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

Federal Aid in Wildlife Restoration Grant W-159-R

Annual report, February 15, 2015

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State: Montana
Agencies: Fish, Wildlife & Parks and Montana State University
Grant: Montana Bighorn Sheep Study
Grant #: W-159-R
Time Period: 1 December, 2013 – 31 January, 2015
Note: All results should be considered preliminary; please contact the authors before citing or referencing these data

Project Background

Bighorn sheep conservation and management has been, and continues to be, a challenge in Montana and across western North America. Approximately a century ago all of the ungulates native to Montana were severely depressed in numbers and distribution due to overexploitation, habitat modification, and a lack of concerted and scientifically-informed management. Regulation of harvest, habitat protection and enhancement, intensive natural history studies, and translocation programs resulted in successful restoration of most ungulate species. Today elk, mule deer, white-tailed deer, and pronghorn are all abundant, broadly distributed across the state, support a robust hunter harvest, and are enjoyed by nearly all Montanans and those visiting our state (Picton and Lonner 2008). While similar management and conservation efforts have been devoted to bighorn sheep, most populations are relatively small and are patchily distributed across the state, with many populations static or periodically experiencing dramatic declines despite the fact that adequate habitat seems to be abundant (Toweill and Geist 1999, Montana Department of Fish Wildlife and Parks 2010).

Wildlife managers routinely make decisions on bighorn sheep population augmentation and restoration, harvest, habitat management, disease prevention and response, and other conservation actions without adequate knowledge of the drivers of demographic processes that inform management of many of Montana's more successfully restored ungulates species. Our better understanding of elk and deer population dynamics and ecology are a consequence of a tradition of investing in long-term field studies focused on understanding basic species ecology and recognized management challenges (Mackie *et al.* 1998, Hamlin and Ross 2002, Hamlin *et al.* 2009). In contrast, field studies of bighorn sheep have been limited primarily to short-term, master's thesis projects focused on a specific herd (e.g., Schallenberger 1966, Erickson 1972, Stewart 1975, Keating 1982, Legg 1996, Ostovar 1998, Enk 1999). While informative, such studies accrue knowledge slowly and cannot make the major advancements in ecological understanding that are realized from coordinated and intensive long-term research involving multiple populations in diverse ecological settings.

As an initial start to establishing a state-wide bighorn sheep research project, Montana Fish, Wildlife and Parks (MFWP) supported a six month contract to 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. This project has recently been completed and a final report has been circulated among all MFWP personnel involved in bighorn sheep management (Butler, Garrott, and Rotella 2013). This effort revealed substantial variation in annual recruitment rates (as indexed by lamb:ewe ratios) within each herd over time. Regression models that considered a wide range of covariates indexing cold and warm season climate conditions, as well as impacts of pneumonia-related disease die-offs provided limited ability to explain the observed variation in recruitment, with top models from only seven of the 25 herd-specific analyses explaining ≥40% of the variation in the annual recruitment estimates. The analyses revealed drastic reductions (averaging 76%) in recruitment rates for multiple years following all-age disease die-

off events. The impact of pneumonia outbreaks on subsequent recruitment rates, however, was highly variable, suggesting there are likely unknown factors involved in the demographic expression of pneumonia die-off events in infected bighorn populations (Butler, Garrott, and Rotella 2013).

While there is strong evidence of ungulate populations occupying temperate and high-latitude ranges having clear associations between annual variation in seasonal climate conditions and demographic attributes (Sæther 1997, Gaillard *et al.* 2000), we only detected these associations in approximately one-quarter (5) of the herd-specific analyses of Montana's bighorn sheep recruitment data. For these five herd-specific analyses, unique sets of climate covariates were found to best explain the variation in recruitment rates and the same climate covariates frequently had opposite relationships with recruitment rates. Thus, these results provide evidence that bighorn populations in Montana, even those in close proximity to each other, can be influenced differently by climate (Butler, Garrott, and Rotella 2013).

The recruitment analyses also revealed substantial variation in the baseline productivity of Montana's bighorn sheep herds, with average recruitment under normal conditions (and no disease-related effects) ranging from approximately 22 to 49 lambs per 100 ewes among herds. While these differences in baseline productivity suggest individual herds experience biologically significant differences in the magnitude and perhaps sources of underlying limiting factors, the variation in baseline productivity did not appear to be associated with ecoregions, patterns of annual precipitation experienced throughout the state, or any other index of potential habitat quality. There was, however, a relationship between mean population size and baseline recruitment, with larger populations generally experiencing higher average lamb:ewe ratios than smaller populations (Butler, Garrott, and Rotella 2013). While these results provide no direct evidence for the mechanisms that may be responsible for such a relationship there is ample literature describing many potential mechanisms (e.g. predation, inbreeding depression, increased effects of stochastic processes) that can contribute to poor demographic performance of small populations (Berger 1990, Boyce 1992, Dennis 2002, Festa-Bianchet *et al.* 2006, Mills 2007, Cassaigne *et al.* 2010). Currently, 72% (35 of 48) of Montana's bighorn populations have less than 100 individuals, suggesting perhaps that many of the state's populations may be marginally viable at their current population sizes. Improving current management actions and/or devising new options aimed at increasing population sizes seems warranted, however, the analysis of Montana's bighorn recruitment data demonstrate that our understanding of the factors affecting demographic vigor of bighorn sheep populations in Montana is currently limited. This research initiative is designed to help improve our ecological understanding of bighorn sheep to help better inform management and conservation.

Project Objective
The overall aim of this research program is to assess the role of herd attributes, annual variation in climate, disease pathogens, and habitat conditions on bighorn sheep recruitment, adult survival, and population dynamics.

Location

Bighorn sheep research conducted under this grant is focused within the range of seven distinct bighorn sheep populations across varying ecological settings in Montana, occupying portions of Deer Lodge, Fergus, Lewis & Clark, Madison, Missoula, Sanders, Stillwater and Teton Counties, as well as the Flathead Indian Reservation. Populations initially planned to be included in the research program included Paradise, Lost Creek, Hilgard, Highlands, Castle Reef, and Fergus. However, due to logistical constraints of capturing animals, the Highlands population was dropped from the research program and replaced by the Petty Creek population.
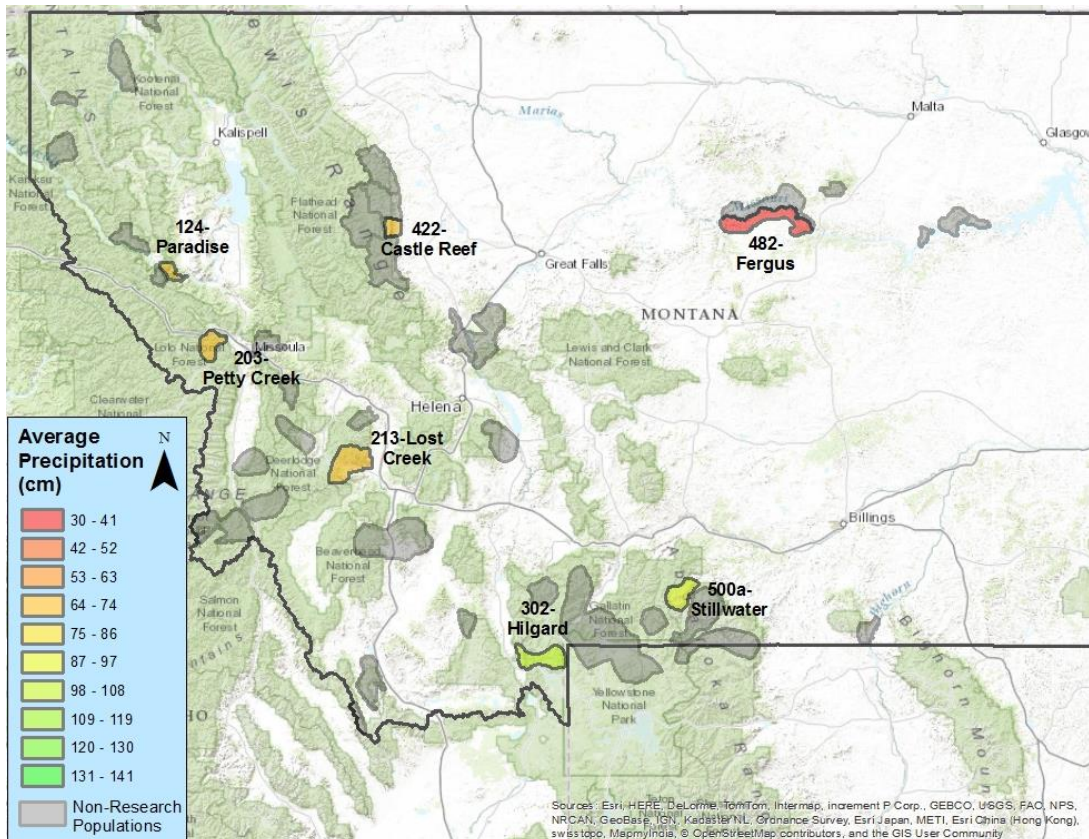

