

## **Chronic carriers and population density in bighorn sheep: implementation and evaluation of management tools**

Kevin Monteith, Professor, Haub School & Coop Unit;  
Brittany Wagler, Research Scientist, Haub School & Coop Unit;  
Rachel Smiley, PhD student, Haub School & Coop Unit;  
Hank Edwards, Supervisor, Wildlife Health Laboratory, Wyoming Game and Fish Department;  
Jennifer Malmberg, Assistant Professor, Veterinary Sciences;  
Aly Courtemanch, Wildlife Biologist, Wyoming Game and Fish Department;  
Gary Fralick, Wildlife Biologist, Wyoming Game and Fish Department;  
Doug McWhirter, Wildlife Coordinator, Wyoming Game and Fish Department;  
Zach Gregory, Wildlife Biologist, Wyoming Game and Fish Department;  
Pat Hnilicka, Wildlife Biologist, US Fish and Wildlife Service;  
Daryl Lutz, Wildlife Coordinator, Wyoming Game and Fish Department;  
Tony Mong, Wildlife Biologist, Wyoming Game and Fish Department;  
Corey Class, Wildlife Coordinator, Wyoming Game and Fish Department;  
Doug Brimeyer, Assistant Chief, Wyoming Game and Fish Department;

The entrance of epizootic pneumonia to bighorn sheep populations muddles the already complicated processes underlying population dynamics and is often the culprit for massive crashes of sheep populations, sometimes followed by chronically low survival of young. Populations of bighorn sheep have been afflicted by respiratory disease for the past century (Grinnell 1928). Although a wealth of research exists on the effects of disease on bighorn sheep (Miller et al. 2012, Cassirer et al. 2018), pneumonia continues to be one of the most poorly understood diseases that afflict wildlife in North America (Plowright et al. 2013a). Although epizootic pneumonia caused by bacterial respiratory pathogens are known to be the underlying driver of the massive mortality events, the frequency and intensity of the dieoffs are inconsistent and infections are not always manifested in disease (Cassirer et al. 2013). Indeed, chronically infected animals continue to persist and appear to develop some level of immunity against pneumonia (Plowright et al. 2013b). Nevertheless, the pathway to how populations are affected, or even regulated by, pneumonia is not ubiquitous. Some appear to face chronic lamb pneumonia and poor population performance, whereas others often experience sporadic fluctuations in abundance with periods of rapid population growth followed by massive population declines (Monello et al. 2001, Cassaigne et al. 2010).

Given the persistence of pneumonia pathogens in chronically infected herds that vary in their effects from year to year, the idea has emerged that some individuals may consistently harbor the pathogens and reinfect others that may otherwise clear them (Besser et al. 2013, Plowright et al. 2013a). Therefore, it has been proposed that pneumonia may be maintained in populations by a few individuals that are chronic carriers and with their removal, could alleviate the prevalence of *Mycoplasma ovipneumoniae*—considered to be the primary pathogenic agent in the pneumonia complex (Besser et al. 2013). In two small populations of bighorn sheep in western South Dakota, Garwood et al. (2020) implemented a test and removal program in one herd, and left the other as a control. Two chronic carriers were identified in each herd. Upon removal of the two chronic carriers from the treatment herd, they failed to detect *Mycoplasma ovipneumoniae* and mortality hazard for lambs was reduced by 72% compared with the control herd (Garwood et al. 2020). Also, there was a 41% reduction in hazard of adult mortality

(Garwood et al. 2020). Similarly, in Oregon, an opportunistic observation of loss of a single *Mycoplasma ovipneumoniae* positive individual in a population improved juvenile survival (Spaan et al. 2021). Before the death of the *Mycoplasma ovipneumoniae* positive sheep, observations indicated juvenile mortality caused by pneumonia was frequent in the population. Indeed, similar evidence of the potential role of chronic carriers, especially in herds with persistent lamb mortality to pneumonia have emerged, further suggesting that a test and removal program may be an effective management action (Cassirer et al. 2018).

Consequences of pneumonia pathogens within populations however, are not consistent across populations or even across time. Therefore, dieoffs are likely dependent upon certain ecological or environmental conditions—understanding these interactions could yield management alternatives to help reduce the frequency of epizootics and dampen fluctuations in abundance. In an extensive analysis of pneumonia epizootics from 99 herds across bighorn sheep range, Monello et al. (2001) noted that 88% of pneumonia-induced dieoffs occurred at or within 3 years of peaks in population size. As density dependence increases with growth of populations, declines in food availability lead to reductions in nutritional condition. In the absence of pneumonia, nutrition underlies demography rates in bighorn sheep (Stephenson et al. 2020), similar to many other wildlife populations. Recent work has shown that better nutrition improves over-winter survival of both infected and non-infected sheep (Dekelaita et al. 2020). Further, nutritionally limited female bighorns may prioritize energetic investment into immune function or pathogen tolerance over recruitment, supporting the notion that improving nutrition in herds may benefit the overall health of the herd in the presence of pneumonia-associated pathogens. Consequently, efforts to maintain populations at moderate density and thus, reduce density dependence and associated nutritional consequences may prove fruitful in avoiding epizootic dieoffs in otherwise productive populations (Monteith et al. 2018).

As we have learned through our efforts associated with the Bighorn Sheep Nutrition-Disease Project, the Jackson and Whiskey sheep populations likely highlight two potential contrasts in how pneumonia may affect populations. The Whiskey sheep continue to be suppressed by chronically low lamb survival, which is partially underpinned by pneumonia-related mortality and is likely complicated by poor summer nutrition and energetic costs of carrying pathogens. In contrast, Jackson sheep continue to thrive with reasonable lamb survival and a growing population. But with occasional reductions in nutritional condition and reaching historic peaks in abundance, Jackson sheep may be sitting on the precipice of an epizootic dieoff. Both populations possess the key pathogens associated with the pneumonia complex. Notably however, chronic carriers are far more apparent in the Whiskey and Upper Shoshone sheep and are not evident in Jackson sheep where most individuals seem to either clear or suppress the pathogens to the extent that we are unable to detect them. Indeed, in our data from the Whiskey population, some individuals appear to be chronic carriers and test positive for the pathogens in almost every sampling instance.

Given what we have learned since 2015 (highlighted in brief below), the Wyoming Game and Fish Department along with collaboration from the Shoshone & Arapaho Fish & Game Department has proposed an implementation and evaluation of 2 management tools: 1) test and cull, and 2) female harvest. Specifically, management prescriptions aim to implement test and cull of chronic carriers in the Red Creek portion of the Whiskey sheep herd, and implement female harvest in a delineated treatment zone of the Jackson sheep herd. Both management

actions will be accompanied by maintenance of our ongoing research to provide a rigorous assessment of the effectiveness of both approaches.

### **What we've learned**

Since March 2015, we have employed a longitudinal design to data collection across the Jackson, Whiskey, and Upper Shoshone sheep herds in northwestern Wyoming to connect seasonal changes in nutritional condition, reproduction, survival of young, presence of respiratory pathogens, immunocompetence, forage quality, and micronutrients. Although efforts are still underway to analyze and process current data, a few meaningful and intriguing patterns have emerged.

