.



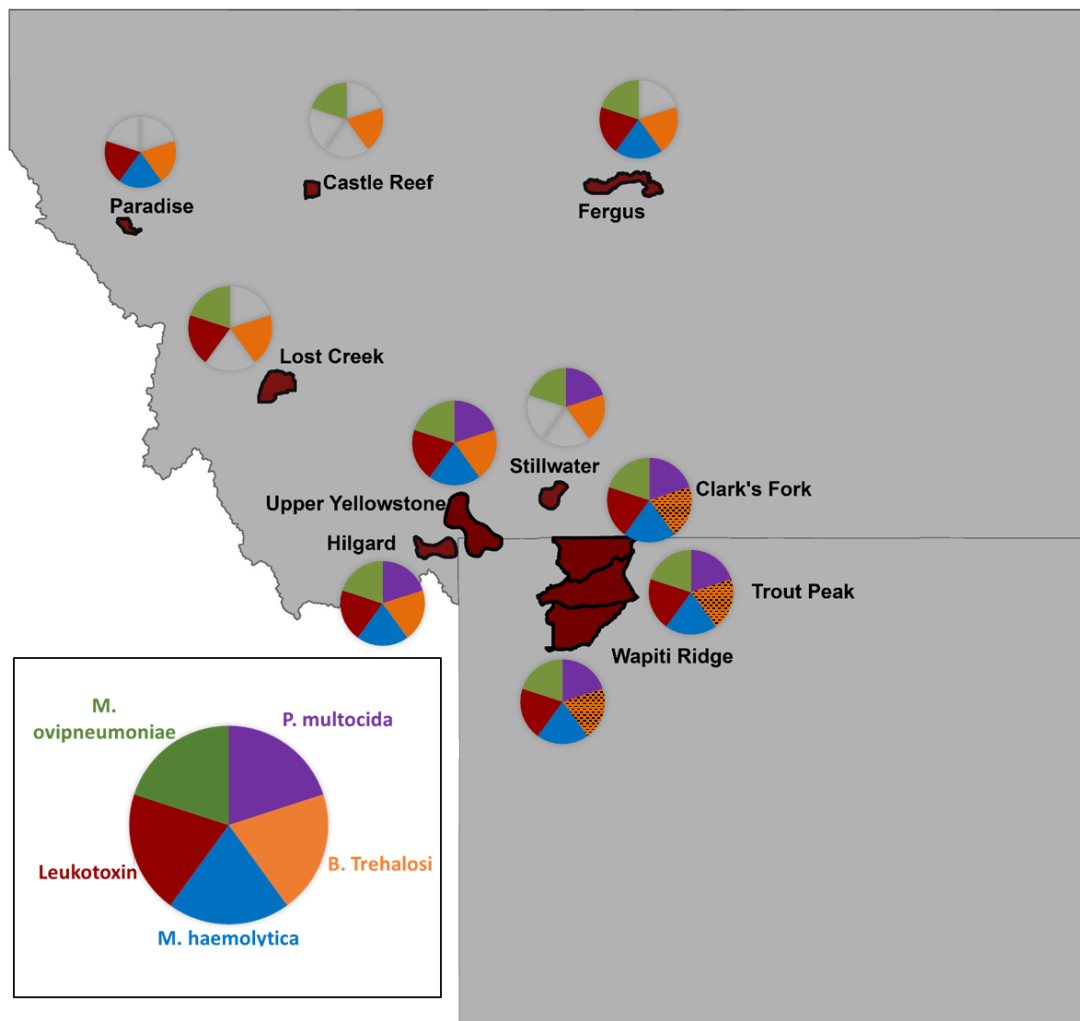
**Figure 5.** *Respiratory pathogen communities that were detected in each of the six sampled study populations. A full pie chart indicates that all five pathogenic agents of interest were detected and missing segments indicate that the pathogenic agent represented by that segment was not detected. The pie charts only represent herd-level detection of the pathogenic agents and not the proportion of sampled animals in each herd that each agent was detected (prevalence).*

The findings from the respiratory pathogen testing from the Montana Bighorn Sheep Project become more insightful when combined with data from other regional efforts. Four additional bighorn populations in Greater Yellowstone Area (Upper Yellowstone, Clark’s Fork, Trout Peak, and Wapiti Ridge) have been sampled for respiratory pathogens using the same protocol for collecting nasal and tonsil swabs as part of a complementary project, the Greater Yellowstone Area Mountain Ungulate Project (MUP). These populations are part of a large metapopulation of bighorn sheep occupying the northern and eastern regions of the Greater Yellowstone Area (GYA). Figure 6 illustrates the pathogen communities that have been detected in populations that are part of both research efforts.

### 2.3 Detection Probability Initiative

Consideration of detection probability has recently received increasing attention in wildlife disease studies, as it can confound inferences regarding pathogen communities if unaccounted for (McClintock et al., 2010; D. A. W. Miller et al., 2012). There is evidence suggesting this may indeed be an issue for bighorn sheep pathogen research as literature suggests that certain pathogens may be more difficult to detect than others (Dassanayake et al., 2010,

Bavananthasivam et al., 2012, Walsh et al. 2012). Beginning in spring 2015, the Montana Bighorn Sheep Project began an initiative to use occupancy modeling to quantify detection probability of the different pathogens under different sampling protocols. The framework requires collecting repeated samples from individual animals at the time of capture and, stated most simply, assessing how frequently lab results from repeated sampling of the same animal agree. Results from this effort will be used in a simulation study to develop recommendations for sampling intensities needed to reliably determine whether a given pathogen is present in a population. The Montana Bighorn Sheep Project has engaged the MFWP Wildlife Health Lab, as well as the WGF wildlife disease lab, in this initiative and both labs have agreed to collect repeated swab samples from bighorns they sample as part of their disease monitoring work in

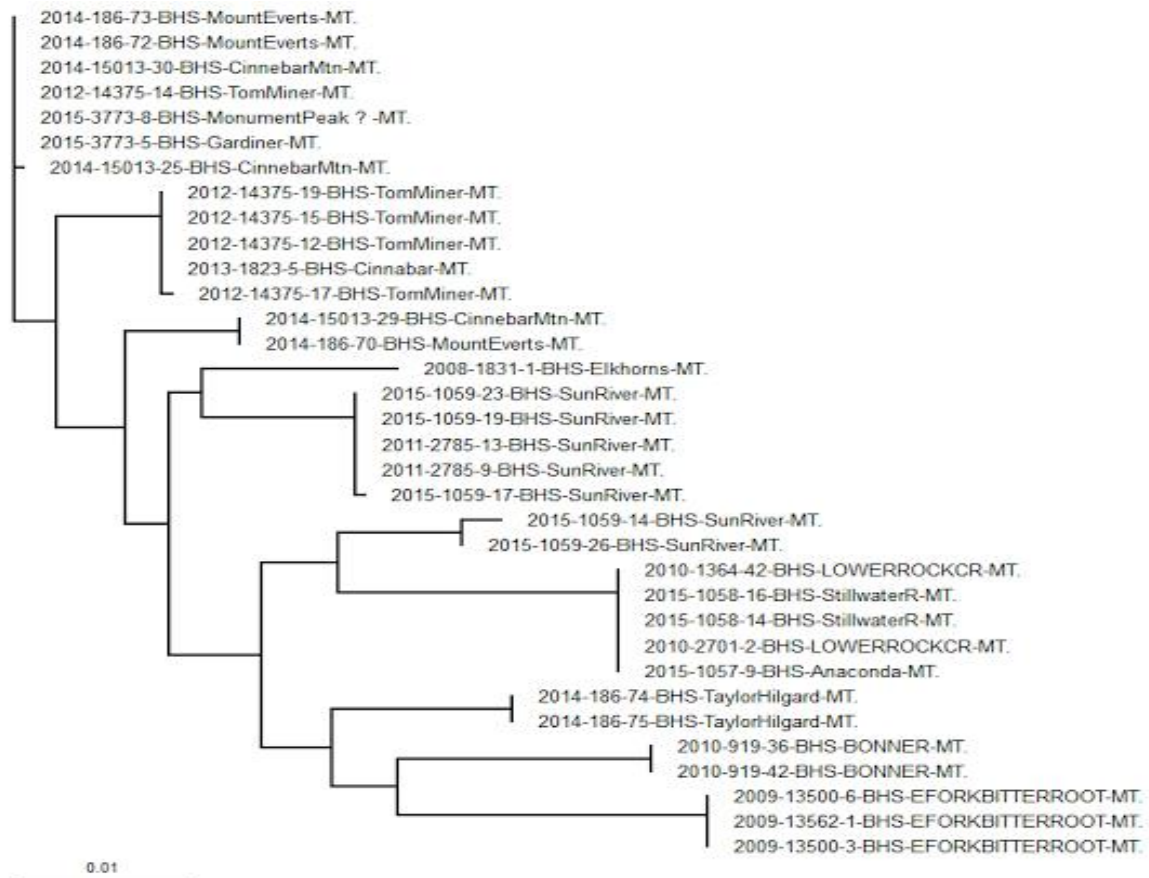


**Figure 6.** Respiratory pathogen communities that were detected in sampled populations in Montana and Wyoming. A full pie chart indicates that all five pathogenic agents of interest were detected and missing segments indicate that the pathogenic agent represented by that segment was not detected. The pie charts only represent herd-level detection of the pathogenic agents and not the proportion of sampled animals in each herd that each agent was detected (prevalence). \*\*The black hash marks present in the “*B. trehalosi*” slices for Clark’s Fork, Trout Peak and Wapiti Ridge indicate that beta-hemolytic isolates (suggestive of leukotoxin production and increased virulence relative to the *B. trehalosi* isolates) of this species were identified.

winter 2015/2016. To date, duplicate tonsil swabs have been collected from 198 bighorns that will be used to assess detection probability of *Pasteurellaceae* organisms and duplicate nasal swabs have been collected from 155 bighorns that will be used to assess detection probability of *Mycoplasma ovipneumoniae*. Incorporating the efforts of MFWP and WGF through winter 2015/2016, we anticipate duplicate tonsil and nasal swabs will be collected from an additional 150-200 animals and incorporated into the analysis. Analysis on this project will begin spring 2016 and we anticipate a manuscript will be submitted for publication by Fall 2016.