Figure 1. *Ranges of, and precipitation experienced by, the seven study populations included in the Montana Bighorn Sheep Study. Polygons shaded in gray show ranges of the other bighorn sheep populations in Montana that are not part of this research effort.*

Study Objectives (Year 1 of 6 year-study)

During the first field season of this bighorn sheep research program, the primary objectives were:
1) Capture, sample, and instrument animals in each study population.
2) Assess variation in respiratory pathogen communities and exposure among sampled populations
3) Assess variation in body condition and physiological status among sampled populations
4) Monitor demographic rates in instrumented populations

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

1.1 Study populations

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.

Paradise: This population in 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 280 animals, experiences moderate recruitment in most years, and is believed to be isolated from other bighorn populations. There are no records of this population experiencing a disease-related die-off.

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 experienced a disease-related die-off.

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 disease-related die off events, the most recent occurring in 2010. The population currently numbers ~60 animals due to the recent die-off, and while post-die-off recruitment was initially low, current recruitment appears to be gradually improving.

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 disease-related die-off 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.

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 disease-related die-offs between 1924 and 1936, a fourth die-off in 1984, and the most recent die-off 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 die-off recruitment has been very low.

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 400 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 disease-related die-offs.

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 is no history of disease-related die offs in the population, but the population has been augmented twice (1970, 1984).

1.2 Animal Capture and Sampling

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). In total, the goal by the end of winter 2014/2015 was to capture 210 animals and deploy 105 pairs of VHF and GPS radio collars. All capture and handling procedures followed protocols approved by the Montana State University Institutional Animal Care and Use Committee (Permit # 2014-32).

1.2.1. Capture Methods

All captures were planned for winter months. Planned capture methods included helicopter net-gunning for Paradise and Fergus and drop-netting for the other five populations. Helicopter net-gunning was performed by Quicksilver Air Inc. The realized method to capture animals in the Stillwater population became chemical immobilization via ground darting due to changes in safety requirements on the privately owned mine property where this population winters. Animals in this population were chemically immobilized using B.A.M. (30 mg Butorphanol/adult, 10 mg Azaparone/adult, 12 mg Metatomidine/adult). Chemically immobilized animals were administered oxygen at two liters/minute and were also subcutaneously administered 5-7 mL Liquamycin LA-200 (oxytetracycline antibiotic). Reversal entailed intramuscularly administering 200mg Tolazaline/adult followed by 31.25 mg Atipamezole /adult.

1.2.2 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, throat 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.

1.2.3 Capture and Sampling Accomplishments

To date 126 bighorn sheep have been captured as part of the Montana Bighorn Sheep Study and 74 pairs of GPS and VHF radio-collars purchased for the project have been deployed (Table 1). Capture and sampling objectives have been satisfied in three of the seven study populations and capture efforts in winter 2014/2015 are ongoing. The Hilgard population was successfully captured and sampled during winter 2013/2014 using an 80' drop net. In this population 29 animals were captured and all 15 pairs of GPS/VHF radio-collars were deployed on adult females. Thirty adult females were captured and sampled in each of the Paradise and Fergus populations during winter 2014/2015 and in each population 15 pairs of GPS/VHF radio-collars were also deployed on adult females. At the time of writing, all animals instrumented with radio-collars as part of this study are alive.

Capture and sampling goals have been partially achieved in three of the remaining four study populations during winter 2014/2015. Twenty animals have been captured and sampled in the Castle Reef population with use of 40' drop nets and 13 pairs of GPS/VHF radio-collars have been deployed. Seven animals have been captured and sampled in the Lost Creek population with use of a 40' drop net and five pairs of GPS/VHF radio-collars have been deployed. Additionally, 10 adult females have been captured and sampled in the Stillwater population via ground darting and each animal was instrumented with a pair of GPS and VHF radio-collars.

Drop-net capture efforts in the Petty Creek population have been unsuccessful to date due to challenges attracting animals to the drop-net sites. We are optimistic that increasing snowpack throughout the winter will increase the attractiveness of bait to the animals in this population and efforts are ongoing through winter 2014/2015 for all populations where samplings goals have not been met. Where possible, ground darting will be considered to capture animals in populations where drop-netting proves unsuccessful.
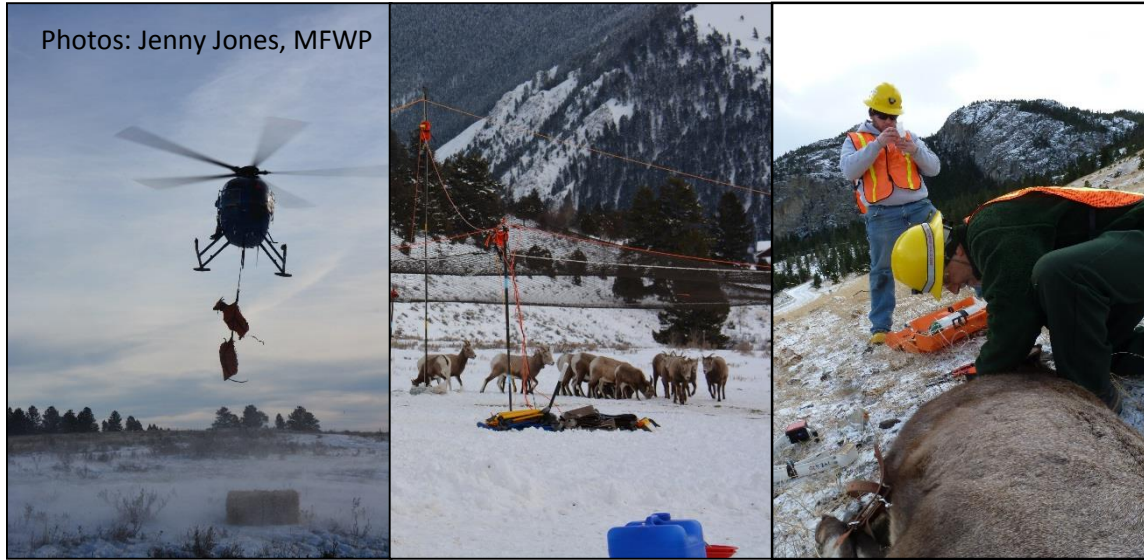
Figure 2. *From left to right: two bighorn ewes from Fergus population captured by Quicksilver Air Inc., a group of bighorns from the Hilgard population under a 80' drop net just prior to capture, and principle investigator Bob Garrott and technician Aaron McGuire processing a chemically immobilized bighorn ewe from the Stillwater population.*

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 capture for the Montana Bighorn Sheep Study in the Fergus bighorn population was a management capture to translocate 30 bighorns out of this population as the herd is above management objective. 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.