In brief:

- 1) Based on seasonal changes in nutritional condition, Whiskey sheep are apparently nutritionally limited on summer range and yet experience adequate winter conditions. Meanwhile, Jackson summer ranges appear robust with winter ranges being less so, whereas Upper Shoshone ranges fall out somewhere in between (Fig. 1).
- 2) Survival and recruitment of young in Whiskey has been exceedingly low for most of the study. Mortality within the first few weeks of life is attributed to an assortment of causes including predation, maternal neglect, and accidents in both Whiskey and Jackson sheep (Fig. 2). Thereafter, Whiskey sheep from about 2 to 5 months of age experience persistent mortalities associated mostly with pneumonia, which does not occur in Jackson.
- 3) Beyond apparent nutritional limitations on summer range in Whiskey, mothers that successfully raise lambs over the summer end up losing fat, meaning successive years of recruitment would severely compromise their survival (Fig. 3).
- 4) Carriage of pathogens comes at a nutritional cost. Adults who contract more pathogens over the summer gain less fat than those who contract fewer or no pathogens (Fig. 3).
- 5) Pneumonia is the leading cause of lamb mortality in the Whiskey herd (Fig 2), but surprisingly, not all lambs that die of pneumonia have detectable levels of *Mycoplasma ovipneumoniae*. Most have heavy burdens of *Pasteurella multocida*, another common pathogen associated with pneumonia, suggesting this pathogen may be equally problematic in some cases. The contributions of other pathogens besides *Mycoplasma ovipneumoniae* suggests that there are several pathogenic factors contributing to poor lamb survival in the Whiskey herd, and begs the question of whether considerations for improving overall condition may be beneficial.
- 6) Adult survival is high in these three herds, but the primary cause of mortality is predation by mountain lions (Fig. 4).
- 7) Two adults in the Upper Shoshone herd died of pneumonia in 2021 – which were the only adults to succumb to disease throughout the study (Fig. 4).
- 8) Chronic carriers of *Mycoplasma ovipneumoniae* carry more pathogens than non-carriers, indicating *Mycoplasma ovipneumoniae* may make adults more vulnerable to other pathogens associated with pneumonia (Fig. 5).
- 9) At each capture event, individual sheep in which *Mycoplasma ovipneumoniae* is detected carry more pathogen species than individuals that are not carrying *Mycoplasma ovipneumoniae* (Fig. 6).

- 10) Population abundance of the Jackson herd has reached the level at which two pneumonia dieoffs have previously occurred at in the 2000s. The herd has surpassed management objectives and we have observed density-dependent behaviors such as dispersing males.

### Next Steps

Given current observations, our aim is to continue to unravel the processes underpinning the dynamics of these herds while implementing 2 management tools. Importantly, current management prescriptions are informed by what we have learned in the past 6 years of research, and are explicitly aimed at alleviating pathogen burden and improving nutritional condition in the hopes of alleviating burdens associated with the pneumonia complex.

Project objectives include:

- 1) Assess efficacy of test-and-cull methods to reduce *Mycoplasma ovipneumoniae* burden on the Red Creek portion of the Whiskey herd.
  - a. We will continue to assess pathogen presence, nutritional condition, immunocompetence, and cause-specific lamb mortality to determine the effects of removal of chronic carriers and if lamb survival improves as a result.
- 2) Assess the effects of female harvest on nutritional condition and pathogen presence in the Jackson herd.
  - a. We will continue to assess pathogen presence, nutritional condition, immunocompetence, and cause-specific lamb mortality to determine the effects of reduction in density in one segment of the Jackson herd, using another segment as a control. Nutritional condition of the population has declined over the course of our study, which is correlated with increasing density.
- 3) Continued assessment of survival and cause-specific mortality of adult female sheep in Jackson, Whiskey, and Upper Shoshone herds.
  - a. We will assess factors that contribute to probability of survival (e.g., nutritional condition, body mass, age, reproductive status), and causes of mortality when it occurs (e.g., disease, predation, accident). To date, leading cause of mortality for adult females has been predation by mountain lions.
- 4) Assess survival and cause-specific mortality of newborn sheep in Whiskey and Jackson herds to provide a comparison of the relative roles of nutrition, habitat, predation, and disease on recruitment of young.
  - a. We will assess factors that contribute to probability of survival and cause of mortality of neonatal sheep, including but not limited to: birth mass, sex, birth date, habitat conditions, maternal nutritional condition, presence of respiratory pathogens, and maternal age.
  - b. We will quantify the effects of nutrition, predation, and disease on survival and recruitment of young to identify which is most limiting to Whiskey and Jackson bighorn sheep.
  - c. Comparisons of lamb survival before, during, and after management actions (test and cull and ewe harvest) will help to determine their efficacy.
- 5) Assess longitudinal changes associated with disease, nutrition, and immunocompetence.
  - a. We will assess seasonal dynamics in nutritional condition and presence of respiratory pathogens to evaluate the interactive role between these two factors.
- 6) Assess early life learning behaviors of bighorn sheep

- a. We will assess how neonate sheep learn their migration routes, summer ranges, and winter ranges and observe their faithfulness to these ranges as they age.

Notably, our objectives correspond directly to needs outlined in The Wyoming Plan by the State-wide Bighorn/Sheep Domestic Sheep Interaction Working Group (2004). Specifically, that group expressed needs to monitor habitat selection and habitat nutritional carrying capacity (pg 10), evaluate if poor nutrition contributes to disease susceptibility (pg 10), and determine if habitat improvements influence nutritional status of populations and thus influence nutritional carrying capacity (pg 15). In addition, the group indicated that knowledge of carrying capacity was necessary to ensure sheep do not exceed the capacity of their habitat, and that understanding how habitat improvements modify nutrition and subsequently influence performance is necessary for sound management (Appendix K).

### **Study areas**

The study area for this project includes the Jackson herd (HA 7), Upper Shoshone portion of the Absaroka herd (HA 1–4), and Whiskey herd (HA 10)—all of which are core, native sheep herds within Wyoming, and are key herds within the Statewide Surveillance Program. Large-scale pneumonia dieoffs have never been documented in Cody herds. The Jackson herd has undergone two pneumonia dieoffs in the past 15 years, but has recovered relatively quickly following good lamb survival and recruitment. In contrast, the Whiskey herd underwent a pneumonia dieoff over 20 years ago and continues to exhibit chronically low lamb recruitment. By contrasting these three herds, we hope to gain insight into why each has responded differently to the same bacterial pathogens.

The Jackson region is located east of Grand Teton National Park, near Jackson, WY. Summer and winter ranges are located in the Gros Ventre Mountain Range. Seasonal ranges for the Whiskey herd are located south and east of Whiskey, WY near the town of Dubois, WY and extends into the northern end of the eastern front of the Wind River Range, and over to the western foothills of the Wind River Range for components of the herd. Seasonal ranges in the Upper Shoshone region occur throughout the Absaroka Range, east of Yellowstone National Park. This region extends throughout the North and South Fork drainages of the Shoshone River.

### **Approach:**

Our aim is to take a multi-pronged approach to evaluating management actions to aid in bolstering population performance in the face of the pneumonia complex, which will include the continuation of our longitudinal study.

*Adult capture and monitoring.*—We will strive to maintain a sample of >70 adult ( $\geq 1$  yr old) female bighorn sheep across the 3 herds, including a marked cohort of 25 animals in Jackson, up to 40 animals in the Red Creek subherd in Whiskey, and 15 animals in Upper Shoshone herds to capture variation in habitat conditions and population densities among the herds. Efforts will include capture and radiomarking males as possible in the Red Creek subherd. Desired samples in each region reflect in part, the goal of having at least 20 pregnant animals in Jackson each spring, and capturing and monitoring as many animals as possible in the Red Creek subherd in Whiskey. Radiocollars will remain on animals that survive the duration of the experiment (3-5 years). When mortalities occur, collars will be recovered and placed on new, unmarked animals during the following capture effort. With our approach to long-term monitoring among the herds

and the potential change in disease frequency and its effect, we hope to be able to elucidate the potential interactions among density, nutrition, and disease.