## 2.4 Mycoplasma ovipneumoniae Strain Typing

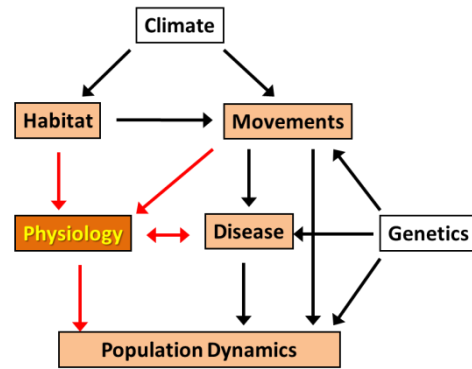
Dr. Tom Besser has conducted strain-typing on *M. ovipneumoniae* isolates from the five study populations it was detected in, as well as from several other populations in Montana (Figure 7). This work has led to several interesting findings, including multiple strains hosted within populations and sharing of strains by populations separated by hundreds of miles. Dr. Besser is initiating a large strain-typing effort across many western states and provinces that aims to, among other things, evaluate the spread, evolution, and clustering of *M. ovipneumoniae* across the United States in bighorn sheep, mountain goats, domestic sheep, and domestic goats.



**Figure 7.** Cladogram showing different *Mycoplasma ovipneumoniae* strains isolated from bighorn sheep in Montana. Listed isolates that fall along the same vertical line are of the same strain and the horizontal distance between branches indicates the amount of genetic base-pairs the strains differ by.

### Objective # 3:

*Assess variation in body condition and physiological status among sampled populations*



Quantity and quality of forage and associated animal nutritional condition influence the survival and reproduction of ungulates (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). 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 was assessed using field-based measurements including ultrasonography and traditional body condition scoring, while physiological and nutritional condition were assessed using both traditional and state-of-the-art serum based assays.

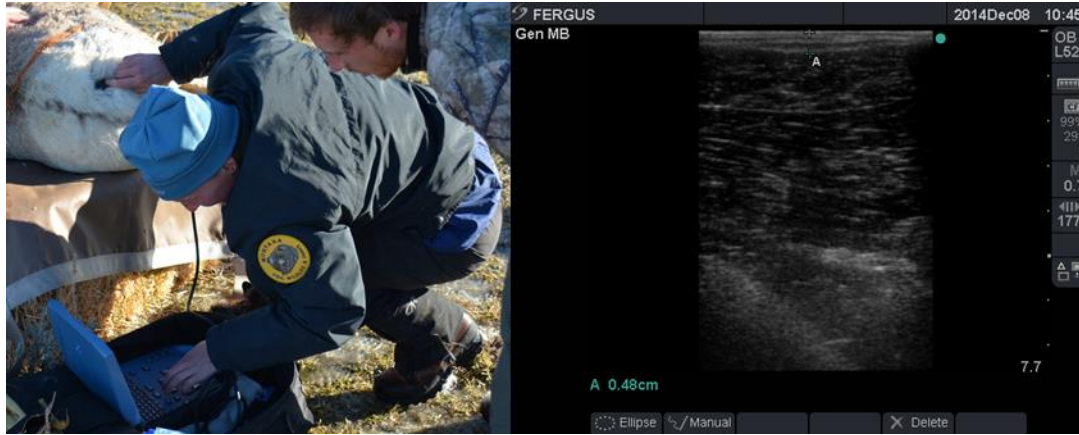
### 3.1 Field-Based Body Condition Assessments

#### 3.1.1 Methods

We used ultrasonography to measure rump fat thickness of adult female bighorn sheep and assessed whether or not they were lactating. For animals captured in December and January we consider lactation to be indicative of recruiting a lamb through the fall. In addition, the lumbar vertebrae, sacrum, base of tail and caudal vertebrae were palpated manually and a body condition score was assigned. The rump fat thickness measurements and body condition scores were used to estimate the percent ingesta-free body-fat of each adult female (%IFBF; personal communication with Tom Stephenson, Sierra Nevada Bighorn Sheep Recovery Coordinator). Body weight and skeletal size (hind foot length) were also measured on all animals. Although these measurements are not direct measures of body condition, differences in skeletal size and body weight across populations may be reflective of nutritional status or other factors related to fitness.

A portion of animals were sampled in late winter, making comparisons of data collected earlier in the winter and determination of lactation status during the previous year impossible. Additionally, some animals were sampled in small numbers in a separate winter resulting in too little data for useful summarization of body condition for that population and year. For these reasons, summaries below primarily incorporate data from December or January in winters 2013/2014 and 2014/2015, however data from Petty Creek are presented, despite being collected later in the winter as they were not available to be reported in the previous annual report





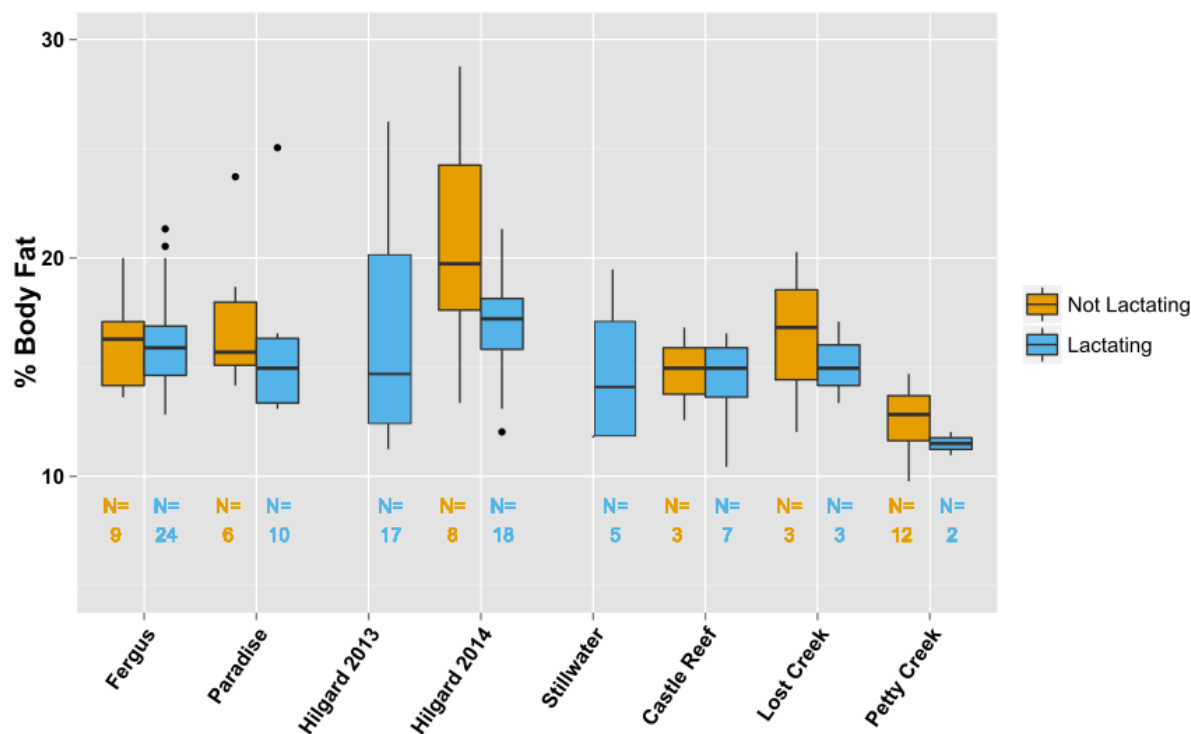
**Figure 8.** Measuring rump fat thickness of a bighorn sheep ewe. The ultrasound screen observed by collectors is shown on the right.

### 3.1.2 Results

Rump fat thickness measurements have been taken from 187 adult females, body weight and skeletal length have been measured on 204 adult females, and serum has been collected from all captured animals.