Sampling and radio-collaring of the Hilgard population has also been enhanced beyond the original research objectives. Just prior to the initiation of this study in winter 2011/2012 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 2013/2014, 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 GPS collars purchased with funds generated by MFWP bighorn sheep auction sales, allowing us to include this newly established population in our routine research monitoring. The increased data and sample collection that has resulted from these collaborations will undoubtedly improve insights that will be obtained from the research program.

	ANIMALS SAMPLED Goal = 30/population		VHF Radio-Collars In Population Goal = 15/population		GPS Radio-Collars In Population Goal = 15/population	
	2013/2014	2014/2015	2013/2014	2014/2015	2013/2014	2014/2015
Paradise	--	30	--	15	--	15
Petty Creek	--	0	--	0	--	0
Lost Creek	--	7	--	6	--	6
Hilgard	29	50	25	31	15	25
Castle Reef	--	20	--	13	--	13
Fergus	--	60	--	30	--	15
Stillwater	--	10	--	10	--	10
TOTAL	**29**	**177**	**25**	**105**	**15**	**84**

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.*

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

Respiratory disease (pneumonia) was identified as a major cause of mortality in bighorn sheep near the turn of the 20th Century and is still an important factor limiting bighorn sheep populations across the western United States and Canada (Rush 1927, Buechner 1960, Douglas 2001, Cassirer and Sinclair 2007, George *et al.* 2008, Edwards *et al.* 2010, Wolfe *et al.* 2010). Respiratory disease is often a complex system, and causative agents are difficult to identify even in domestic animals (Yates 1982, Wehausen *et al.* 2011). A great amount of research effort has been invested into identifying the causative agents of respiratory disease in bighorn sheep; however, less emphasis has been placed on assessing the relationship between presence of suspected respiratory pathogens and demographic performance of wild bighorn populations.

One very clear finding that has stemmed from observation and experimentation is that domestic sheep are asymptomatic carriers of the pathogen(s) responsible for respiratory disease epizootics in bighorn sheep, as commingling experiments with the two species have collectively resulted in a 98% mortality rate in bighorn sheep (Besser *et al.* 2013). This strong association has had a major influence on bighorn sheep management practices and has also helped narrow the focus on potential pathogens responsible for respiratory disease epizootics in bighorn sheep to a relatively

short list of bacterial species: (1) members of the *Pasteurellaceae* family, specifically leukotoxin producing strains of *Mannheimia haemolytica* (*M. haemolytica*) and *Biberstenia trehalosi* (*B. trehalosi*) as well as *Pasteurella multocida* (*P. multocida*; Foreyt 1989, 1990, Foreyt *et al.* 1994, Dassanayake *et al.* 2009, 2010b, 2013, Lawrence *et al* 2010, Wolfe *et al.* 2010, Shanthalingam *et al.* 2014); and (2) *Mycoplasma ovipneumoniae* (*M. ovipneumoniae;* Besser *et al.* 2008, 2012a, 2012b, 2013). However, there is continued debate in the literature over the roles these pathogens play in the expression of respiratory disease in wild bighorn populations. In addition to uncertainty in the pathogen(s) primarily responsible for respiratory disease epizootics, there is uncertainty as to whether respiratory disease epizootics result from opportunistic endemic pathogenic agents that become virulent for unknown reasons, or if they result from introduction of novel pathogens (Miller *et al.* 2012a). Accordingly, improving our knowledge of pathogen communities in healthy bighorn populations has been recognized as a research necessity (Besser *et al.* 2012b). Thus, a major aim of the Montana Bighorn Sheep Study is to assess respiratory pathogen communities in both robust and struggling bighorn populations and determine to what extent variation in pathogen communities explains variation in demographic rates of different populations.

2.1 Disease Sampling Methods
The Montana Bighorn Sheep Study adopted sampling methodologies that improve knowledge of both the *Pasteurellaceae* and *M. ovipneumoniae* communities in study populations. To detect *Pasteurellaceae* pathogens, sampling strategies similar to those employed by Wyoming Game and Fish Department (WGF) were adopted to allow for future comparison of respiratory pathogen communities in bighorn sheep populations that are outside of this research effort. The *Pasteurellaceae* sampling methodology entailed taking two throat swabs from each animal. One swab from each animal was immediately froze in TSB transport media and shipped to Washington Animal Disease Diagnostic Laboratory (WADDL) for detection of *Pasteurellaceae* pathogens via routine culture methods. These swabs were also used to take advantage of a new leukotoxin PCR test being offered by WADDL to assess whether the *Pasteurellaceae* organisms present in each population possess the gene to produce leukotoxin; the agent responsible for severe *Pasteurellaceae* respiratory infections in bighorn sheep (Dassanayake *et al.* 2009, Dassanayake *et al.* 2013). The other throat swab was used to inoculate a culture plate at the animal, which was then incubated in a custom built incubator in order to facilitate growth of more *Pasteurellaceae* organisms and increase detection rate of individual species. This method follows WGF protocol and also allows the project to cryogenically preserve bacterial cells and DNA, resulting in a collection of samples for future disease testing. For a subset animals that were sampled in each population, a swab of bacterial growth from the culture plate was also shipped to WADDL to test for *Pasteurellaceae* organisms via routine culture in order to assess whether this method increases detection rate of *Pasteurellaceae* species.

Exposure of study populations to *M. ovipneumoniae* was assessed by sending serum from each animal to WADDL to detect antibodies against *M. ovipneumoniae*. To assess presence of *M. ovipneumoniae* in study populations, one nasal swab was collected from each animal and sent

frozen in TSB transport media to WADDL for PCR detection. Collaboration with the Hells Canyon Initiative has led to Dr. Tom Besser at WADDL agreeing to conduct genetic strain typing of *M. ovipneumoniae* detected in PCR tests and allow exploration of whether different strains of this species display different pathogenicity. Additionally, samples were collected using the same methods as swabs that were previously collected from animals as part of the Greater Yellowstone Area Mountain Ungulate Project (MUP), which allows data collected as part of the two efforts to be directly comparable.