We will continue to recapture the same radiomarked females during both autumn and late winter to allow assessment of seasonal ranges, and provide links to seasonal pulses in mortality commonly observed with pneumonia (i.e., breeding and lactation seasons). Animals in Jackson and Whiskey will be recaptured via helicopter netgunning (Barrett et al. 1982, Wagler et al. 2022), captures in Red Creek will be supplemented with dropnetting and ground darting, and given logistical circumstances, sheep will be captured via ground darting in the Upper Shoshone (Smith et al. 2015). We will fit each sheep with an Iridium satellite GPS collar programmed to obtain a location at least every 1 hr throughout the year. In addition, the Iridium collars will be programmed to transmit location data and status (live or in mortality) remotely via the satellite system at least every 3 days, or in the instance of a mortality, immediately. Sheep in the Jackson and Whiskey regions will be fit with Iridium GPS collars (825g; ~1% body weight) equipped with a communication UHF system to allow remote monitoring of vaginal implant transmitters and fawn collars by mom's collar. Because the radiocollars are live satellite collars, all animals will be monitored live relative to survival, and mortalities will be recovered as quickly as possible following death in an effort to ascertain cause of death.

We will determine age of each female via incisor replacement patterns and horn annuli. We will measure body weight using an electronic platform scale, and assess relative size of animals using morphometric measurements. We will conduct field ultrasonography with a 5-MHz transducer to determine nutritional condition of each captured animal using standard protocols developed for bighorn sheep (Stephenson et al. 2020). During late-winter captures we will again use ultrasound and transabdominal scanning with a 3-MHz transducer to determine pregnancy status (Stephenson et al. 1995).

Upon completion of ultrasonography in March, pregnant female sheep will be fitted with VITs. Chemical immobilization is not necessary to fit females with VITs (Bishop et al. 2007), thus all animals will remain physically restrained during processing. We will insert a VIT using the technique described in detail by Bishop et al. (2007), which has become the standard in studies utilizing VITs to identify birth sites and capture neonatal ungulates (Monteith et al. 2014, Smith et al. 2014b). We will sterilize all VITs in chlorhexidine solution prior to installment. We will insert VITs using smoothed and sterilized plastic tubing and wooden dowels. The plastic tubing is approximately 20cm long, with 2.5-cm diameter (Bishop et al. 2007). We will place tubing and dowel in chlorhexidine solution between use. To insert a VIT, we will fold the silicone wings of the VIT together and place the VIT in the end of the tubing. We will apply liberally nonspermicidal lubricating jelly to the tubing, and will insert it into the vaginal canal until the tip of the wings of the VIT are pressed firmly against the cervix. We will then use the dowel, which extends through the tubing, to firmly hold the VIT in place while the tubing is gently pulled out from the vagina. The transmitter antenna is usually flush with the vulva, but sometimes extends outward in smaller animals. Fitting pregnant animals with VITs takes <1min to complete and will be coincident with other data collection and monitoring of our animals during capture. Therefore, fitting females with VITs will not increase our processing time more than 1 min. We will use standard VITs that have been sufficiently described in detail elsewhere (Bowman and Jacobson 1998, Carstensen et al. 2003, Johnstone-Yellin et al. 2006, Carstensen et al. 2009, Smith et al. 2014b), with the exception that ours will have the addition of a new

chipboard with technology to communicate with the dam's Iridium collar. The new chipboard comes at no additional weight and does not alter the shape of the VIT.

VITs have flexible, silicone wings that induce pressure against the vaginal wall to retain the transmitter, thus eliminating sutures and facilitating a quick, nonsurgical insertion process (Bishop et al. 2007). Vaginal implant transmitters have been employed without any reproductive problems or effects on female survival (Bowman and Jacobson 1998, Carstensen et al. 2003, Bishop et al. 2007, Carstensen et al. 2009, Monteith et al. 2014, Smith et al. 2014b) and are a practical technique for locating birth sites and subsequently, the capture of neonates (Carstensen et al. 2003, Johnstone-Yellin et al. 2006, Bishop et al. 2007, Monteith et al. 2014, Smith et al. 2014b). In a study focused on animal welfare, Johnson et al. (2006) found that VITs caused minimal tissue irritation and did not influence reproductive performance. Antennas of the VIT's will be approximately 9.5 cm in length and the tips are encapsulated in a resin bead to avoid fraying of the antenna. Vaginal implant transmitters will be equipped with a temperature-sensitive and light-sensitive switch that will increase pulse rate from 40 pulses to 80 pulses per minute when the temperature decreases below 32° C representative of the VIT being expelled by the sheep, a signal that will be received by the mothers Iridium GPS collar, activating an email to be sent indicating birth has occurred along with the current location. Alternatively, we will monitor VITs via aerial or ground-based telemetry. We will then recover the VIT immediately to determine if the location the VIT was expelled was indicative of a birth site, and thereby begin searches for the neonate(s). All neonate capture, handling and monitoring is described below.

*Disease monitoring.*—We will collect a suite of nasal (n = 2), throat (n =2), and ear swabs (n = 2) to culture and identify the various potential pathogens associated with pneumonia. Nasal and throat (pharyngeal) bacteriological swabs will be collected using sterile polyester tipped applicators and placed immediately in Hardy glycerol-buffered broth, and frozen as soon as possible. We take care to not contaminate samples by contacting non-target areas. We will enhance ability to collect tonsil swabs using a lighted extender and tongue depressor to sample the tonsillar crypts. Nasal swabs will be collected by simply rotating swabs inside each nostril. A replicate will be collected and included in a cryovial to be frozen. All culture and pathogen identification work will be conducted by the Wildlife Health Laboratory of the Wyoming Game and Fish Department using their standardized protocols. Nasal swabs will be cultured specifically for *Mycoplasma ovipneumoniae* with a PCR assay used to determine if this pathogen is present in each sampled population. An index of the relative exposure of each sampled population to *Mycoplasma ovipneumoniae* will also be obtained by submitting a small volume of serum for anti-*M. ovipneumoniae* antibody detection by ELISA. Similarly aerobic culture of throat swabs will be performed to determine presence of typical Pasteurella pathogens including *Pasteurella multocida*, *Mannheimia haemolytica*, *Bibersteinia trehalosi*, and *Arcanobacterium pyogenes*. If *Mannheimia haemolytica* is detected in culture we may also perform leukotoxin gene testing. The extensive and standardized methodologies stem from a broad and detailed collaboration to develop the most robust sampling design for bighorn sheep (Butler et al. 2017).

We will evaluate whether previous experiences of individual animals (i.e., nutritional condition, reproduction, etc.) affects their probability of becoming infected, becoming symptomatic, and the subsequent influence of infection on vital rates.

*Lamb survival and monitoring.*—During the springs, and the beginning of the parturition season (approximately 20 May), adult females and VITs will be monitored remotely via UHF and

satellite technology that links mom's GPS collar with the VIT. The technology will take advantage of the Iridium satellite system and Iridium GPS collars to uplink data that correspond to the status of the VIT and neonate collars directly. Upon evidence of an expelled VIT, we will use field crews and ground-based telemetry to locate the VIT and the radiocollared female as quickly as possible. The location of the VIT, and location and behavior of the female will be used to identify search areas for the newborns. When searches fail to produce neonates, personnel will evaluate whether the location of VIT was an actual birthsite and confirm that supposition by observing pregnancy status and behavior of female (Monteith et al. 2014, Smith et al. 2014a, Smith et al. 2014b). If the female appears to have undergone parturition and if personnel are available, we will attempt to observe the female from a safe distance to avoid disturbance (>500m if habitat and topography allow) and utilize postpartum behavior of female to locate neonates (Carstensen et al. 2003, Monteith et al. 2014, Smith et al. 2014b).