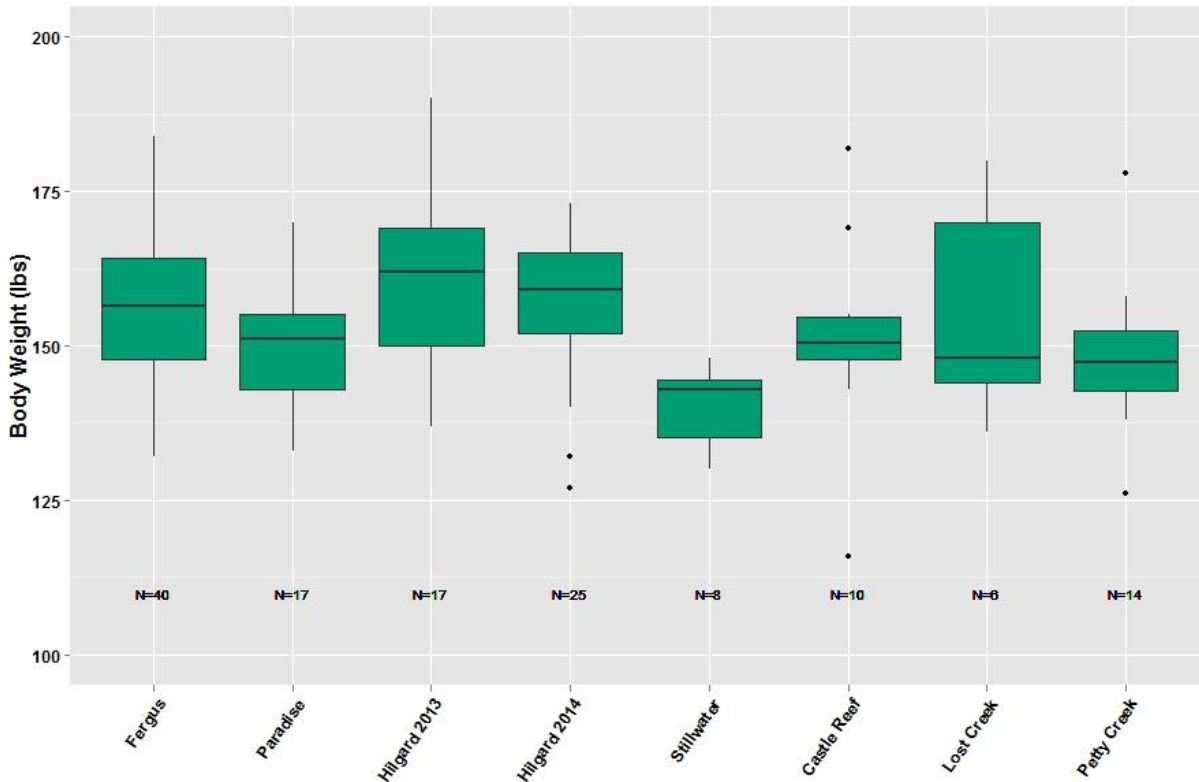
Rump fat measurements of adult females ( $\geq 3.5$  years) varied from 0.15 – 1.58 cm, corresponding to %IFBF estimates ranging from 9.8% to 28.8%. As would be expected, %IFBF of lactating adult females captured in December or January was lower than non-lactating females (lactating = 15.7 %IFBF, non-lactating = 16.5 %IFBF). Median %IFBF of lactating adult females (captured in December or January) across populations varied from 11.9% IFBF (Stillwater) to 17.2% IFBF (Hilgard 2014), though sample sizes for several populations were relatively small (Figure 9). Median %IFBF for adult females at Petty Creek, which were sampled in February 2016, was 12.3%. Due to the late timing of sampling at Petty Creek (early February), lactation (or lack thereof) may not be indicative of lamb rearing the previous year as the cessation of lactation may have occurred prior to sampling.

Given that the estimated threshold level of winter %IFBF for bighorn sheep to maintain pregnancy is around 10% (Stephenson *et al.* 2012), preliminary evidence suggests that body condition entering winter does not routinely limit pregnancy rates in the study populations. The data also suggest considerable annual variability in body condition in the Hilgard population (and likely other populations as well), as the median %IFBF of lactating females ( $\geq 3.5$  years) notably changed between the successive years of sampling (winter 2013/2014 = 14.7 %IFBF, winter 2014/2015 = 17.2 %IFBF; Figure 9).



**Figure 9.** Boxplot illustrating distribution of percent ingesta-free body-fat (measured using ultrasonography) of lactating and non-lactating adult female bighorn sheep ( $\geq 3.5$  years) across the sampled study populations. Horizontal lines through boxes represent median values, length of box represents the middle 50% of observations (IQR), vertical lines (whiskers) represent observations outside the IQR that are within 1.5x the range of the IQR, and points outside the whiskers represent observations greater than 1.5x the range of the IQR. The numbers of animals sampled in each category are shown below the boxes. **\*\*Note that Petty Creek was sampled in February, which likely resulted in decreased fat measurements compared to measurements taken earlier in the winter. Additionally, lactation, or lack thereof, in February may not be indicative of lamb production the previous year\*\***

Body weight of adult females ( $\geq 3.5$  years) varied from 116-190 lbs. Overall, median body weights of lactating and non-lactating adult females were similar (lactating = 155 lbs, non-lactating = 156 lbs). Median body weight of adult females across populations varied from 143 lbs (Stillwater) to 162 lbs. (Hilgard 2013), though sample sizes in some populations were relatively small (Figure 10). Median body weight of adult females at Petty Creek (measured in February 2016) was 147 lbs. Median body weight of adult females in the Hilgard population was similar both sampling events (Winter 2013/2014-162 lbs, Winter 2014/2015-159 lbs).



**Figure 10.** Boxplot illustrating distribution of body weight measurements of adult female bighorn sheep (3.5 years old or greater) across the sampled study populations. Horizontal lines through boxes represent median values, length of box represents the middle 50% of observations (IQR), vertical lines (whiskers) represent observations outside the IQR that are less than 1.5x the range of the IQR, and points outside the whiskers represent observations greater than 1.5x the range of the IQR. The numbers of animals sampled in each category are shown below the boxes.

### 3.2 Lab-Based Serum Assays

#### 3.2.1 Methods

Given the value of measuring body condition and understanding its relationship to nutritional status, combined with the challenge in attaining the equipment and expertise that are required to assess body condition in the field, we are collaborating with animal physiologist (Dr. Jim Berardinelli) and animal geneticist (Dr. Jennifer Thomson) at Montana State University (MSU) to develop a suite of traditional serum-based metabolite assays to assess body condition, as well as nutritional physiological, and reproductive status of bighorn sheep similar to those being used successfully in the livestock industry. Traditional metabolite assays for concentrations of non-esterified fatty acids (NEFA) and total protein (TP) are completed for animals sampled as part of the Montana Bighorn Sheep Study. NEFA and TP are indicative of the animals' energy reserves being utilized, with higher NEFA concentrations indicative of mobilization of fat reserves while low TP concentrations can be indicative of malnutrition or acute infection. Whereas fat measurements are indicative of the stored energy available to animals, these metrics are indicative of resources that are available and being utilized by the animals at the time of capture.

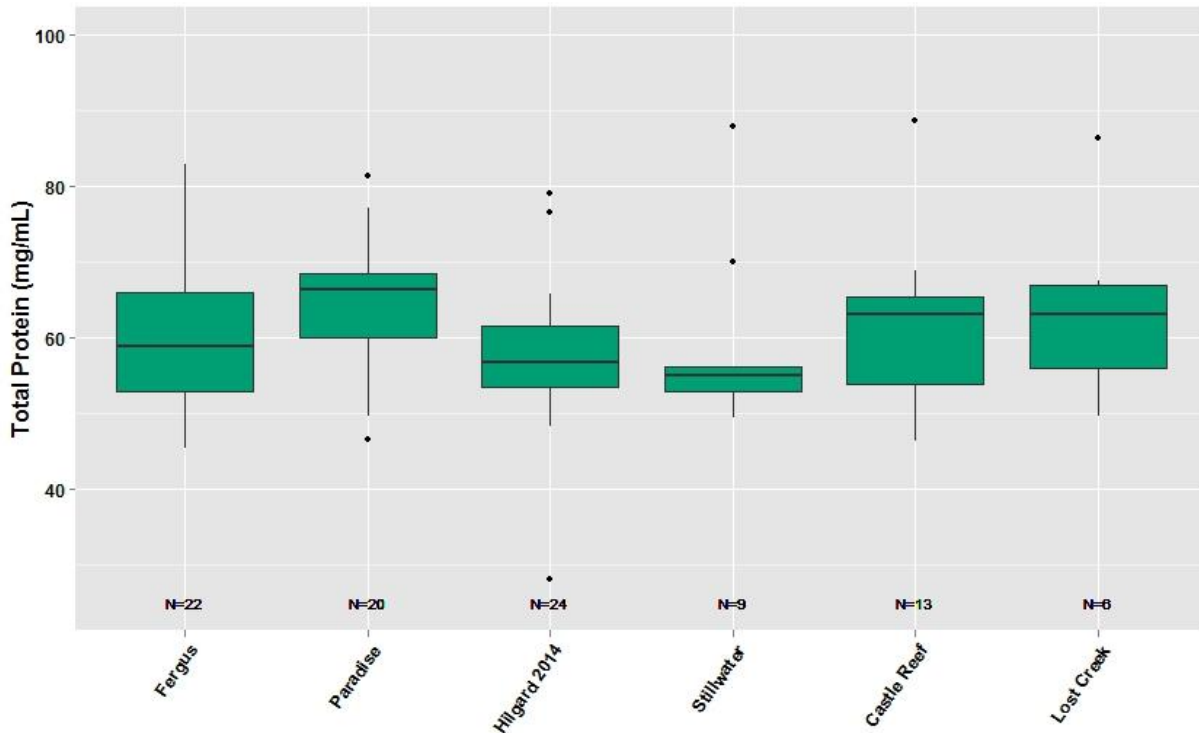
Additionally, this team is taking advantage of technological advances in the field of metabolomics. Nuclear magnetic resonance spectroscopy (NMR) is a tool that has been utilized in the medical and veterinary world to identify and quantify a multitude of different biological molecules in a given sample. The Department of Chemistry & Biochemistry at MSU has invested in an NMR instrument that our team has acquired access and training to use. As opposed to traditional serum assays which test for a single biological molecule, NMR can identify and quantify many biological molecules in a 1-mL serum sample, providing ample opportunity to assess nutrition, body composition, stress, reproductive status, and various other physiological functions. Specific metabolites and compounds detected through NMR are linked to known metabolic pathways and thus are capable of providing an extremely detailed description of animals' physiological state at the time of sampling. Given the link between survival/reproduction and physical characteristics, and between physical characteristics and physiology, there is great potential for this work to provide insights into physiological drivers of demographic processes in bighorn sheep.