Figure 3. *Field sampling techniques. A. Collecting nasal swab for M. ovipneumoniae detection. B. Collection of blood for detection of M. ovipneumoniae antibodies. C. Collecting throat swab for detecting Pasteurellaceae species. D. Plating throat swab onto Columbia Blood Agar plate at the animal.*

2.2 Disease Sampling Results
2.2.1 Sampling Effort
Serum, as well as nose and throat swabs that were placed immediately in transport media, were collected from 125 animals in six of seven study populations as part of the Montana Bighorn Sheep Study (n = 29 in 2013/2014, n = 96 in 2014/2015, Table 2). All samples were sent to WADDL for diagnostic testing and results have been received. However, leukotoxin PCR results are still pending. Eighty additional sets of samples were collected from two study populations in winter 2014/2015 (N=30 from Fergus, N=50 from Hilgard) as part of management activities by MFWP. These samples were also sent to WADDL for the same diagnostic tests and results from these tests will be included in the Montana Bighorn Sheep Study. However, at the time of writing results from these samples are not available and data from the sampling of the Hilgard population during winter 2014/2015 are not shown. Additionally, throat swabs from 134 animals

captured in winter 2014/2015 (n = 96 research captures, n = 38 management captures) were used to directly inoculate culture plates and the bacteria cultured from each plate have been cryogenically preserved for future testing. Swabs of bacterial growth from 76 of the 134 plates were sent to WADDL for *Pasteurellaceae* culture testing.

SAMPLE TYPE	Serum	Nasal Swab – TSB	Throat Swab- TSB	Throat Swab- Plated	
(Test)	(M.ovi Serology)	(M.ovi PCR)	(Pasteurellaceae Culture / PCR)	(Pasteurellaceae Culture / PCR)	
	Collected & Submitted	Collected & Submitted	Collected & Submitted	Collected	Submitted
Paradise	30	30	30	30	15
Lost Creek	7	7	7	7	7
Hilgards 2013/2014	29	29	29	-	-
Hilgards 2014/2015	48	49	49	38	18
Castle Reef	20	20	20	20	12
Fergus	30	30	30	30	15
Stillwater	9	9	9	9	9
TOTAL	173	174	174	134	76

Table 2. *Number of samples collected and submitted for respiratory pathogen identification in each study population of the Montana Bighorn Sheep Study. All serum, nasal swabs in TSB, and throat swabs in TSB were submitted to Washington Animal Disease Diagnostic Lab (WADDL) for diagnostic tests. TSB refers to the transport media swabs were placed in. The general diagnostic tests that have been or will be run for each sample type is shown in italic within the table header. M.ovi is an abbreviation of Mycoplasma ovipneumoniae. A portion of plated throat swabs (throat swabs that are used to directly inoculate culture plates at the time of capture) are submitted to WADDL to assess if the method improves detection of Pasteurellaceae pathogens. Samples of bacterial growth from all plated throat swabs are cryogenically preserved for future respiratory pathogen testing.*

2.2.1 Disease Sampling Results
Diagnostic tests detected seven types of potential respiratory pathogens. These pathogens include *M. ovipneumoniae* (35 detections in five populations)*, M. haemolytica* (16 detections in three populations), *B. trehalosi* (124 detections in six populations)*, P. multocida* (one detection in one population)*, unidentified Mannheimia species* (13 detections in four populations), unidentified *Pasteurella species* (23 detections in four populations)*, and Trueperella pyogenes* (13 detections

in five populations). Of these seven groups of pathogens, those currently of most interest are *M. ovipneumoniae*, *M. haemolytica*, *B. trehalosi,* and *P. multocida*, and the following summaries will be restricted to these groups (Figure 4).
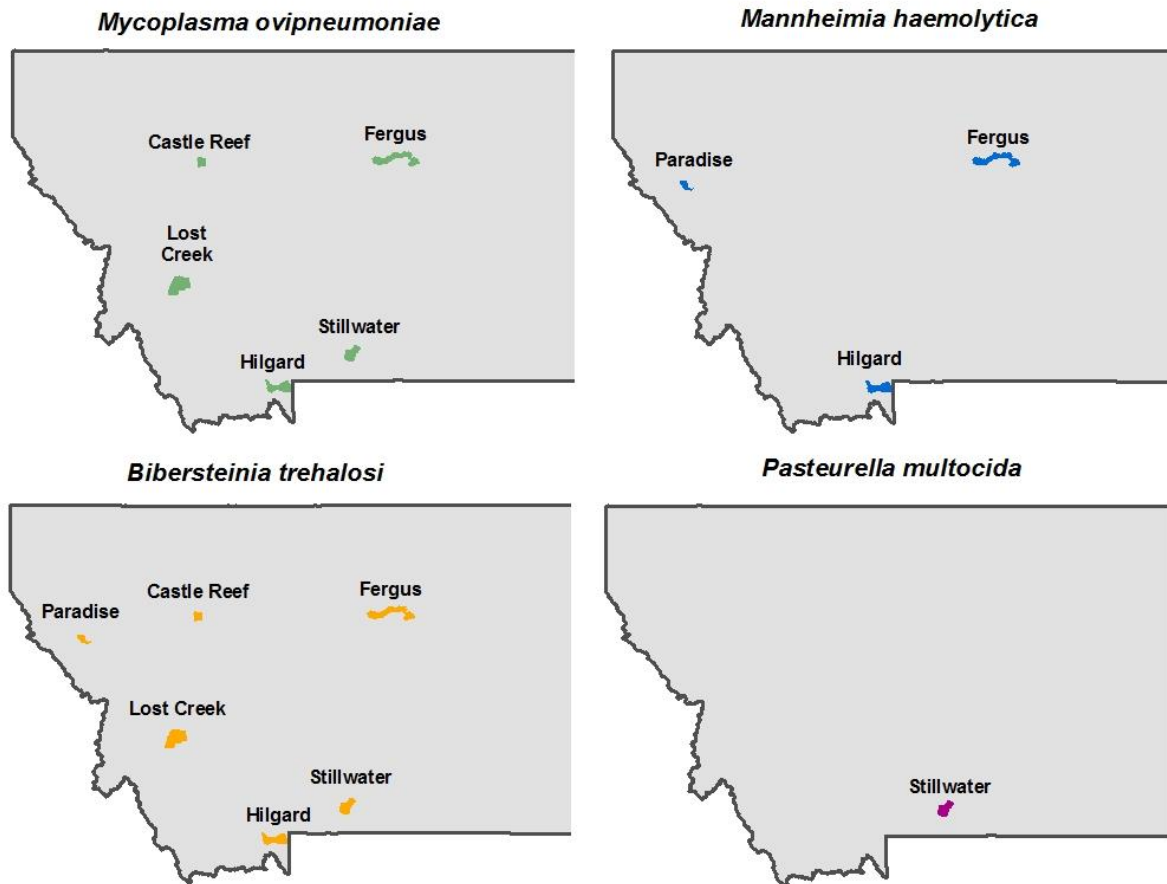

Figure 4. *Maps indicating the study populations in which respiratory pathogens of interest were detected. Populations where pathogens of interest were not detected are not shown.*