We will capture neonatal sheep by hand and place them in a cloth bag containing local vegetation to facilitate restraint, although added restraint is rarely necessary, and to minimize scent transfer, although that will have little influence on potential abandonment (Livezey 1990, Carstensen et al. 2003, Monteith et al. 2014, Smith et al. 2014b). We will determine sex of each neonate and acquire a measurement of new hoof growth using dial calipers (Robinette et al. 1973, Brinkman et al. 2004, Monteith et al. 2014). We will measure length of hind foot, and total body length using a cloth tape (Sams et al. 1996), collect blood via venipuncture of the jugular, and determine body mass of each neonate within the cloth bag to the nearest 0.1 kg using a hand-held electronic scale (Smith et al. 2014b), and fit neonate with an expandable GPS collar.

*Chronic carrier removal.*— In the Red Creek portion of the Whiskey herd we will attempt to capture every animal, test them for *Mycoplasma ovipneumoniae* via previously outlined methods, and lethally remove individuals identified as a chronic carrier. Similar to other protocols (Garwood et al. 2020), we will define a chronic carrier as an animal that tests positive for *Mycoplasma ovipneumoniae* twice in a 3-capture sequence. Given our capture sequence of March and December, that means an animal tests positive in sequential capture events, or 2 out of 3 sequential capture events that occur within an 18-month period. For animals identified as chronic carriers, if they are subsequently captured, they will be euthanized via planned methods of humane killing according to IACUC protocol and AVMA guidelines.

For animals identified as chronic carriers after a capture event (e.g., pathogen results not available until after capture), animals will be euthanized by either Wyoming Game and Fish personnel or Tribal wardens (Red Creek sheep reside on reservation property) via a high-powered rifle.

The chronic carriers that are removed will be immediately brought to the Wyoming State Veterinary Laboratory for thorough necropsy, in efforts to document factors that may contribute to chronic carriage of *Mycoplasma ovipneumoniae*, such as nasal tumors.

We will continue to monitor individual nutritional condition, reproductive status, pathogen presence, and immune function to identify if removal of chronic carriers reduces prevalence of *Mycoplasma ovipneumoniae* in the rest of the herd, to determine if recruitment rates improve, and to determine if nutritional condition of the herd is improved. We also will continue lamb survival monitoring, which will help us to determine if and why mortality rates are influenced as a result of chronic carrier removal.



*Female Harvest.*—We identified subherds of sheep within the Jackson herd that have minimal interaction using GPS data to outline control and experimental areas for ewe harvest (Fig. 7). Spatial separation between the experimental and control groups will aid in our ability to detect any changes in the experimental group. The Wyoming Game and Fish Department has implemented a female harvest season to begin Fall 2022. We will continue to monitor nutritional condition, pathogen detection, and adult and lamb survival between the control and experimental subherds to document effects of female harvest on bighorn sheep demographics and herd health.

### **Net benefits**

Respiratory disease has afflicted populations of bighorn sheep for the past century and, despite substantial research on the topic, pneumonia continues to be one of the most poorly understood diseases that afflict wildlife in North America. Although we have learned much in recent years, most research has been myopically focused on identifying the primary infectious agent associated with pneumonia. Nevertheless, evidence continues to support multiple primary and perhaps secondary infectious agents, and in most instances, we now manage herds that are chronically infected as opposed to those subject to new exposure. Management, however, remains difficult and there are few strategies that have proven effective in recovering chronically infected populations. Moreover, fundamental components underlying any large ungulate population including, habitat quality and quantity, and density dependent interactions remain operational and yet, are often neglected when considering disease dynamics. Our work to date has demonstrated that indeed, infected populations are not immune from fundamental nutritional dynamics and instead, suggests that nutrition may well be a key explanatory factor, along with disease, of the disparity in performance across sheep herds in northwest WY.

Our work seeks to understand how we can more effectively manage chronically infected populations – both through nutritional strategies and pathogen-reduction strategies. In doing so, we have the opportunity to learn how to more effectively manage sheep and their habitat through strategies rooted in science. Benefit of this continued research will come two-fold: implementation of these management strategies is intended to directly benefit the populations that they target, and intensive monitoring of the effect of these strategies will inform management of populations of bighorn sheep throughout their range.

## Timeline

This project began in 2015, with the goal of maintaining longitudinal data collection through 2021. Currently, we aim to enter into the tool implementation and evaluation phase of this work as outlined in the proposal herein during spring 2022.

Year	Time	Task
2022	March	Adult recapture; Begin test and cull
	Summer	Neonate capture and monitoring
	December	Recapture adults
	Ongoing	Lab, data analyses, and writing
2023	March	Adult recapture; Continue test and cull
	Summer	Neonate capture and monitoring
	December	Recapture adults
	Ongoing	Lab, data analyses, and writing
2024	March	Adult recapture; Continue test and cull
	Summer	Neonate capture and monitoring
	December	Recapture adults
	Ongoing	Lab, data analyses, and writing
2025	March	Adult recapture; Continue test and cull
	Summer	Neonate capture and monitoring
	December	Recapture adults
	Ongoing	Lab, data analyses, and writing
2026+	Ongoing	Evaluate trajectory, reinform way forward

## Projected Budget:

Description	2022	2023	2024	2025	2026
<b>Radiocollars</b>					
20 Satellite uplink GPS collars at \$2400/collar	48,000	0	0	0	0
Satellite uplink for GPS collars (\$500/collar/year)	35,000	35,000	35,000	35,000	0
35 Expandable GPS lamb collars at \$600/collar	21,000	21,000	21,000	21,000	0
40 Vaginal implant transmitters at \$275/transmitter	11,000	11,000	11,000	11,000	0
70 Rebattery GPS collars at \$300/collar	21,000	21,000	21,000	21,000	0
<b>Sheep Captures</b>					
Spring helicopter capture 40 sheep at \$850/per	34,000	34,000	34,000	34,000	0
Autumn helicopter capture 40 sheep at \$850/per	34,000	34,000	34,000	34,000	0
Ground captures 40 sheep per year at \$150/per	6,000	6,000	6,000	6,000	
<b>Lab Analyses</b>					
Immune function, hormone, and pathogen-based analyses	7,000	7,000	7,000	7,000	0
<b>Personnel, Travel, Supplies</b>					
Field equipment and supplies	12,000	5,000	5,000	5,000	500
Travel expense	21,000	21,000	21,000	21,000	6,000
PhD Student (n = 1)	32,000	32,960	33,949	34,967	17,484
MSc Student (n = 1)	25,200	25,956	26,735	27,537	13,768
Field Technicians (n = 4)	25,000	25,000	25,000	25,000	0
Accounting and technical support	23,604	19,874	19,998	20,125	2,993
Publications and outreach	5,000	5,000	5,000	5,000	5,000
	<b>360,804</b>	<b>303,790</b>	<b>305,681</b>	<b>307,629</b>	<b>45,745</b>
	<b>Total Projected Cost:</b>				<b>1,323,649</b>