NMR metabolic profiling requires only a small amount of serum from each animal. This technology could be applied in the future to nearly all animals captured for management activities as blood samples are routinely collected from all captured animals. In addition, there would be the opportunity to conduct retrospective studies of animals and herds that have been sampled in the past whose serum has been preserved. Bighorn sheep serum samples collected by collaborators in Wyoming over the past several years as part the GYA Mountain Ungulate Research Initiative have already been provided for NMR metabolic profiling and results will be incorporated with those from the Montana Bighorn Sheep Study, increasing the overall scope of the research.

### 3.2.2 Results

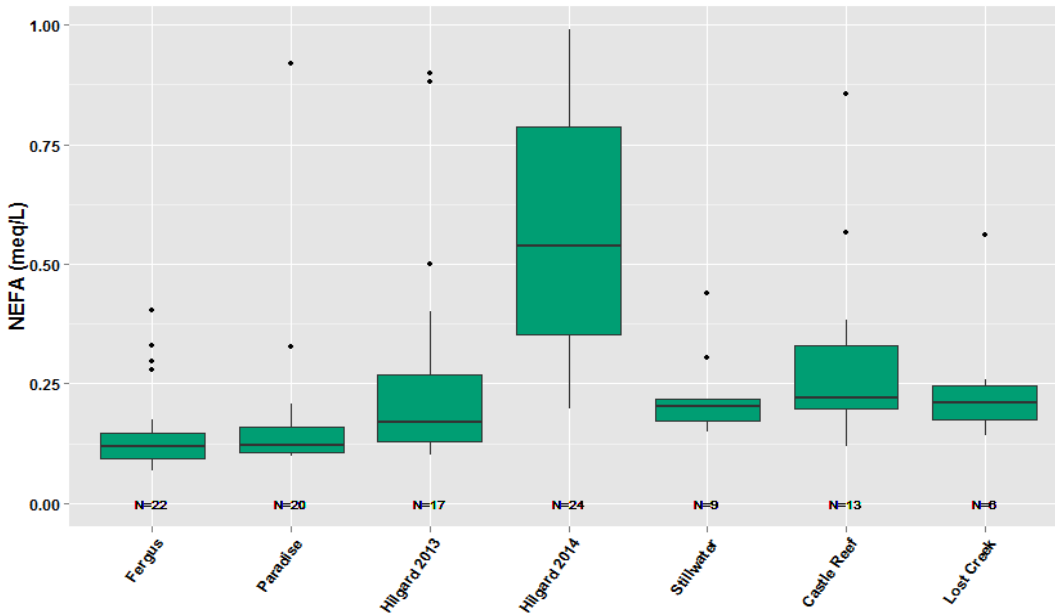
For adult females sampled in December and January, NEFA concentrations were similar for lactating and non-lactating animals (median concentration: 0.19 meq/L for lactating females vs. 0.20 meq/L for non-lactating females), as were TP concentrations (median concentration: 56.7 mg/mL for lactating females vs. 59.0 mg/mL for non-lactating females). There was some variability in TP across populations, with median December and January concentrations ranging from 54.9 mg/mL at Stillwater to 66.4 at Paradise (Figure 11). These values are comparable to previously reported total protein concentrations in Rocky Mountain bighorn sheep (Franzmann 1971), though slightly lower, as Franzmann reported a median concentration of 68 mg/mL from 65 animals sampled from Alberta, Montana and Wyoming.

There was considerable variability in NEFA among animals sampled from the different study populations in December and January, with median concentrations ranging from 0.12 meq/L in the Fergus population to 0.52 meq/L in the Hilgard population in Winter 2014/2015 (Figure 12). At that time, the animals in the Hilgard population also exhibited a wide range of variability relative to the animals sampled from other populations as well as from the Hilgard population the previous winter



**Figure 11.** Boxplot illustrating distribution of total protein concentrations in serum of adult female bighorn sheep (3.5 years old or greater) across the sampled study populations, restricted to animals sampled in December and January. Hilgard 2013 is not shown because the same assay was not run on serum from this sampling event. Horizontal lines through boxes represent median values, length of box represents the middle 50% of observations (IQR), vertical lines (whiskers) represent observations outside the IQR that are less than 1.5x the range of the IQR, and points outside the whiskers represent observations greater than 1.5x the range of the IQR. The numbers of animals sampled in each category are shown below the boxes.

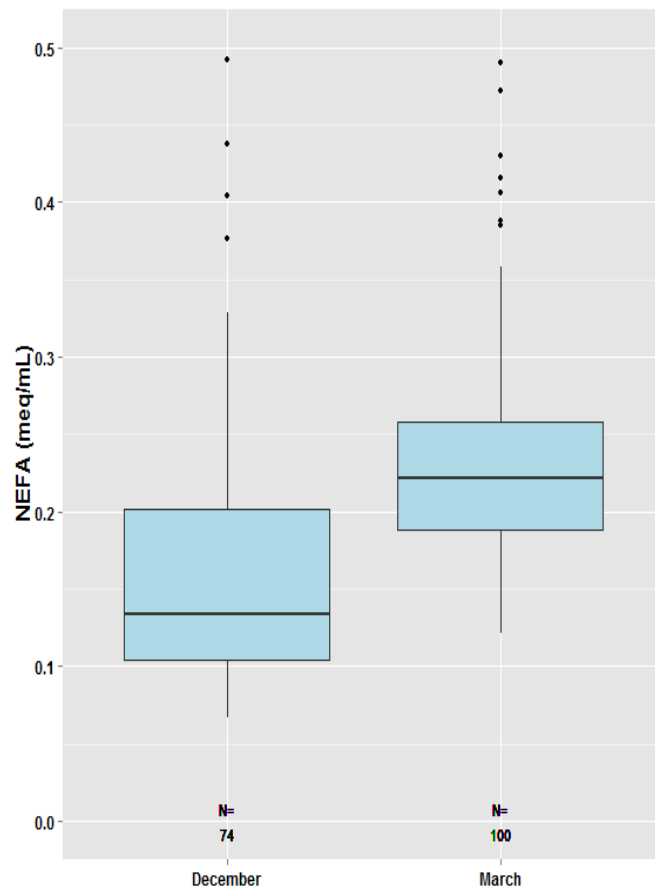
The biological significance of the difference in the concentrations of the metabolites among the study populations is not currently clear, and deepening this understanding is a goal of this research project. However, we have collected serum from animals across Montana and Wyoming at the beginning of winter (December) as well as at the end of winter (March) and it is clear that wintering ungulates should and, indeed, do have different physiological profiles when they enter winter compared to when they exit winter. While controlled studies with captive animals would provide the most rigorous methodology for developing metabolite ‘health panels’ for bighorn sheep, such studies are beyond the scope of this study. However, we can take advantage of known declines in body condition and physiological state from fall through winter due to prolonged sub-maintenance diets. Comparing metabolite concentrations in serum collected from animals in December to those in March offers an initial means to evaluate how real physiological change is reflected in the metabolite data.



**Figure 12.** Boxplot illustrating distribution of non-esterified fatty acids (NEFA) concentrations in serum of adult female bighorn sheep (3.5 years old or greater) across the sampled study populations, restricted to animals sampled in December and January. Horizontal lines through boxes represent median values, length of box represents the middle 50% of observations (IQR), vertical lines (whiskers) represent observations outside the IQR that are less than 1.5x the range of the IQR, and points outside the whiskers represent observations greater than 1.5x the range of the IQR. The numbers of animals sampled in each category are shown below the boxes.

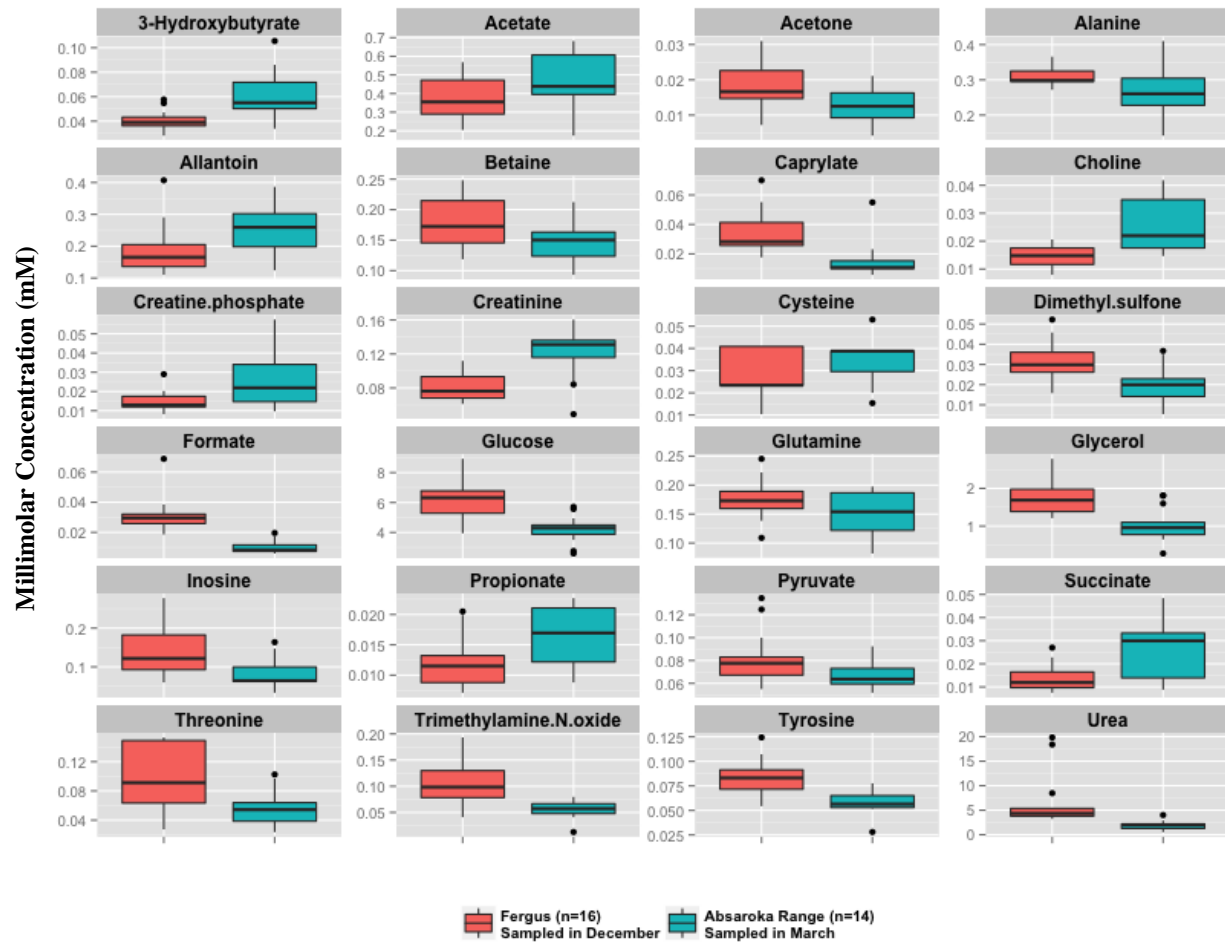
Total protein concentration didn't differ between animals sampled in December (median concentration: 62.2 mg/mL) and animals sampled in March (median concentration 59.9 mg/mL), suggesting that, in general, the animals sampled in March were not lacking energy reserves. NEFA concentration, however, did differ between animals sampled in December (median concentration: 0.13 meq/L and March (median concentration 0.23 meq/L), suggesting that fat reserves were not depleted by March and that animals were drawing more heavily from them during this time (Figure 13).