B. trehalosi was detected in every sampled population at high prevalence, with detection ranging from 71% 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 3% 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 79% of animals sampled (Castle Reef). Interestingly, minimum PCR prevalence and prevalence of animals with serum antibodies for *M. ovipneumoniae* do not appear to be related (Figure 5). *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) populations. *P. multocida* was only detected in a single animal from the Stillwater population. Results from the leukotoxin PCR tests will help clarify the potential for the pathogens communities to cause respiratory disease. It should be noted that the number of animals sampled in several populations is rather modest and also that the true prevalence of these pathogens is higher than the detection rate. Figure 5 illustrates minimum prevalence of the different pathogens across the study populations.
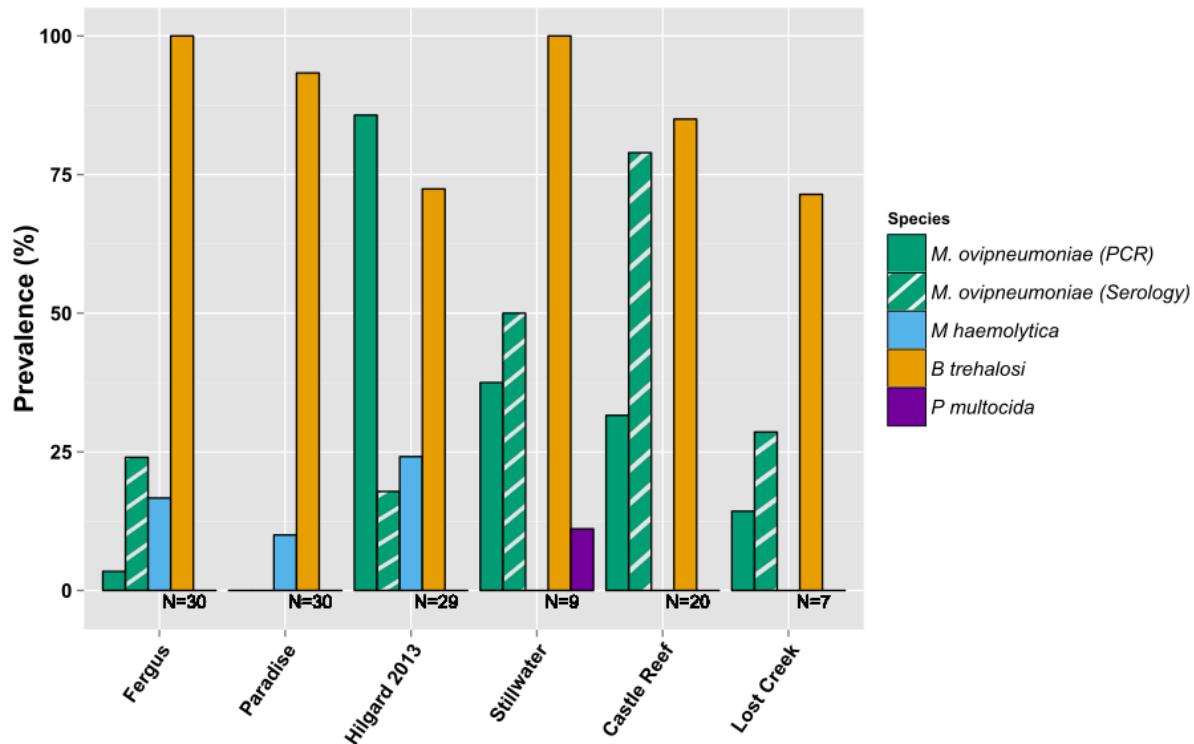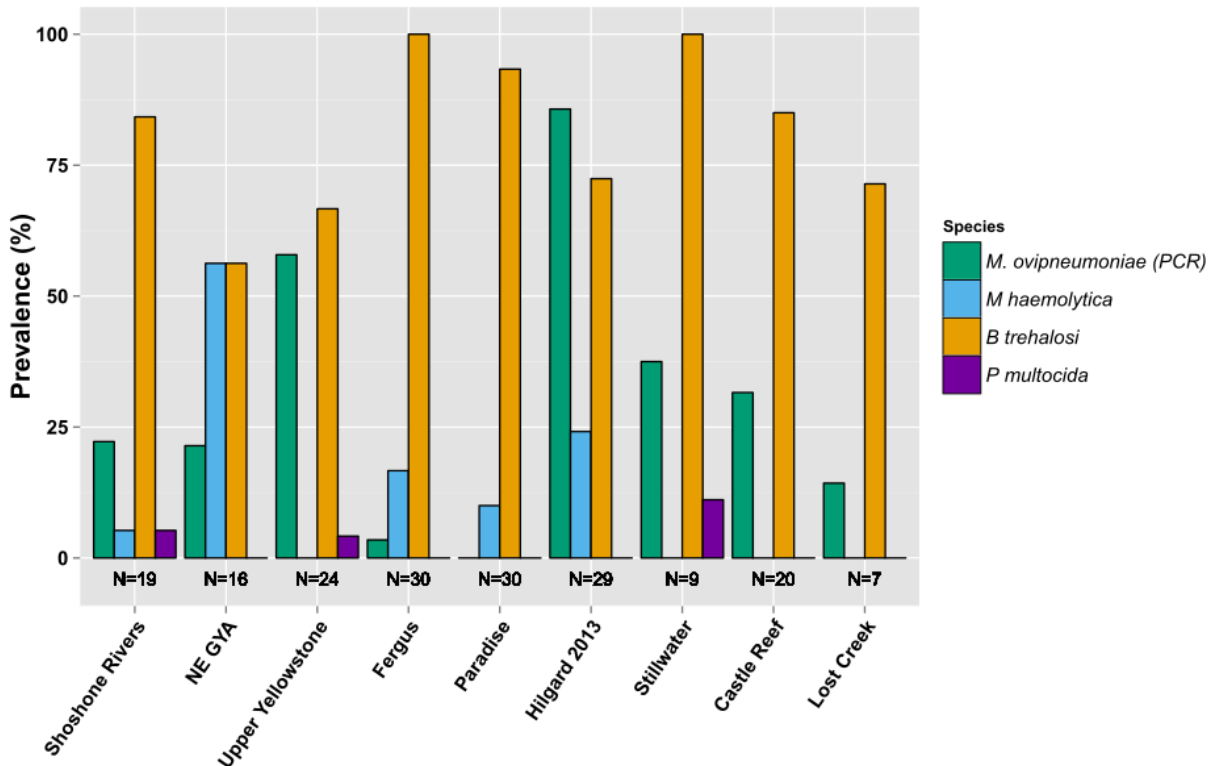

Figure 5. *Minimum prevalence of respiratory pathogens of interest across populations sampled as part of the Montana Bighorn Sheep Study. Minimum prevalence represents the number of animals a pathogen was detected in relative to how many were animals were tested. Presence of M. ovipneumoniae was assessed using PCR tests, while exposure to the pathogen was assessed using serology antibody tests. Presence of the other pathogens was assessed with culture tests.*

2.2.1 Synergy with Greater Yellowstone Area Mountain Ungulate Project Disease Sampling
The findings from the respiratory pathogen testing from the Montana Bighorn Sheep Project become more insightful when combined with data from other regional efforts. Three additional bighorn populations in Montana and Wyoming (Upper Yellowstone, Northeast GYA, and Shoshone Rivers) were previously sampled for respiratory pathogens using the same protocol for collecting nasal and throat swabs as part of a sister project, the Greater Yellowstone Area Mountain Ungulate Project (MUP). These populations are part of a large meta-population of bighorn sheep occupying the northern and eastern regions of the Greater Yellowstone Area (GYA). Detection rates for the pathogens of interest from these additional populations are shown in Table 3. Pathogen data of both the Montana Bighorn Sheep Study populations and additional populations sampled in the GYA are shown in Figure 6.