**Figures**

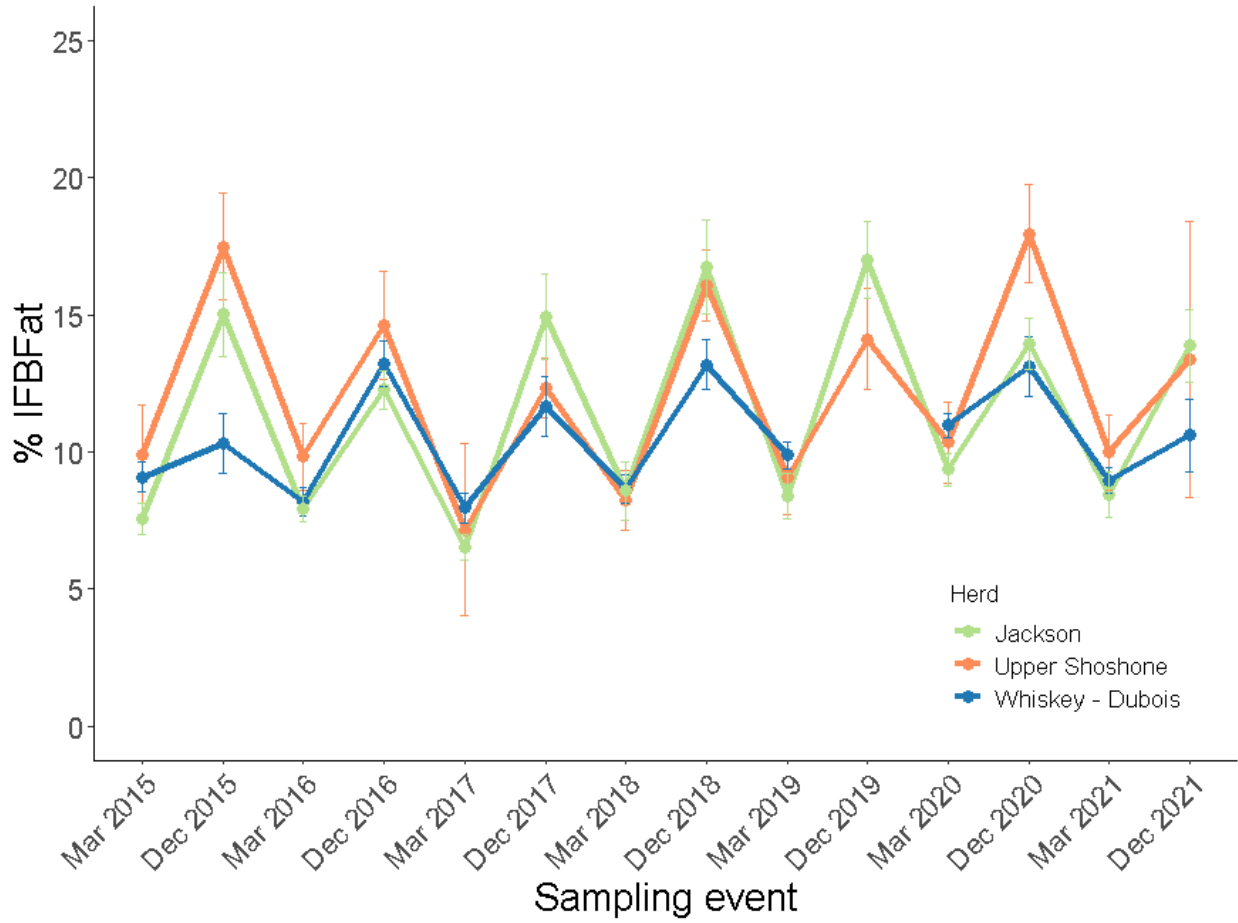


Figure 1. Mean percent ingesta-free body fat (IFBFat) levels in Whiskey, Jackson, and Upper Shoshone bighorn sheep herds between 2015-2021. Seasonal changes represent fat gains over the summer (reflected in December) and fat loss over winter (reflected in March).

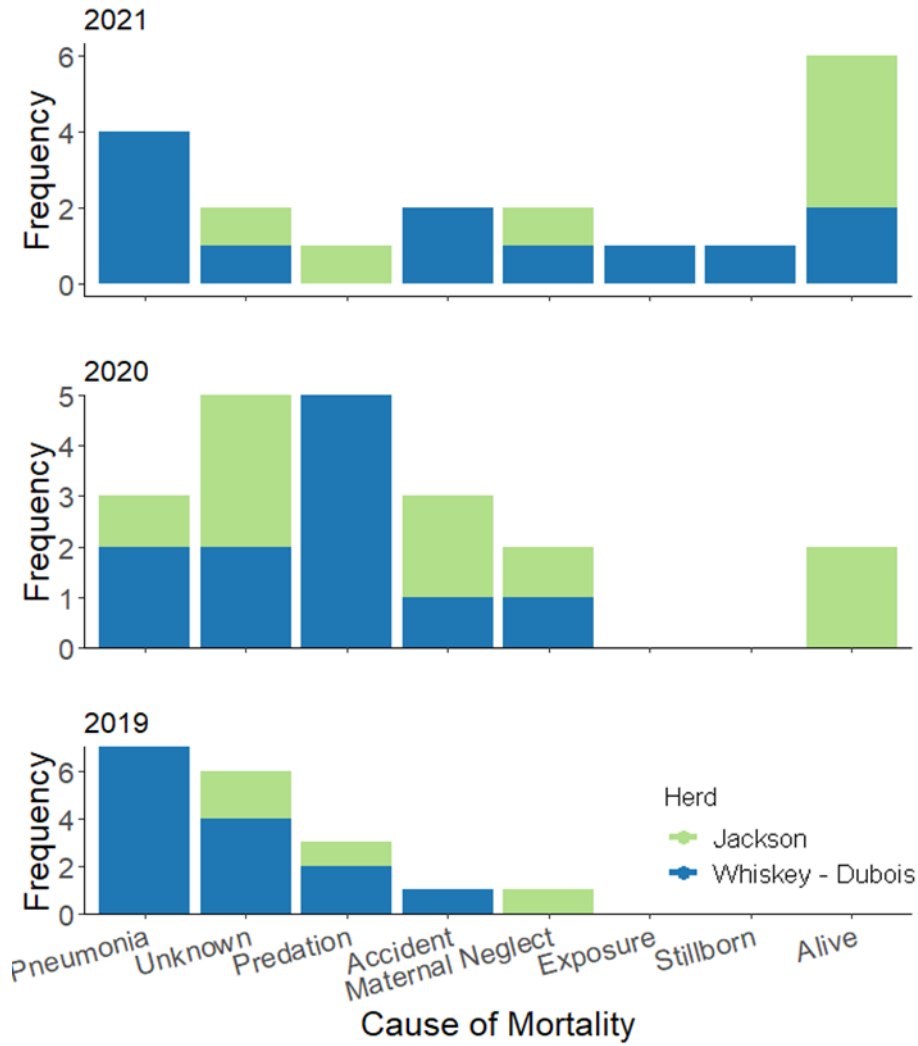


Figure 2. Cause-specific lamb mortality of bighorn sheep collared as neonate in Jackson and Whiskey herds 2019-2021. Lamb survival reflects survival rates up to one year of age for 2019 and 2020, and to current in 2021 (lambs are approximately 7 months old).