**Figure 13.** *Boxplot illustrating distribution of NEFA concentrations in serum of adult female bighorn sheep (3.5 years old or greater) sampled across Montana and Wyoming in December and January. Horizontal lines through boxes represent median values, length of box represents the middle 50% of observations (IQR), vertical lines (whiskers) represent observations outside the IQR that are less than 1.5x the range of the IQR, and points outside the whiskers represent observations greater than 1.5x the range of the IQR. The numbers of animals sampled in each category are shown below the boxes.*



Fifty small molecular weight metabolites and compounds were identified and quantified in bighorn sheep samples using NMR technology. Twenty-four of these metabolites and compounds were found to be significantly different between the two populations (Fergus and Absaroka Range), indicating a distinct metabolic shift in various metabolic pathways used for energy production at the time of sampling (Figure 14). The identification and quantification of these 50 metabolites and compounds in bighorn sheep serum, which correspond to known physiological processes, is an unprecedented accomplishment in the study of bighorn sheep. Though the entire metabolic profile cannot immediately be interpreted (as most of it has not been previously described), this technology has led to breakthroughs in human medicine and will likely prove to be of significant benefit in wildlife studies as well. Nevertheless, comparing specific molecules, as given in the present report, can provide important biological insight. For example, pyruvate and acetate are two molecules, quantified using NMR, that are byproducts of glucose and fatty acid metabolism, respectively. The mean concentration of pyruvate was 0.082

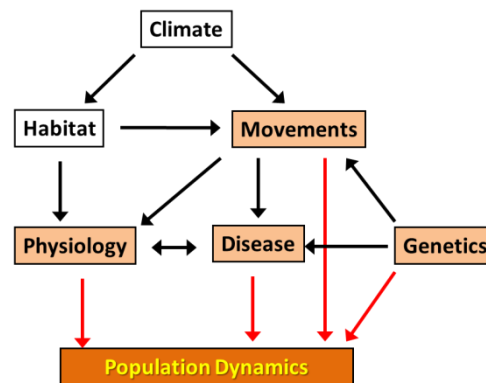
mM in animals sampled from the Fergus population and was lower in animals sampled from the Absaroka Mountains (0.066 mM). Conversely, the mean concentration of acetate in animals sampled from the Fergus population, 0.379 mM was lower than that for animals sampled from the Absaroka Mountains (0.466 mM; Figure 14). Together, these results indicate that the animals sampled from the Fergus population in December were metabolizing more glucose as an energy source than the animals sampled from the Absaroka Mountains in March. Conversely, animals sampled from the Absaroka Mountains were metabolizing more fat reserves as an energy source than the animals sampled from the Fergus population. While these preliminary findings are not surprising, they do illustrate the ability of this technology to detect physiological differences between animals.



**Figure 14.** Boxplot illustrating distribution of 24 metabolites in serum of adult female bighorn sheep (3.5 years old or greater) determined through nuclear magnetic resonance spectroscopy (NMR) to be significantly different between the two populations. Horizontal lines through boxes represent median values, length of box represents the middle 50% of observations (IQR), vertical lines (whiskers) represent observations outside the IQR that are less than 1.5x the range of the IQR, and points outside the whiskers represent observations greater than 1.5x the range of the IQR.



**Objective # 4:** *Monitor demographic rates in instrumented populations*



Accurate estimates of population size and demographic vital rates of wildlife populations are fundamental to guiding management actions because they elucidate demographic health and predict 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 annual recruitment.

#### **4.1 Adult Female Survival**

Adult female survival is being monitored in the seven 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 was inferred from GPS collar data

To date, 129 adult females in all seven study populations have been radio-collared and monitored for survival (Table 1), 14 of which have died. Animals instrumented with radio-collars in winter 2015/2016 (n=21) are not included in the following discussion, however all were found to be alive in follow up survival checks in January and February 2016. Causes of death have included hunter-harvest (n=3), cougar predation (n=2), trauma (n=1), and disease (n=1), however; the cause of half of the mortalities were undetermined (n=7, Table 2). In 2015, five mortalities occurred March-April, four mortalities occurred May-June as well as October-November, two mortalities occurred December-January and no mortalities occurred July-September. The percentage of instrumented adult females captured in the study populations in Winter 2014/2015 or previous that entered 2015 alive and survived to present, ranged from approximately 75% at Lost Creek to approximately 94% at Hilgard (Figure 15), but herd specific summaries are presented below. Another year (2016/2017) of monitoring of the instrumented animals will provide adequate data to begin formal analyses of the survival data.

**Table 2.** Cause of death for mortalities of adult female bighorn sheep in the six study populations which have been monitored since winter 2014/2015. Petty Creek is not shown due to the very recent implementation of survival monitoring.

Cause of Death	Study Population					
	<i>Fergus</i>	<i>Paradise</i>	<i>Hilgard</i>	<i>Stillwater</i>	<i>Castle Reef</i>	<i>Lost Creek</i>
Hunter Harvest	3	0	0	0	0	0
Disease	0	0	0	0	0	1
Trauma/Accident	0	0	1	0	0	0
Predation	0	1	0	0	0	1
Undetermined	0	1	1	2	2	1
<b>TOTAL MORTALITIES</b>	<b>3</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>3</b>

*Paradise:*

Two of the 15 adult females that were radio-collared in December 2014 died in 2015, leaving 87% of these animals alive and in the population. One died March 16<sup>th</sup>, likely the result of mountain lion predation, and the second died October 4<sup>th</sup> with the cause of this death unknown.

*Lost Creek:*

Three of the 12 adult females that were radio-collared in January and March 2015 died in 2015, leaving 75% of these animals alive and in the population. One died April 15<sup>th</sup> due to disease, the second died May 23<sup>rd</sup> for unknown reasons, and the third died December 25<sup>th</sup> from mountain lion predation.

*Hilgard:*

Seventeen radio-collared adult females were alive and present in 2015 as a result of sampling efforts in January 2012 (n=5) and December 2013 (n=15). Previously, a single adult female (radio-collared January 2012) died in April 2014. Fifty-two animals were translocated out of the population on January 6<sup>th</sup>, 2015 and two of the remaining radio-collared adult females from the 2012 sampling were among them. One of the 17 remaining radio-collared adult females died May 18<sup>th</sup> for unknown reasons, leaving 94% of these animals alive and in the population.