	Mycoplasma ovipneumoniae (PCR)	Mannheimia haemolytica (Culture)	Bibersteinia trehalosi (Culture)	Pasteurella multocida (Culture)
Upper Yellowstone (N=24)	58%	0%	67%	4%
Northeast GYA (N=16)	21%	56%	56%	0
Shoshone Rivers (N=19)	22%	5%	*84%	5%

Table 3. *Minimum prevalence of respiratory pathogens from bighorn populations sampled as part of the Greater Yellowstone Area Mountain Ungulate Project using the same protocol to collect and assay the nasal and throat swabs as is used in Montana Bighorn Sheep Study. Minimum prevalence represents the number of animals a pathogen was detected in relative to how many were animals were tested.*

* 11 of 16 B. trehalosi isolates detected from the Shoshone Rivers population were beta-hemolytic, which is indicative of leukotoxin production and virulence. Beta hemolytic B. trehalosi isolates were not detected in other populations sampled using these methods

Figure 6. *Minimum prevalence of respiratory pathogens across populations sampled as part of the Montana Bighorn Sheep Study and the GYA Mountain Ungulate Initiative. Minimum prevalence represents the number of animals a pathogen was detected in relative to how many were animals were tested and the pathogen was not detected in. Presence of M. ovipneumoniae*

was assessed using PCR testing and presence of the other pathogens was assessed with culture tests.

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.

3.1 Body Condition Assessment Methods

3.1.1 Field-Based Measurements of Body Condition
We used ultrasonography to measure rump fat thickness. In addition, the lumbar vertebrate, sacrum, base of tail and caudal vertebrate 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 some other factors related to fitness.
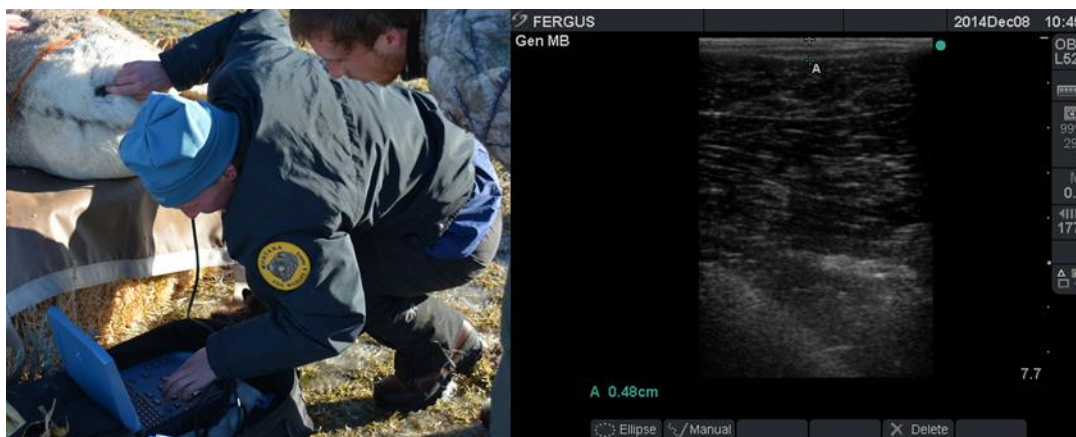

Figure 7. *Measuring rump fat thickness of a bighorn ewe. The ultrasound screen observed by collectors is shown on the right.*

3.1.2 Physiological Assessment of Nutritional Status

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 physiologists (Drs. Jim Berardinelli and Jennifer Thomson) at Montana State University to develop a suite of 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.

Reproductive processes are regulated primarily via the endocrine and neuroendocrine systems interacting with homeostatic mechanisms of the central nervous system. It is well-known that negative energy balance disrupts reproductive processes to limit and significantly reduce reproductive rates and efficiency in livestock, wildlife, and humans. It is also well-known that certain energy-related metabolites and metabolic hormones are related to changes in body composition and reproductive functions in domestic ruminants, such as cattle and sheep. Thus, by providing information on both energy-related and reproduction-related metabolites and hormones, these assays have the potential to vastly improve our understanding of wildlife nutrition and reproduction. Researchers have recently been able to predict body composition and energy balance in cattle using an 'index' associated with circulating concentrations of metabolic hormones and Dr. Thomson is currently developing a similar metabolic profile for domestic sheep. The relatedness of domestic and wild sheep suggests that extending this research from domestic sheep to wild sheep is biologically reasonable.

Given that these metabolite assays require only a small amount of serum from each animal, they 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 and archived by MFWP. 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 these assays and results will be incorporated with those from the Montana Bighorn Sheep Study, increasing the overall scope of the research. Drs. Berardinelli and Thomson are currently working to extend their metabolite research from domestic sheep to bighorn sheep and once a promising hormone/metabolite panel has been identified for bighorn sheep, we will begin assaying bighorn sheep samples.

3.2 Body Condition Assessment Findings

3.2.1 Sampling Effort

Rump fat thickness measurements have been taken from 153 adult females, body weight and skeletal length have been measured on 164 adult females, and serum has been collected from all captured animals.

3.2.2 Rump Fat and Body Weight Measurements

Rump fat measurements of adult females (≥3.5 years) varied from 0.20 – 1.60 cm, corresponding to %IFBF estimates ranging from 10% to 26%. As would be expected, median %IFBF of lactating adult females was lower than non-lactating females (lactating = 15.7 %IFBF, non-lactating = 16.7 %IFBF), though this relationship appears to vary across populations (Figure 8). Median %IFBF of lactating adult females across populations varied from 11.9 %IFBF (Stillwater) to 17.2 %IFBF (Hilgard 2014), though sample sizes for several populations were relatively small (Figure 8). 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 (≥ 3.5 years) notably changed between the successive years of sampling (winter 2013/2014 = 14.7 %IFBF, winter 2014/2015 = 17.2 %IFBF; Figure 8).
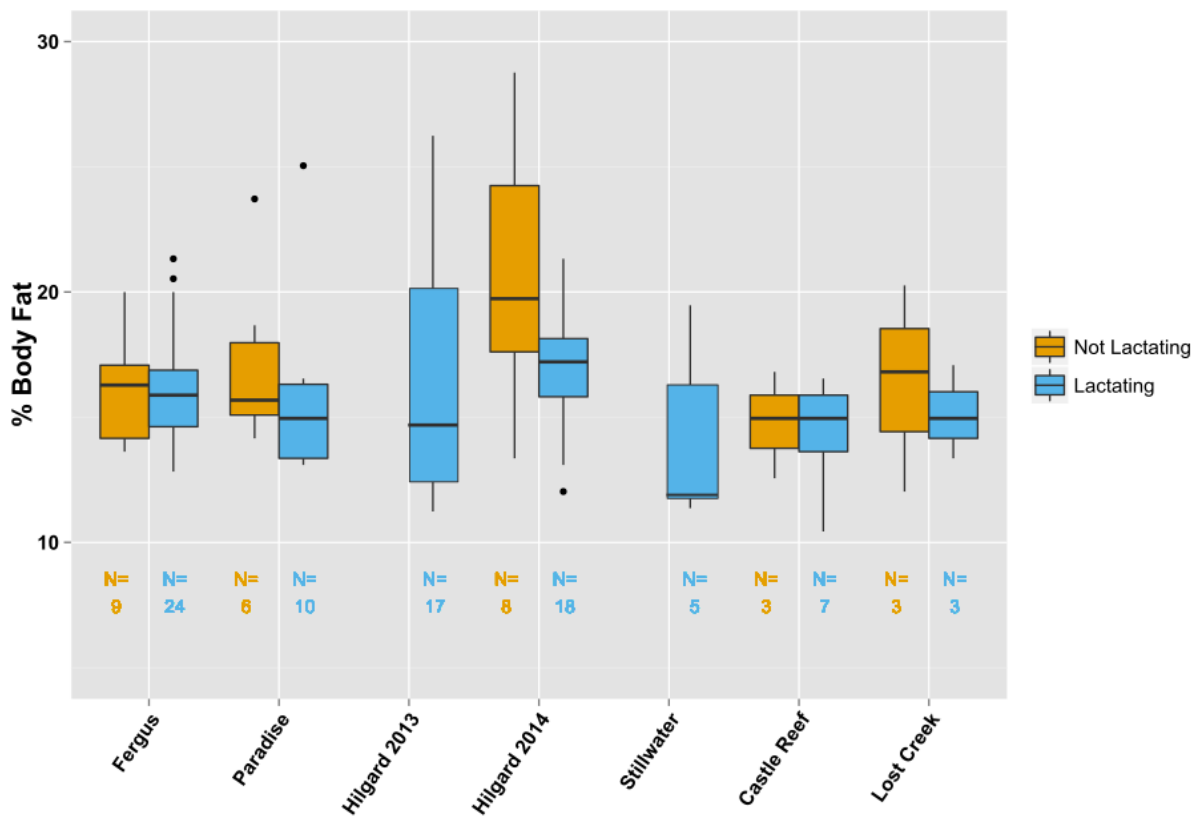
Figure 8. *Boxplot showing distribution of percent ingesta-free body-fat (measured using ultrasonography) of lactating and non-lactating adult female bighorn sheep (≥3.5 years) across the sampled study populations. Horizontal lines through boxes represent median values, length of box represents the middle 50% of observations (IQR), vertical lines 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.*

