Highland Bighorn Sheep Population Management Evaluation

Annual interim report, Dec 2022

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Executive Summary

Bighorn sheep herd recovery in Montana following disease-related die-off events has had mixed success, and in some cases, augmentations have failed to recover herd performance following all-age pneumonia die-offs. The purpose of this project is to test the efficacy of management actions designed to improve performance of struggling bighorn sheep populations. In the Highland Mountains of southwest Montana, we initiated a management experiment to evaluate the effects of test and removal of *Mycoplasma ovipneumoniae* (*M. ovi*) positive animals and mineral supplementation on lamb survival and population growth. This annual report summarizes work during the first year of this management experiment.

We captured and sampled a total of 54 bighorn sheep in the Foothills, Notch Bottom, La Marche, and Sheep Mountain sub-herds during winter 2021-2022. We were unable to locate Red Mountain animals, and this sub-herd was not sampled. PCR testing detected *M. ovi* presence in Foothills, Notch Bottom, La Marche, and Sheep Mountain sub-herds, suggesting active infections in each of these sub-herds.

A total of 32 bighorn sheep (31 females and 1 male) were also collared in winter 2021-2022. We have collected 54,965 locations from bighorn in the LaMarche, Sheep Mountain, Foothills and Notch Bottom sub-herds. No bighorn in the Red Mountain sub-herd were sampled or collared. Six ewes have died since capture and 1 ewe collar has malfunctioned. The causes of mortality include mountain lion predation (n=1), vehicle strike (n=1), capture related (n=1), pericarditis (n=1), and 2 mortality causes are pending disease sampling results. We are currently monitoring 24 collared ewes and 1 ram. To date, some interchange between the Highland Mountains sub-herds has been documented. A juvenile ram from Sheep Mountain has visited the Foothills and La Marche sub-herd range. Multiple ewes from Sheep Mountain crossed the Big Hole River in October and visited the LaMarche sub-herd range.

During spring 2022, we captured and collared a total of 24 neonatal lambs. We monitored lamb survival throughout the summer and fall. To date, 5 lambs have died, 5 collars have fallen off, and 0 collars have malfunctioned. The causes of lamb mortalities included captured related (n =1), mountain lion predation (n=1), natural causes (n=1), and unknown cause (n=2). We are currently monitoring the survival of 14 lambs. During spring and summer 2022, we also conducted visual observations of adults and lambs in all five sub-herds to monitor for signs of disease, including coughing, droopy ears, lethargy, or fluids from their mouth or noses. We observed signs of disease in the Foothills, Notch Bottom and Sheep Mountain sub-herds but not in the Red Mountain or La Marche sub-herds.

Project Background

Bighorn sheep populations across Montana and western North America are infected with a suite of pathogens including *Mycoplasma ovipneumoniae* (*M. ovi*), which can cause epizootics, chronic disease, and may limit long-term population growth (Butler et al. 2017, Cassirer et al. 2018). Disease expression may also be related to mineral or nutritional deficiency. In particular, chronic disease may limit lamb survival and population performance for long periods of time hindering bighorn sheep conservation. This project addresses a key need to develop strategies to increase lamb survival and improve population performance in bighorn sheep herds infected with *M. ovi*.

Respiratory pathogens are commonly hosted by bighorn sheep populations and may reduce recruitment rates for some time post die-off (Butler et al. 2017, 2018). *M. ovi* has been associated with all-age die-offs resulting in high levels of mortality, and are often followed by annual epizootics among juveniles and sporadic pneumonia mortality among adults (Cassirer et al. 2013, Smith et al. 2014). However, due in part to *Pasteurellaceae* testing protocols with poor detection probability, the association between *Pasteurellaceae* species and die-offs is unknown, but all-age die-offs are currently believed to be a polymicrobial disease process initiated by *M. ovi* causing ciliostasis and ciliary sloughing in the trachea and bronchi, inhibiting the mucociliary escalator's ability to clear pathogens such as *Pasturellaceae* from the respiratory tract (Niang et al. 1998, Cassirer et al. 2018). Regardless, environmental and individual factors such as use of mineral licks, nutritional status, social interactions (Manlove et al. 2014), movement behaviors (Lowrey et al. 2020), and overall immune response may interact with pathogens in a way that influence disease expression. Trace mineral deficiencies may lead to immunological deficiencies and increase susceptibility to bacterial infections (Flueck et al. 2012, Garrott 2021), as well as physiological stress (Ayotte et al. 2006). In particular, selenium (Se) deficiencies may result in poor reproductive success and reduced immune responses that may affect susceptibility to pneumonia, as observed in domestic sheep (*Ovis aries*) (Rooke et al. 2004). Elucidation of such factors could provide insights for management approaches that alleviate effects of potentially limiting factors and improve the performance of bighorn sheep herds.

In the Highland Mountain bighorn sheep population, we suspect population recovery following an all-age pneumonia event has been limited by persistent disease. Bighorn sheep in this area were extirpated in the early 1900's and reestablished by transplants from the Sun River herd in 1967 and 1969. The population increased to 300-400 animals over the ensuing decades until the 1994-95 die-off reduced the population by 90%. Since then, multiple augmentations from five MT populations (Sun River, Bonner, Sula, Ruby Mountains, and Fergus herds) added 131 sheep to the Highlands herd. The last augmentation occurred in 2014. Despite these efforts, the Highland population continues to struggle with both low lamb survival and population growth.