*Castle Reef:*

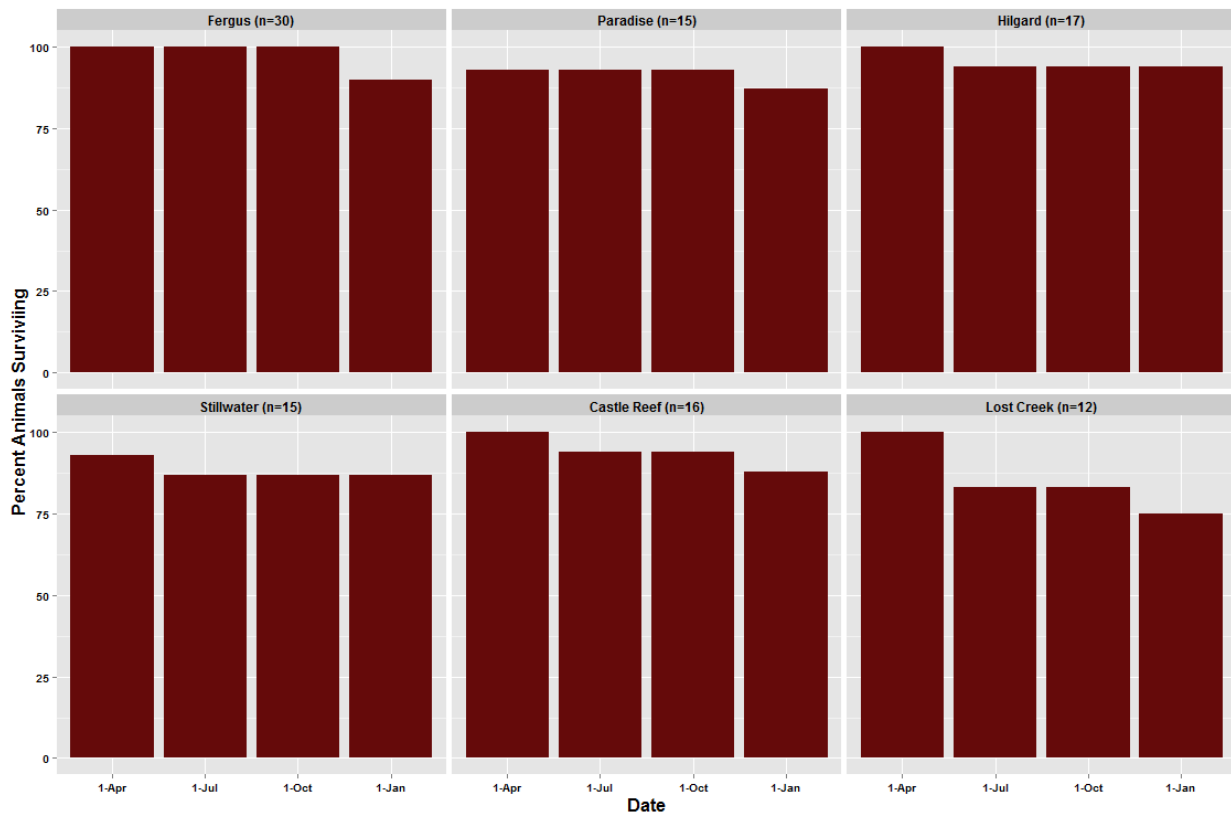
Two of the 16 adult females that were radio-collared December 2014 - March 2015 have died, leaving 88% of these animals alive. One died April 29<sup>th</sup> and the second died at an unknown date in January 2016, both for unknown reasons.

*Fergus:*

Three of the 30 adult females that were radio-collared in December 2014 died in 2015, leaving 90% of these animals alive and in the population. All three mortalities were due to hunter harvest.

Stillwater:

Two of the 15 adult females that were radio-collared December 2014 - March 2015 died in 2015, leaving 87% of these animals alive and in the population. One died April 1<sup>st</sup> and the second died at an unknown date, but likely in June. The cause of both mortalities remains unknown.



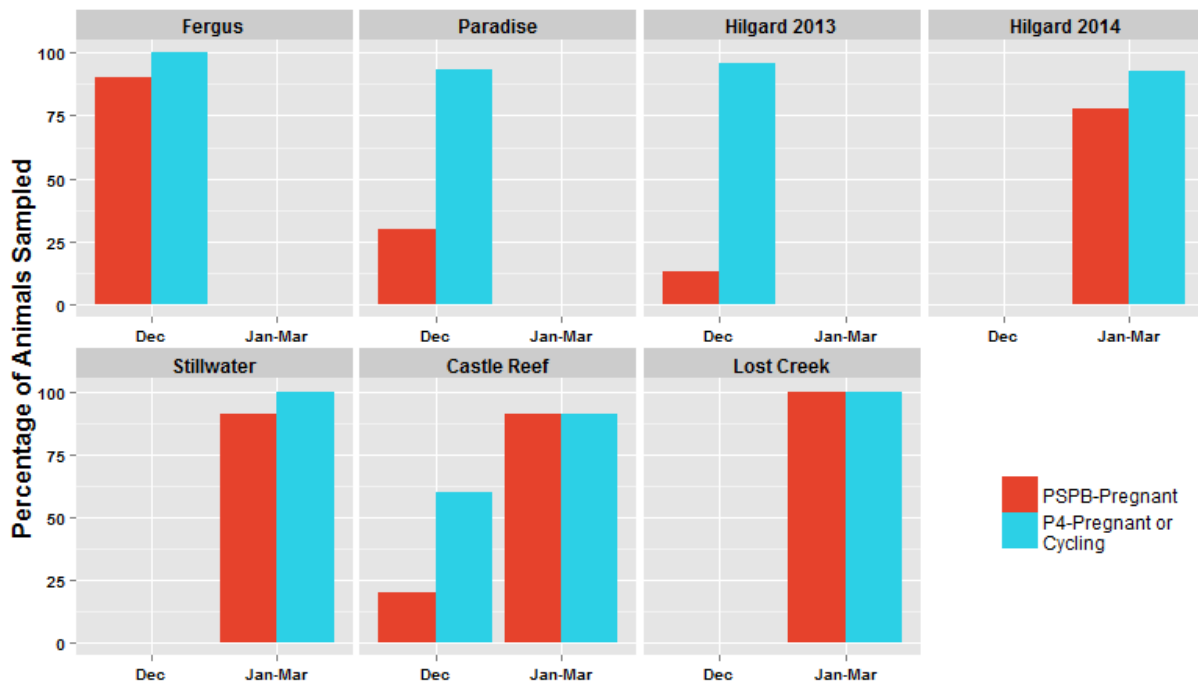
**Figure 15.** Barplots illustrating the percentage of adult female bighorn sheep radio-collared in Winter 2014/2015 or previous surviving to April 1<sup>st</sup> 2015, July 1<sup>st</sup> 2015, October 1<sup>st</sup> 2015, and January 1<sup>st</sup> 2016.

#### 4.2 Pregnancy

Pregnancy rates of adult female animals ( $\geq 1.5$  years old) in study populations as well as numerous populations in Wyoming were assessed using serum assays that measure concentrations of pregnancy specific protein B (PSPB) and progesterone (P4). PSPB concentrations indicate whether an animal is or recently was pregnant; however, detectible concentrations require up to a month following fertilization to reliably indicate pregnancy. P4 concentrations greater than 1.5 ng/mL indicate whether the animal is exhibiting regular estrous cycles (reproductively active) and an opportunity of becoming pregnant during the breeding season. Pregnancy is defined by sustained high concentrations of progesterone and the presence of detectible concentrations of PSPB. For animals sampled in December (near the end of the breeding season) PSPB may not be a reliable criterion to assess pregnancy; whereas, P4 reliably indicates whether or not an animal is cycling and has an opportunity to become pregnant. The combination of high concentration of both PSPB and P4 are unequivocal indicator of a

successful pregnancy. However, a reduced concentration of P4 and PSPB can indicate pregnancy failure (i.e. abortive process).

For animals sampled from study populations between January and March ( $n = 61$ ), the overall pregnancy rate was estimated at 87%. For comparison, the overall pregnancy rate estimated for animals sampled in Wyoming between January and March ( $n = 102$ ) was 95%. For animals sampled in December ( $n = 93$ ), only 43% of animals were found to be pregnant (though this did vary among populations); however, 89% of animals were cycling. The high percentage of reproductively active animals in December combined with the generally high pregnancy rates in animals sampled between January and March, indicate that most animals in December were pregnant or became pregnant despite the limitation of the PSPB assay to reliably detect pregnancy soon breeding and fertilization (less than 20 days). This pattern 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, there still was some variability in the test results among populations and interesting idiosyncrasies that may indicate ecological differences among populations (Figure 16). For example, the PSPB test was able to detect pregnancy in 90% of the animals from the Fergus population, which were sampled in early December, while only able to detect pregnancy in 30% of the animals from the Paradise population, which were sampled during the same week. This difference likely reflects regional differences in timing of breeding and, therefore, parturition.



**Figure 16.** Percentage of adult female bighorn sheep in the different study populations found to be pregnant by serum PSPB test (red) or found to be cycling (reproductively active) or pregnant by serum P4 test (blue), separated by whether the animals were sampled in December (when PSPB is expected to be low) or January-March (when PSPB test is expected to be high). Note that 5 animals were sampled from the Stillwater population in December and PSPB and P4 assays did not find evidence that any of the animals were pregnant or were cycling.

Interestingly, the combination of PSPB and P4 assays suggested that four animals sampled by collaborators in Wyoming had aborted pregnancies in January and February (out of a total of 313 adult female bighorn sheep tested across Wyoming and Montana), suggesting that some percentage of pregnancies in bighorn sheep are lost each year. This interpretation is based on reduced concentrations of PSPB and P4 levels that are less than 1.5 ng/mL. However, the data do not allow a strong inference as to how frequently this occurs because many aborted pregnancies may occur after March as animals' energy reserves are depleted.

#### **4.3 Recruitment**

Recruitment was estimated by calculating lamb:ewe ratios based on the number of lambs and adult females (ewes) observed during classification surveys. Classification surveys were conducted by students, volunteers, and FWP biologists and were conducted from the ground, helicopter, or fixed-wing aircraft. Lamb:ewe ratios were obtained during early winter (defined here as December and January) as an index of lambs entering winter as well as during spring (defined here as March-May) as the estimate of lambs surviving through winter and recruiting to the next age class. Data from 53 early winter and spring classification surveys conducted on the study populations since 2014 are available. The number of surveys conducted in each population varied among populations depending on accessibility of the animals and availability of personnel to conduct surveys.