Body weight of adult females (≥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), however there did appear to be a pronounced difference in several populations (Figure 9). Median body weight of lactating adult females across populations varied from 143 lbs (Stillwater) to 162 lbs. (Hilgard 2013), though sample sizes in some populations were relatively small (Figure 9). Body weight of lactating adult females in the Hilgard population was similar for both sampling events (winter 2013/2014-162 lbs, winter 2014/2015-156 lbs; Figure 9)
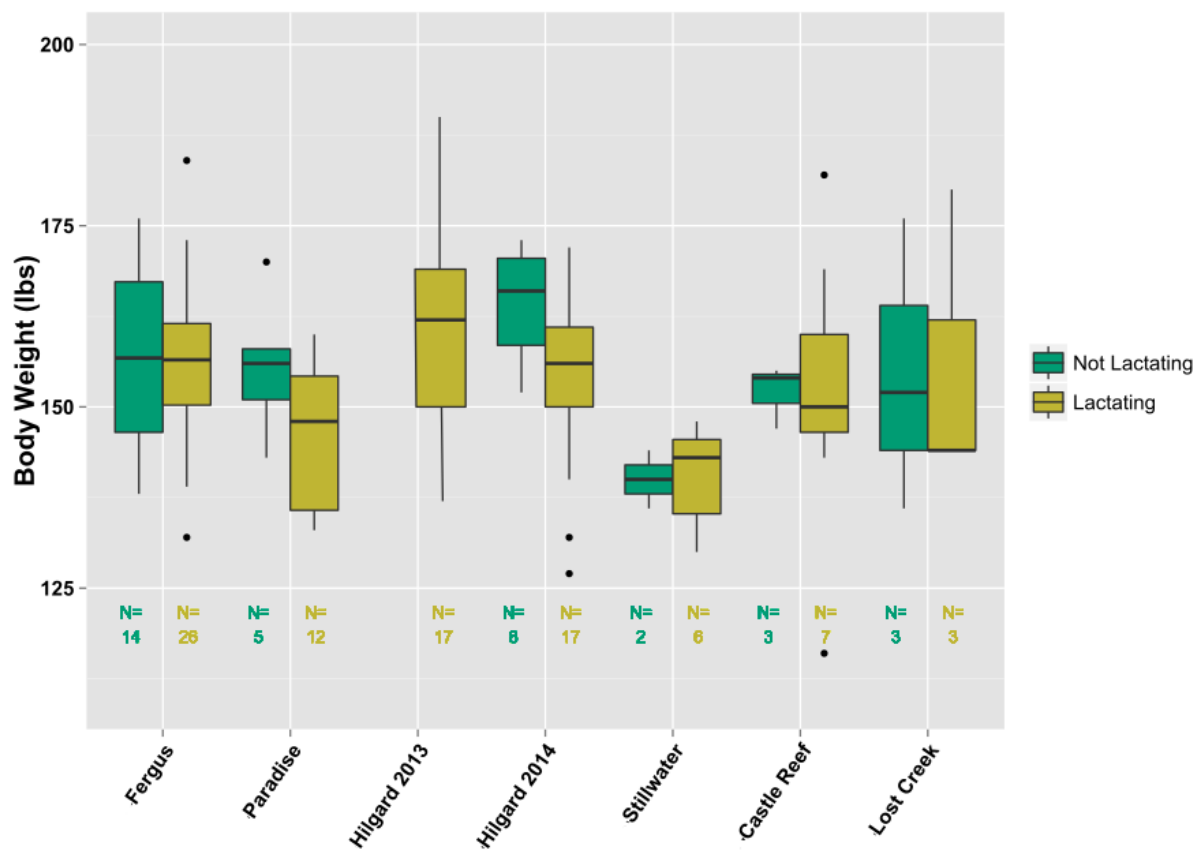
Figure 9. *Boxplot showing distribution of body weight measurements of lactating and non-lactating adult female bighorn sheep (3.5 years old or greater) across the sampled study populations. Horizontal lines through boxes represent median values, length of box represents the middle 50% of observations (IQR), vertical lines 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.*

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 recruitment,

4.1 Demographic Monitoring Methods
The Hilgard population was the only study population that was instrumented during the initial winter of the study to permit implementation of the demographic monitoring plan. The general methods that have been used to monitor the Hilgard population are described below and will also be used to monitor the other study populations.

4.1.1 Population Size
Eighteen Lincoln Petersen mark-resight surveys were conducted on the Hilgard population January - May 2014. Participation of the local biologist and availability of MSU students to conduct surveys allowed for greater effort and resources to conduct population surveys then what we anticipate to be available for the other study populations in the future. Observers visited wintering grounds that the bighorn population is known to frequent and surveys were conducted in a way to avoid double counting groups. When groups of animals were observed, the number of individuals in each age and sex class was recorded, as was the number of collared animals observed. Animals whose age, sex, or presence/absence of a radio-collar could not be verified were recorded as such. After surveys were completed, radio-telemetry was used to assess the number of marked animals available for detection.

From these data a series of population estimates were calculated using the joint hypergeometric estimator in program NOREMARK to compare estimates from simple counts with those obtained from various numbers of replicate mark-resight survey data. First, population estimates for the different age/sex classes were calculated using data from all 18 mark-resight surveys and these were considered our nearest estimate of the true numbers of animals in each age/sex class. Subsequently 20 sets of two, three, four, and five mark-resight surveys (out of the 18 total surveys) were randomly selected and the numbers of animals of each age/sex class were estimated for each set. Additionally population estimates from each mark-resight survey, as well

as the simple total counts were obtained for each survey. The distribution of each series of population estimates were compared to the estimates obtained by using data from all 18 surveys in order to assess how few replicate mark-resight surveys could have been conducted to obtain reasonably accurate population estimates.

4.1.2 Adult Female Survival

Survival of adult females was assessed by instrumenting animals with radio-collars that are equipped with motion-based mortality sensors. The VHF signals from radio-collared animals were monitored at regular intervals, not exceeding three months.