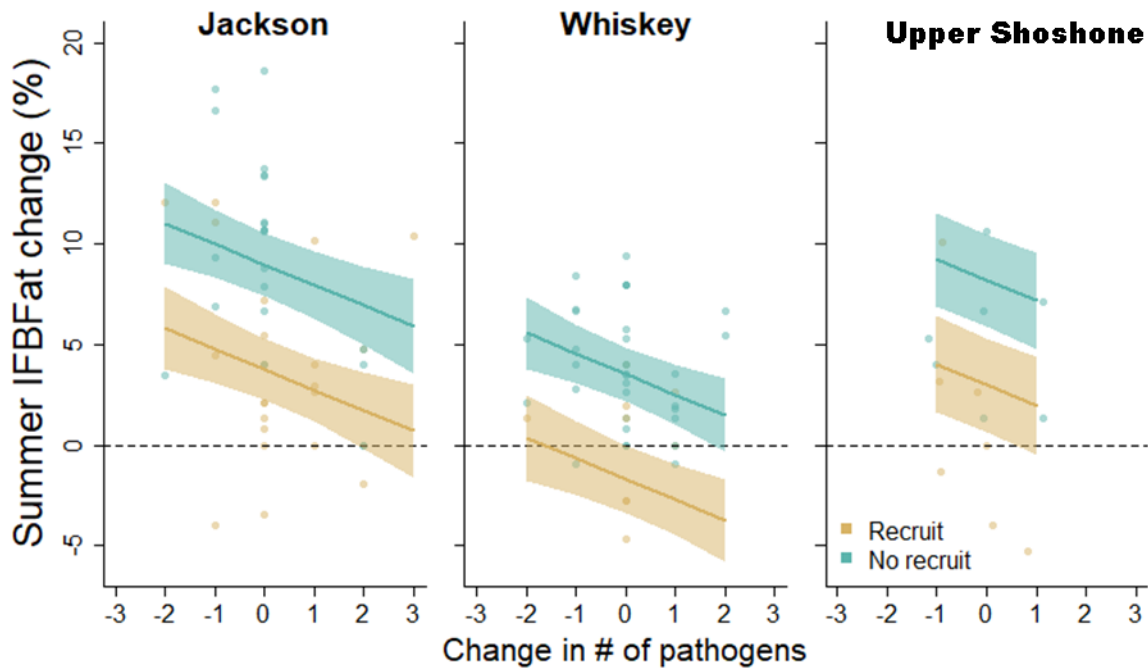


Figure 3. Negative relationship between change in pathogen richness and change in percent ingesta-free body fat (% IFBFat) in Jackson, Whiskey, and Upper Shoshone bighorn sheep herds in 2015-2018. Note, most sheep gain fat over summer with the exception of sheep that recruit lambs in the Whiskey herd.

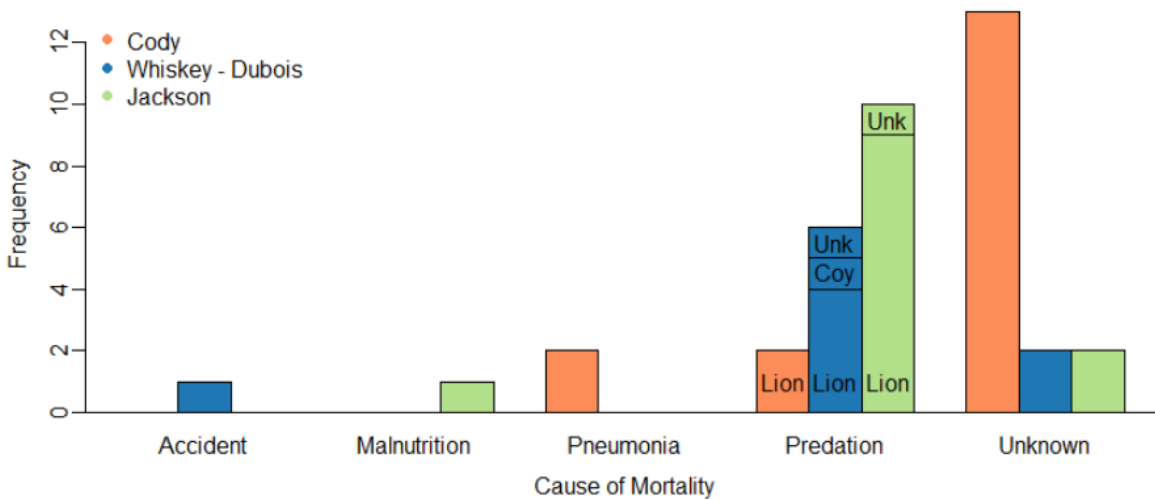


Figure 4. Cause specific mortality of adult bighorn sheep in the Jackson, Whiskey, and Upper Shoshone herds from 2015-2021.

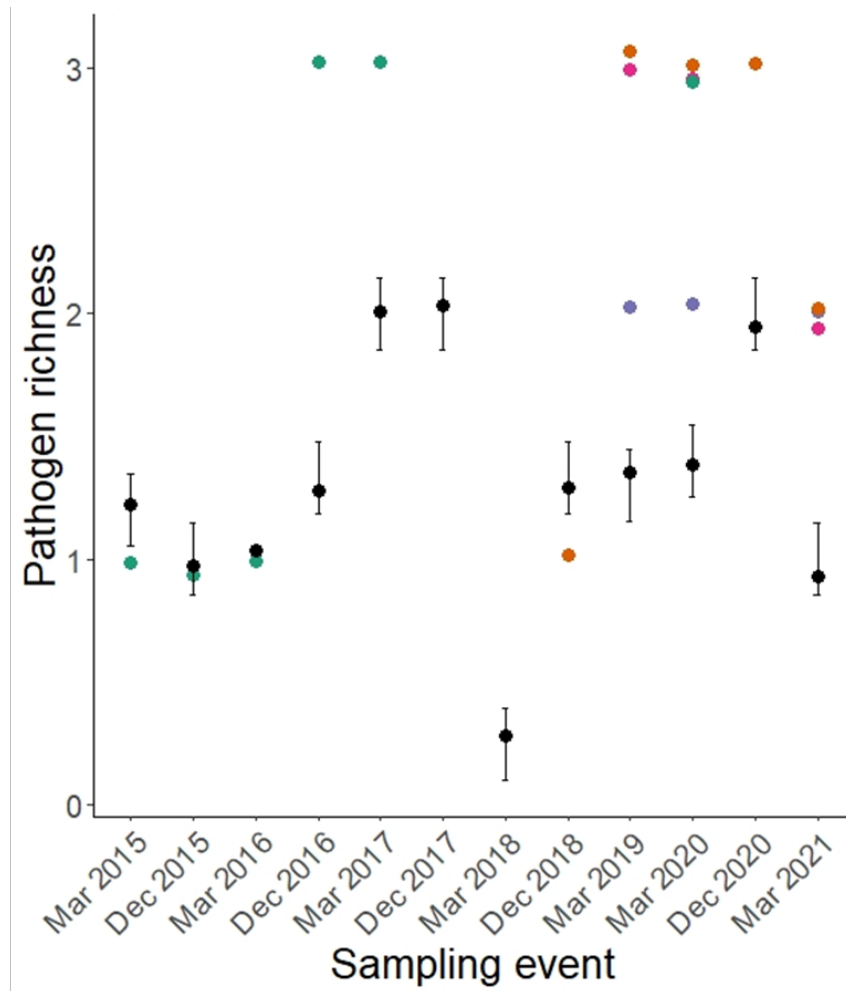


Figure 5. Comparison of pathogen richness (i.e., number of pathogen species not including *Mycoplasma ovipneumoniae*) carried by individuals identified as chronic carriers (colored data points) and all individuals that are not chronic carriers in the Red Creek segment of the Whiskey herd from 2015-2021.