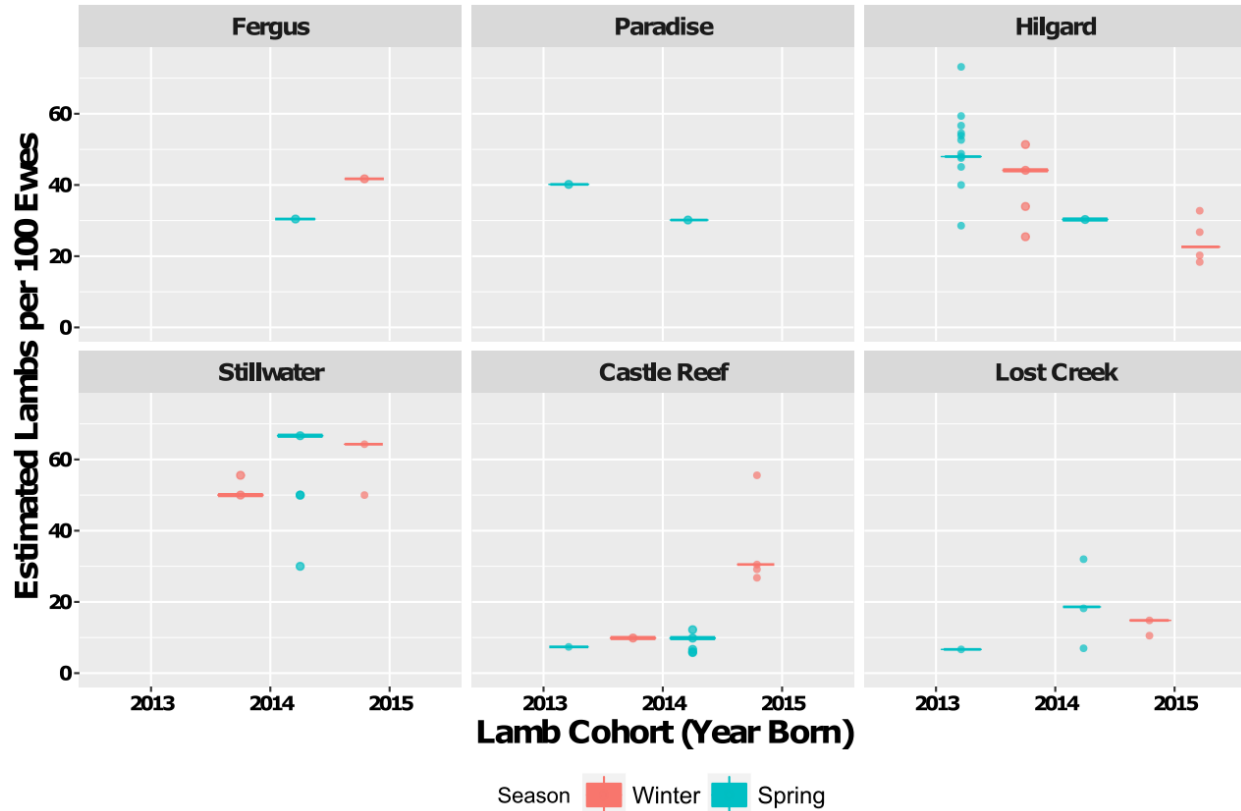
Lamb:ewe ratios varied greatly among study populations and among years within study populations. Additionally, observed lamb:ewe ratios varied among surveys within the season, attributable to variability in observability (Figure 16). The small size of both the Stillwater and the Lost Creek populations make lamb:ewe ratios obtained from these populations subject to greater amounts of variability due to observability. Seasonal lamb:ewe ratios reported here were calculated using the largest lamb count from a survey within the season and the largest ewe count within the same season. Herd specific summaries (excluding Petty Creek) follow:

##### Paradise

Two spring classification surveys have been conducted in the Paradise population as part of annual population monitoring by FWP. The lamb:ewe ratios from these surveys were 40 lambs per 100 ewes in 2014 and 30 lambs per 100 ewes in 2015, based on classifications of 122 and 126 ewes, respectively. A winter classification survey was scheduled for 2015/2016, however, helicopter maintenance, poor weather, and personnel availability have postponed this survey.

##### Lost Creek:

One spring classification survey was conducted in Lost Creek population in 2014, three spring classifications were conducted in 2015 and two surveys were conducted in early winter 2015/2016. The spring 2014 classification survey was conducted as part of annual population monitoring by FWP and the lamb:ewe ratio from this survey was 7 lambs per 100 ewes, based on classification of 45 ewes. The three surveys conducted in spring 2014 resulted in an estimated lamb:ewe ratio of 19 lambs per 100 ewes based on classification of 43 ewes. The two surveys conducted in early winter 2015/2016 resulted in an estimated lamb:ewe ratio of 15 lambs per 100 ewes entering the winter.



**Figure 17.** Observed recruitment data from study populations for lamb cohorts born since 2013 separated by timing of survey. Red represents data collected in December or January, indexing lamb recruitment entering winter, and turquoise represents data collected in March, April, or May, indexing lamb recruitment near the end of winter. For example, the winter recruitment data for the 2013 cohort were collected in December 2013 or January 2014 and the spring data for this cohort were collected March, April or May 2014. Horizontal lines are the estimated lamb:ewe ratios for each season, estimated using the maximum lamb count and maximum ewe count within the season. Dots represent the observed lamb:ewe ratios for each survey within a season.

Hilgard:

Twelve spring classification surveys were conducted in the Hilgard population in 2014, followed by four classification surveys in early winter 2015/2016, one survey in spring 2015, and four surveys in winter 2015/2016. The spring 2014 classification surveys resulted in an estimated lamb:ewe ratio of 48 lambs per 100 ewes, based on classification of 123 ewes. Lamb recruitment entering winter 2014/2015 was estimated to be 44 lambs per 100 ewes, based on classification of 68 ewes, and lamb recruitment at the end of that winter was estimated to be 23 lambs per 100 ewes based on classification of 66 ewes. Lamb recruitment entering winter 2015/2016 was estimated as lower than in previous years, at 23 lambs per 100 ewes, based on classification of 84 ewes.

Castle Reef:

One spring classification survey was conducted in the Castle Reef population in April 2014 as part of annual population monitoring by FWP, one was also conducted in early winter 2014/2015 as part of annual population monitoring by FWP, five were conducted in spring 2015, and four were conducted in early winter 2015/2016. The spring 2014 classification survey resulted in an

estimated recruitment rate of 7 lambs per 100 ewes, based on classification of 95 ewes. Lamb recruitment entering winter 2014/2015 was estimated to be 10 lambs per 100 ewes, based on classification of 71 ewes, and lamb recruitment leaving that winter was estimated to be 10 lambs per 100 ewes based on classification of 61 ewes. Lamb recruitment entering winter 2015/2016 appears higher than in previous years and was estimated to be 31 lambs per 100 ewes based on classification of 59 ewes.

#### Fergus:

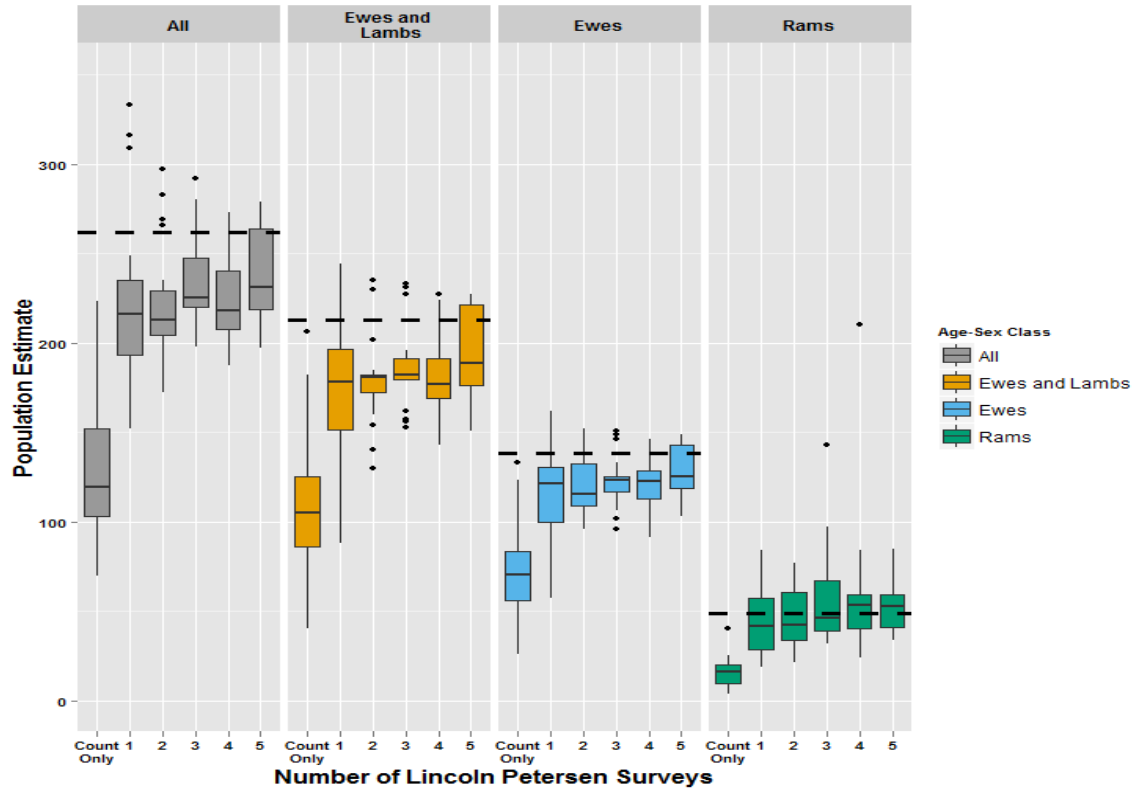
One spring classification survey was conducted in the Fergus population in May 2015 and one survey was conducted from a fixed-wing aircraft in early winter 2015/2016. Lamb:ewe ratios from May 2015 were calculated from the number of yearlings, as the new lamb cohort had already been born. Lamb recruitment was estimated at 30 lambs per 100 ewes in May 2015, based on classification of 69 ewes and lamb recruitment of the next cohort entering winter 2015/2016 was estimated to be 42 lambs per 100 ewes based on classification of 139 ewes.