4.1.3 Pregnancy

Pregnancy status of adult females was assessed by collaborating animal physiologist, Dr. Berardinelli, who performed assays to quantify progesterone and PSPB concentrations in the serum. These assays cannot accurately diagnose pregnancy status during the first 4-6 weeks of gestation when many of the study herds are expected to be captured and sampled. A recently developed assay of a pre-implantation protein in cattle, however, holds potential for earlier detection of pregnancy in bighorn sheep and Dr. Berardinelli is currently working to gain permission to begin evaluating this new assay for use in the Montana Bighorn Sheep Study.

4.1.4 Recruitment

Recruitment of the cohort of lambs born spring 2013 was assessed spring 2014, prior to the lambing season, and fall recruitment of lambs born spring 2014 was assessed in early December 2014. Recruitment was estimated by calculating lamb:ewe ratios based on the number of lambs and adult females (ewes) observed during multiple classification surveys.

4.2 Demographic Monitoring Results

4.2.1 Population Size

Twenty radio-collared adult females and five radio-collared adult males were available for Lincoln-Petersen mark-resight population estimation. The population estimates for ewes, ewes and lambs, and rams in the Hilgard population based on all 18 mark resight surveys were, respectively, 138 (95% CI = 136 – 143), 213 (95% CI = 209-220) and 49 (95% CI = 43-64), resulting in a total population estimate of 262 animals in winter 2013/2014. Although biased slightly low, the distribution of population estimates from the simulations (20 random sets of two, three, four, and five surveys drawn from the 18 total surveys) demonstrated that population estimates from multiple mark-resight surveys were considerably more accurate and less variable than the estimates obtained from simple counts (Figure 10). Accuracy and precision of population estimates increased slightly as the number of surveys increased from two to five (Figure 10).
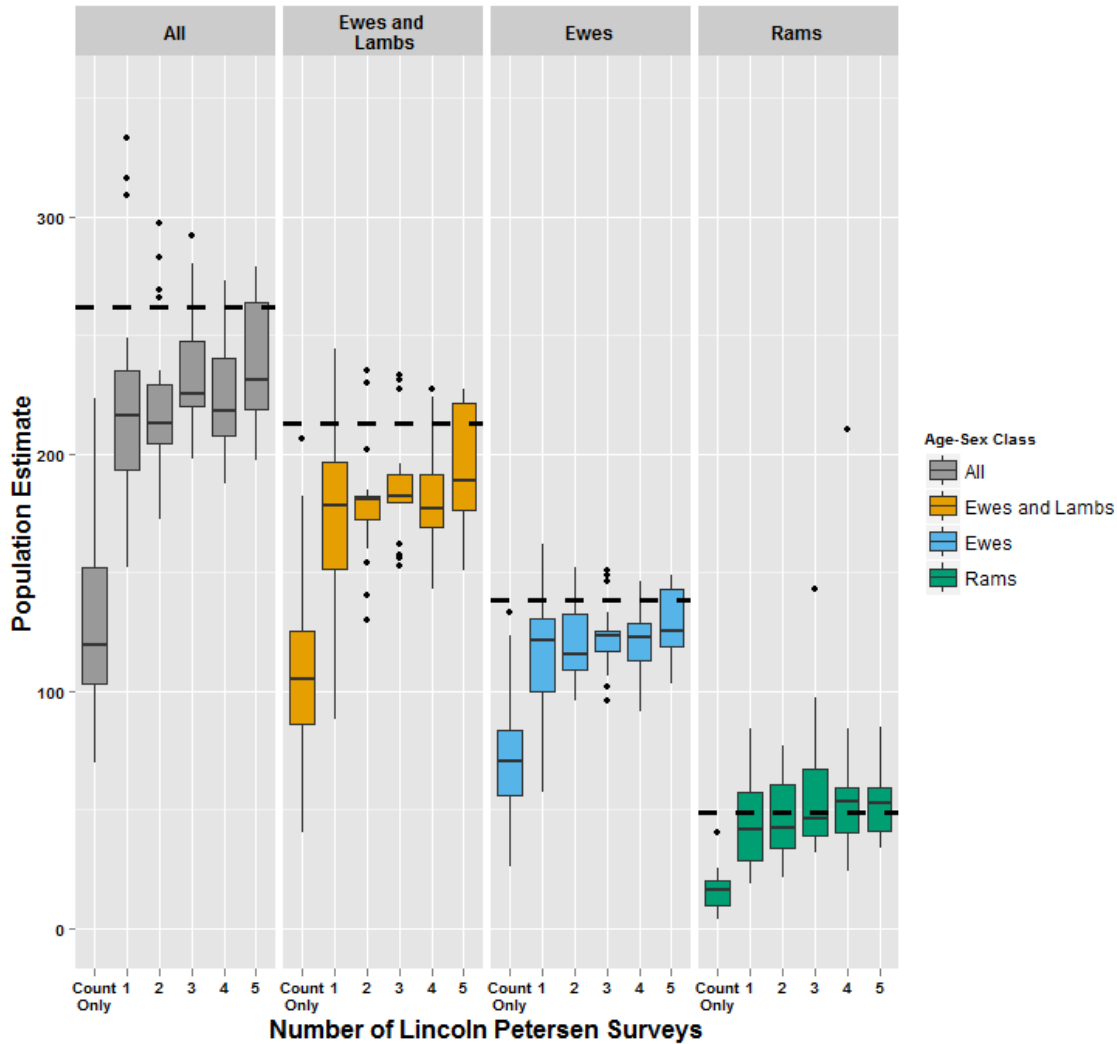
Figure 10. *Boxplots illustrating the distribution of population estimates (y-axis) vs. number of surveys used to calculate population estimates for different age-sex classes of bighorn sheep in the Hilgard population. Eighteen mark-resight population surveys were conducted winter 2014. "Count Only" 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.*

4.2.2 Adult Female Survival

One radio-collared adult female (instrumented previous to this research program) in the Hilgard population died in April 2014. She was found near the highway and a necropsy revealed emaciated body condition and trauma, but no evidence of pneumonia. This resulted in 95% adult female survival in the Hilgard population over the first year of the study.

4.2.3 Recruitment

The mean lamb:ewe ratio obtained from the 18 mark-resight surveys conducting during winter and spring 2014 was 51 lambs:100 ewes, with ratios from individual surveys ranging from 29 to 73 lambs:100 ewes. These data suggest very strong recruitment of the cohort of lambs born in 2013 and, combined with high adult female survival, suggests a growing population. In December 2014 two classification surveys were conducted to index pre-winter recruitment of the 2014 lamb cohort. The mean lamb:ewe ratio from these two surveys was 48 lambs:100 ewes, suggesting another strong year of recruitment in the Hilgard population.

Deliverables
1. This annual report, dated 15 February, 2015, details preliminary results of this multi-year research program.

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, Hank Edwards, Mary Wood, and Jessica Jennings Gaines. Yellowstone National Park staff, including PJ White, Doug Smith, Keri Gunther, Travis Wyman, and Chris Geremia have also contributed to animal capture and sampling as part of the Greater Yellowstone Area Mountain Ungulate Project. This project has benefited from interactions with scientists involved in the Hells Canyon Initiative including Frances Cassirer with Idaho Fish and Game, Kezia Manlove, Ph.D. student at Pennsylvania State University, and Tom Besser with Washington State University. 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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