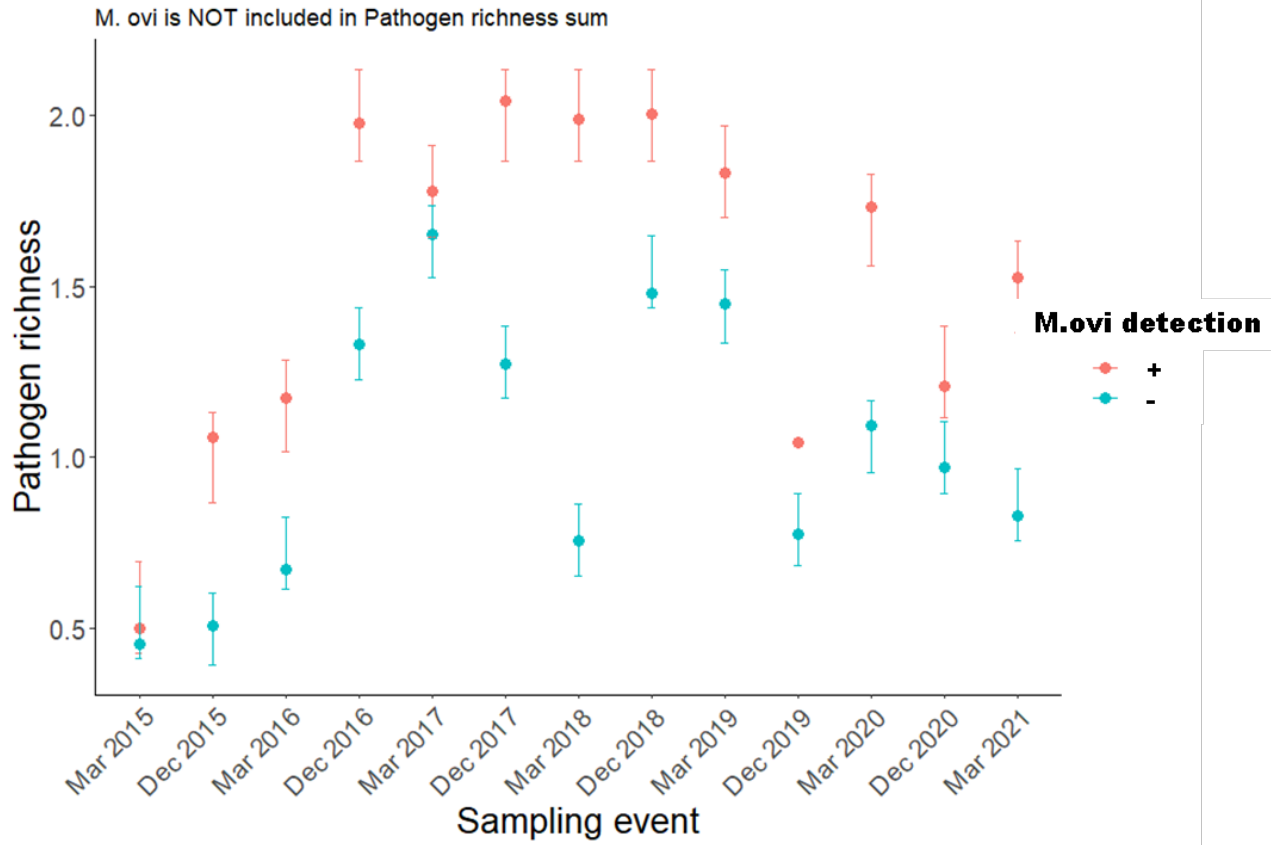


Figure 6. Comparison of pathogen richness between sheep that had positive detections of *Mycoplasma ovipneumoniae* (*M.ovi*) in Jackson, Whiskey, and Upper Shoshone herds from 2015-2021.



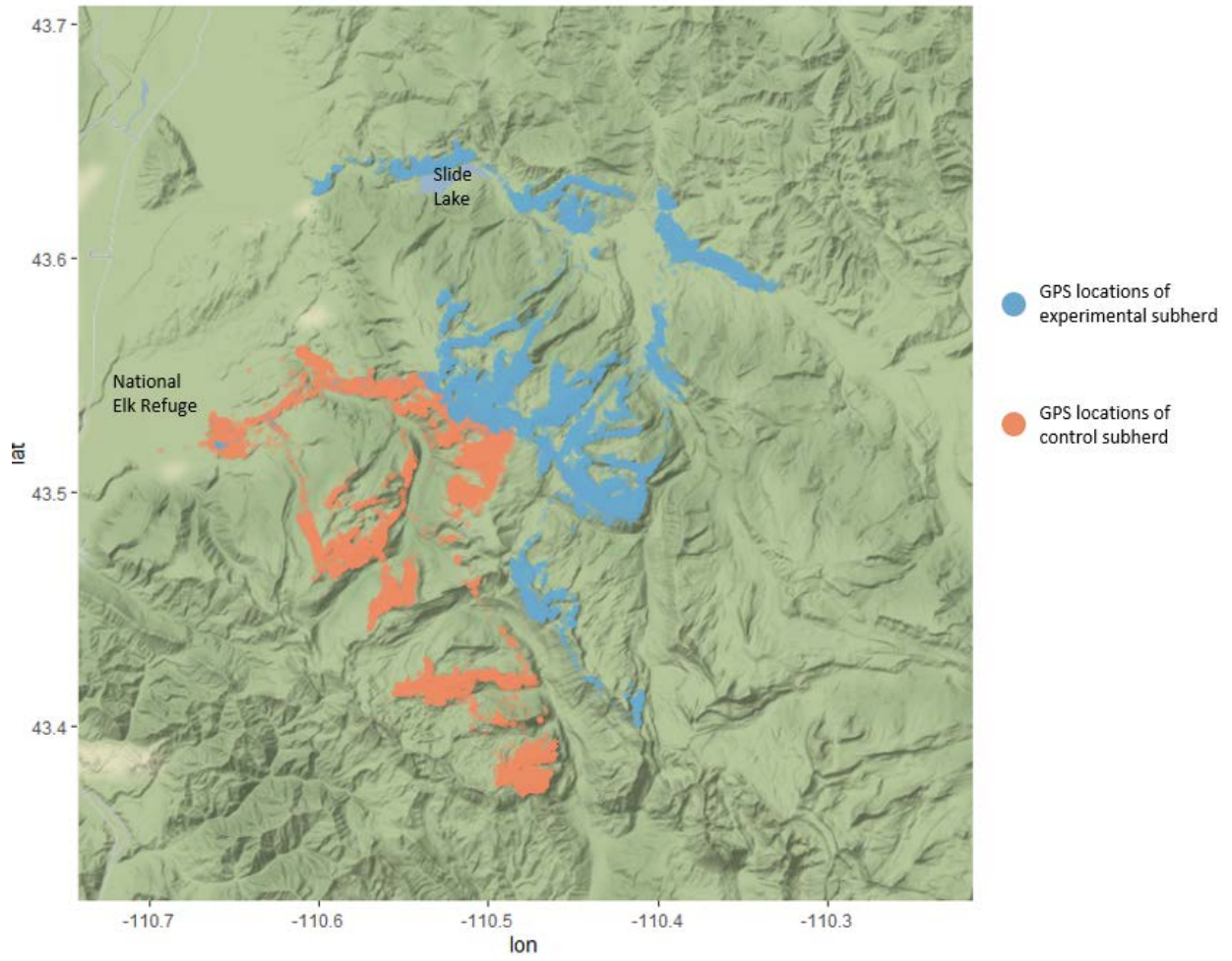


Figure7. GPS locations collected 2015-2020 that will be used to delineate the control (orange) and experimental (blue) subherds.