#### Stillwater:

Four classification surveys were conducted in the Stillwater population in early winter 2014/2015, four were conducted in spring 2015 and two were conducted in early winter 2015/2016. Lamb recruitment entering winter 2014/2015 was estimated to be 50 lambs per 100 ewes, based on classification of 20 ewes and lamb recruitment near the end of that winter was estimated to be 67 lambs per 100 ewes, based on classification of 18 ewes. Recruitment of the next cohort was estimated to be 64 lambs per 100 ewes entering winter 2015/2016, based on classification of 14 ewes.

### **4.4 Population Size**

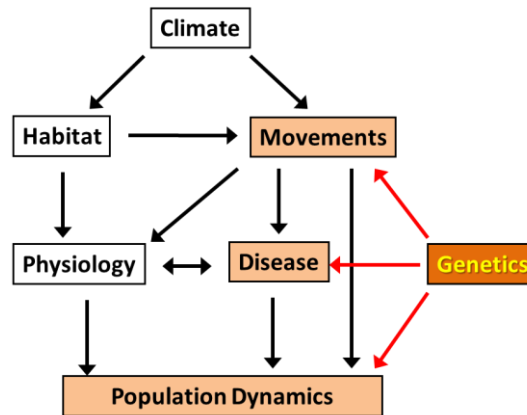
Mark-resight surveys were typically conducted in conjunction with the classification surveys when those surveys occurred after adult females were instrumented with radio-collars, and effectively “marked”. Surveyors conducting classification surveys recording the number of marked and unmarked animals in the survey and after the survey was completed used telemetry to assess how many marked animals were alive. Given known numbers of marked (and alive) animals in a population, mark-resight surveys offer a simple way to gain improved estimates of population size by estimating the number of animals in the population that were not observed in the survey. Because, repeated mark-resight surveys increase the precision of population estimates, our goal has been to conduct at least two mark-resight surveys in each study population each year. However, due to differences among the populations in accessibility and personnel availability, the number of mark-resight surveys conducted in each population has varied from a single survey, up to 18 surveys in a single winter. While data for mark-resight analyses are collected, analyses for most study populations have yet to be initiated. Lacking more rigorous population estimates for most populations, the population estimates described in section 1.2 suffice as the best currently available. Mark-resight analysis has been conducted on data from the Hilgard population, which was surveyed 18 times in winter 2014/2015, to demonstrate the utility of the method. The analysis clearly showed that even a single mark-resight survey typically results in a notable increase in the estimated population size (Figure 18).



**Figure 18.** Boxplots illustrating the distribution of population estimates (y-axis) vs. number of surveys used to calculate population estimates for different age-sex classes of bighorn sheep in the Hilgard population. Eighteen mark-resight population surveys were conducted winter 2014. “Count Only” boxes display the distribution of simple counts for each age-sex class from the 18 surveys. For boxes showing distributions of single survey estimates ( $x=1$ ), the distribution represents Lincoln-Petersen population estimates for each of 18 independent surveys that were conducted. For boxes displaying the distribution of population estimates calculated using data from 2/3/4/5 replicate surveys, the distribution represents population estimates from 20 randomly selected combinations of 2/3/4/5 individual Lincoln-Petersen surveys. Center lines of boxes represent median population estimates, the colored box represent the middle 50% of population estimates, lines extending from boxes and black dots show population estimates outside the middle 50%. Horizontal dashed lines depict “best” estimates of various age-sex classes of the population, calculated using data from all 18 Lincoln Petersen surveys. Twenty adult females and five adult males were marked in this population permitting the calculation of Lincoln-Petersen population estimates for each population segment.



**Objective # 5: Collect and Provide Samples for Bighorn Sheep Genetics Pilot Study**



While the Montana Bighorn Sheep Study project did not include funding to include genetic investigations we think this is 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 about 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.

Since the inception of the statewide bighorn sheep research project we have been laying the groundwork to add a genetic component to the integrated studies. High quality DNA has been collected and archived from all bighorn sheep herds sampled in Montana, as well as all herds sampled in the companion GYA Mountain Ungulate Project. We have also secured a total of \$168,000 to begin study of bighorn sheep genetics in Montana and Wyoming, utilizing the Ovine array. We have a National Science Foundation graduate research fellowship to support salary and cost of education for Ph.D. student Elizabeth Flesch for three years (\$138,000). In addition, we have grants from the Montana and Midwest Chapters of the Wild Sheep Foundation to fund genetic analysis of three bighorn sheep herds in Montana and Wyoming (\$20,000) and ancient DNA extraction from bighorn sheep samples collected from ice patches to serve as baseline information for modern genomes (\$5,000). We also have a grant from the Conservation Trust

Advisory Board of the National Geographic Society to fund genetic analysis of bighorn sheep in Glacier National Park (\$5,000).

As part of a modest pilot study, we plan to quantify genetic attributes of bighorn sheep populations with a range of different herd histories in Montana and Wyoming to investigate genetic similarity and differences, genetic heterogeneity, and genetic distance. We will analyze approximately fifteen individuals from each of four different populations that we predict would differ in genetic characteristics, due to population attributes that potentially impacted their genetics, including origin (native/reintroduced), population size, bottleneck history, degree of connectivity, and augmentation history. We selected four populations that provided a spectrum of these herd attributes, including the Tendoy, Stillwater, and Glacier National Park in Montana and the northeastern Greater Yellowstone Area in Wyoming. We will examine expected and observed heterogeneity and genetic distance estimates to evaluate the potential for links between genetics and herd demography. The pilot study will help us evaluate the potential effectiveness of the Ovine array and whole genome genotyping in addressing genetics research goals for bighorn sheep in Montana and elsewhere. If results of the pilot study are promising, we will attempt to secure funding to formally integrate genetic studies into the statewide bighorn sheep studies.

## **Deliverables**

### **1. Annual Reports**

2014, 2015. R.A. Garrott, K.M. Proffitt, J.J. Rotella, C.J. Butler. 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

### **2. Professional Presentations**

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

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

R.A. Garrott, J.J. Rotella, K.M. Proffitt, J. Ramsey, C.J. Butler. Montana's new statewide bighorn sheep research initiative. 2014. 19<sup>th</sup> 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. 19<sup>th</sup> 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 53<sup>rd</sup> 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 53<sup>rd</sup> 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

Herrygers, M. R., R. Garrott, C. Butler, and J. G. Berardinelli. 2014. Pregnancy rate and metabolites in bighorn sheep (*Ovis canadensis*) at the end of breeding season and the first trimester of pregnancy. Montana Academy of Sciences Annual Meeting, Butte, MT.

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FWP personnel who have assisted in the first years of these studies include, but are not limited to, Bruce Sterling, Jim Williams, Ben Jimenez, Liz Bradley, Tyler Smucker, Ray Vinkey, Mike Thompson, Brent Lonner, Mark Schlepp, Tim McWilliams, Stan Buresh, George Larson, Sonja Smith, Graham Taylor, Julie Cunningham, Jenny Jones, Tyler Park, Howard Burt, Shawn Stewart, Justin Paugh, Ray Mule, Brent Cascadden, Kevin Hughes, Jennifer Ramsey, Emily Almberg, Neil Anderson, Keri Carson, Nick DeCesare, Quentin Kujala, and Justin Gude. In addition, Vickie Edwards, a former FWP biologist, was instrumental in laying the groundwork for including the Petty Creek herd in the studies. The FWP Wildlife Health Lab has been instrumental to this project in all the support service and cooperation they have provided throughout the study. MSU personnel who have assisted in the studies include Aaron McGuire, Jesse DeVoe, Blake Lowrey, Elizabeth Flesch, Jim Berardinelli, Jennifer Thomson, Dave Willey, Rashelle Herrygers, Jesse White, Tawnya Gilstrap, Cheyenne Stirling, John Landsiedel, Heather Brown, Jasmine Cutter, Aubrey Power, Samuel Allen, and numerous other student volunteers.

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Program, traveled to Montana to train MSU and FWP personnel in the use of ultrasonography to quantify rump fat thickness in bighorn sheep. Important Wyoming Game and Fish collaborators on the Greater Yellowstone Area Mountain Ungulate Project that contributes to the regional scope of these studies include Doug McWhirter, Doug Brimeyer, Allyson Courtemanch, Gary Fralick, Hank Edwards, Mary Wood, Hally Killion, and Jessica Jennings Gaines. The Wyoming Game & Fish Wildlife Disease Lab has been instrumental in shaping our respiratory pathogen research and contributing to sample collection outside of Montana. Tom Besser of Washington State University has provided insights and advice on pathogen sampling and has conducted initial strain-typing of *Mycoplasma ovipneumoniae* isolates from Montana and Wyoming bighorn sheep. This project has benefited from interactions with scientists involved in the Hells Canyon Initiative Consortium including Frances Cassirer with Idaho Fish and Game, Kezia Manlove, Ph.D. student at Pennsylvania State University. Kezia Manlove conducted field work monitoring demographics of the Fergus population in summer 2015 and provided valuable information on lamb production and survival that would not otherwise have been available. Tom Besser has also provided insights and advice on pathogen sampling and has conducted initial strain-typing of *Mycoplasma ovipneumoniae* isolates from Montana and Wyoming bighorn sheep.

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