## Literature cited

- Barrett, M. W., J. W. Nolan, and L. D. Roy. 1982. Evaluation of a hand held net gun to capture large mammals. *Wildlife Society Bulletin* 10:108-114.
- Besser, T. E., F. E. Cassirer, M. A. Highland, P. Wolff, A. Justice-Allen, K. Mansfield, M. A. Davis, and W. Foreyt. 2013. Bighorn sheep pneumonia: Sorting out the cause of a polymicrobial disease. *Preventive Veterinary Medicine* 108:85-93.
- Bishop, C. J., D. J. Freddy, G. C. White, B. E. Watkins, T. R. Stephenson, and L. L. Wolfe. 2007. Using vaginal implant transmitters to aid in capture of mule deer neonates. *Journal of Wildlife Management* 71:945-954.
- Bowman, J. L., and H. A. Jacobson. 1998. An improved vaginal-implant transmitter for locating white-tailed deer birth sites and fawns. *Wildlife Society Bulletin* 26:295-298.
- Brinkman, T. J., K. L. Monteith, J. A. Jenks, and C. S. DePerno. 2004. Predicting neonatal age of white-tailed deer in the Northern Great Plains. *Prairie Naturalist* 36:75-82.
- Butler, C. J., W. H. Edwards, J. E. Jennings-Gaines, H. J. Killion, M. E. Wood, D. E. McWhirter, J. T. Paterson, K. M. Proffitt, E. S. Almberg, P. J. White, J. J. Rotella, and R. A. Garrott. 2017. Assessing respiratory pathogen communities in bighorn sheep populations: Sampling realities, challenges, and improvements. *PLoS ONE* 12:e0180689.
- Carstensen, M., G. D. DelGiudice, and B. A. Sampson. 2003. Using doe behavior and vaginal-implant transmitters to capture neonate white-tailed deer in north-central Minnesota. *Wildlife Society Bulletin* 31:634-641.
- Carstensen, M., G. D. Delgiudice, B. A. Sampson, and D. W. Kuehn. 2009. Survival, birth characteristics, and cause-specific mortality of white-tailed deer neonates. *Journal of Wildlife Management* 73:175-183.
- Cassaigne, G. I., R. A. Medellin, and O. J. Guasco. 2010. Mortality during epizootics in bighorn sheep: Effects of initial population size and cause. *Journal of Wildlife Diseases* 46:763-771.
- Cassirer, E. F., K. R. Manlove, E. S. Almberg, P. L. Kamath, M. Cox, P. Wolff, A. Roug, J. Shannon, R. Robinson, R. B. Harris, B. J. Gonzales, R. K. Plowright, P. J. Hudson, P. C. Cross, A. Dobson, and T. E. Besser. 2018. Pneumonia in bighorn sheep: Risk and resilience. *The Journal of Wildlife Management* 82:32-45.
- Cassirer, E. F., R. K. Plowright, K. R. Manlove, P. C. Cross, A. P. Dobson, K. A. Potter, and P. J. Hudson. 2013. Spatio-temporal dynamics of pneumonia in bighorn sheep. *Journal of Animal Ecology* 82:518-528.
- Dekelaita, D. J., C. W. Epps, K. M. Stewart, J. S. Sedinger, J. G. Powers, B. J. Gonzales, R. K. Abella-Vu, N. W. Darby, and D. L. Hughson. 2020. Survival of adult female bighorn sheep following a pneumonia epizootic. *The Journal of Wildlife Management*.
- Garwood, T. J., C. P. Lehman, D. P. Walsh, E. F. Cassirer, T. E. Besser, and J. A. Jenks. 2020. Removal of chronic mycoplasma ovipneumoniae carrier ewes eliminates pneumonia in a bighorn sheep population. *Ecol Evol* 10:3491-3502.
- Grinnell, G. B. 1928. Mountain sheep. *Journal of Mammalogy* 9:1-9.
- Johnson, B. K., T. McCoy, C. O. Kochanny, and R. C. Cook. 2006. Evaluation of vaginal implant transmitters in elk (*Cervus elaphus nelsoni*). *Journal of Zoo and Wildlife Medicine* 37:301-305.
- Johnstone-Yellin, T. L., L. A. Shipley, and W. L. Myers. 2006. Effectiveness of vaginal implant transmitters for locating neonatal mule deer fawns. *Wildlife Society Bulletin* 34:338-344.

- Livezey, K. B. 1990. Toward the reduction of marking-induced abandonment of newborn ungulates. *Wildlife Society Bulletin* 18:193-203.
- Miller, D. S., E. Hoberg, G. Weiser, K. Aune, M. Atkinson, and C. Kimberling. 2012. A review of hypothesized determinants associated with bighorn sheep (*Ovis canadensis*) die-offs. *Veterinary Medicine International* 2012:796527.
- Monello, R. J., D. L. Murray, and E. F. Cassirer. 2001. Ecological correlates of pneumonia epizootics in bighorn sheep herds. *Canadian Journal of Zoology* 79:1423-1432.
- Monteith, K. L., V. C. Bleich, T. R. Stephenson, B. M. Pierce, M. M. Conner, J. G. Kie, and R. T. Bowyer. 2014. Life-history characteristics of mule deer: Effects of nutrition in a variable environment. *Wildlife Monographs* 186:1-62.
- Monteith, K. L., R. A. Long, T. R. Stephenson, V. C. Bleich, R. T. Bowyer, and T. N. Lasharr. 2018. Horn size and nutrition in mountain sheep: Can ewe handle the truth? *Journal of Wildlife Management* 82:67-84.
- Plowright, R. K., K. Manlove, E. F. Cassirer, P. C. Cross, T. E. Besser, and P. J. Hudson. 2013a. Use of exposure history to identify patterns of immunity to pneumonia in bighorn sheep (*Ovis canadensis*). *PLoS ONE* 8:e61919.
- Plowright, R. K., K. Manlove, E. F. Cassirer, P. C. Cross, T. E. Besser, and P. J. Hudson. 2013b. Use of exposure history to identify patterns of immunity to pneumonia in bighorn sheep (*Ovis canadensis*). *PLoS One* 8.
- Robinette, W. L., C. H. Baer, R. E. Pillmore, and C. E. Knittle. 1973. Effects of nutritional change on captive mule deer. *Journal of Wildlife Management* 37:312-326.
- Sams, M. G., R. L. Lochmiller, C. W. Qualls, D. M. Leslie, Jr., and M. E. Payton. 1996. Physiological correlates of neonatal mortality in an overpopulated herd of white-tailed deer. *Journal of Mammalogy* 77:179-190.
- Smith, J. B., T. W. Grovenburg, K. L. Monteith, and J. A. Jenks. 2015. Survival of female bighorn sheep (*Ovis canadensis*) in the black hills, South Dakota. *The American Midland Naturalist* 174:290-301.
- Smith, J. B., J. A. Jenks, T. W. Grovenburg, and R. W. Klaver. 2014a. Disease and predation: Sorting out causes of a bighorn sheep (*Ovis canadensis*) decline. *PLoS ONE* 9:e88271.
- Smith, J. B., D. P. Walsh, E. J. Goldstein, Z. D. Parsons, R. C. Karsch, J. R. Stiver, J. W. C. Iii, K. J. Raedeke, and J. A. Jenks. 2014b. Techniques for capturing bighorn sheep lambs. *Wildlife Society Bulletin* 38:165-174.
- Spaan, R. S., C. W. Epps, R. Crowhurst, D. Whittaker, M. Cox, and A. Duarte. 2021. Impact of mycoplasma ovipneumoniae on juvenile bighorn sheep (*Ovis canadensis*) survival in the northern basin and range ecosystem. *PeerJ* 9:e10710.
- Stephenson, T. R., D. W. German, E. F. Cassirer, D. P. Walsh, M. E. Blum, M. Cox, K. M. Stewart, and K. L. Monteith. 2020. Linking population performance to nutritional condition in an alpine ungulate. *Journal of Mammalogy* 101:1244-1256.
- Stephenson, T. R., J. W. Testa, G. P. Adams, R. G. Sasser, C. C. Schwartz, and K. J. Hundertmark. 1995. Diagnosis of pregnancy and twinning in moose by ultrasonography and serum assay. *Alces* 31:167-172.
- Wagler, B. L., R. A. Smiley, A. B. Courtemanch, G. Anderson, D. Lutz, D. McWhirter, D. Brimeyer, P. Hnilicka, C. P. Massing, D. W. German, T. R. Stephenson, and K. L. Monteith. 2022. Effects of helicopter net-gunning on survival of bighorn sheep. *The Journal of Wildlife Management*.