The bighorn sheep population objective for the Highland Mountains (Hunting District 340) is a minimum viable population of 125 animals (FWP Conservation Strategy, 2010). The estimated abundance is approximately 120 animals, and animals appear to be arranged into a meta-population structure that includes five sub-herds (Figure 1). A brief background and an estimate of number of individuals in each sub-herd within the Highland metapopulation follows.

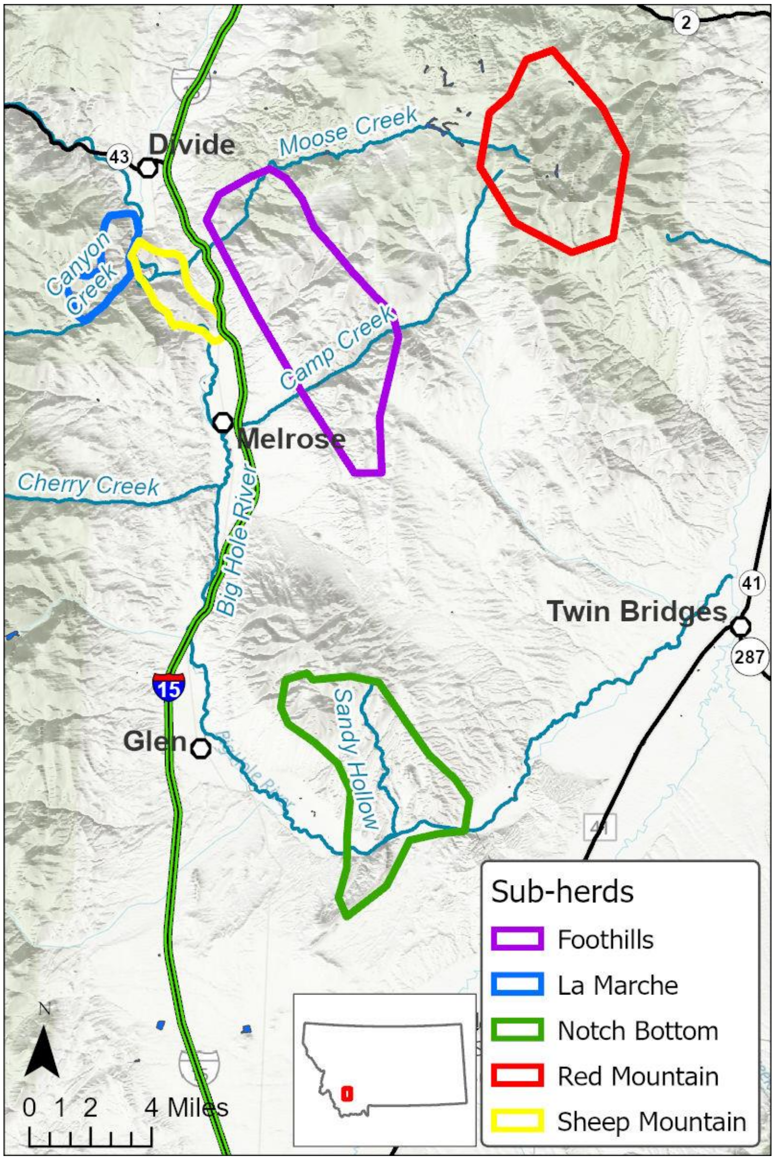

Figure 1. The study area includes the annual range of the Highland Mountains bighorn sheep population in southwest Montana. The population is distributed into five semi distinct sub-herds named Foothills (purple), Red Mountain (red), Notch Bottom (green), Sheep Mountain (yellow), and La Marche (blue).

<u>Foothills sub-herd</u> – This sub-herd is comprised mainly of sheep that were transplanted in January 2008 from the Sun River herd and their offspring. This sub-herd experienced a pneumonia event in the summer of 2008, with confirmed mortalities of ewes and lambs. Several pneumonia-related mortalities have been documented since. This sub-herd does not migrate, based on VHF-tracking 2008-2017. Year-round range is located in the big sagebrush/mountain mahogany foothills east of Melrose, mainly on public land. Tracking data has suggested lamb production >50 lambs: 100 ewes while annual lamb recruitment appeared to have been <20

lambs: 100 ewes. During a 2016 health assessment, four sheep were captured and tested from the Foothills sub-herd (3 ewes, 1 yearling ram). *M. ovi* was detected both from PCR and serology; nasal mucus was observed on two of the ewes. Recent observations suggest that this sub-herd may have cleared itself of pneumonia after 10+ years, perhaps due to adult carriers dying. as evidenced by >50 lambs per 100 ewes as of August 2021.

<u>Notch Bottom sub-herd</u> – This sub-herd was formed in 2008 by two collared adult ewes from the 2007 Ruby transplant, their lambs of the year, and a yearling ewe when they left the Melrose area where they had been released and colonized the SE flank of McCartney Mountain along the Big Hole River near Notch Bottom (a local landmark). The group has remained there ever since. There appears to be some elevational, albeit in reverse, migration between seasonal ranges with the sub-herd wintering and lambing on the flanks of the McCartney Mountain then migrating to lower elevation in the summer and fall to feed on irrigated hay meadows at the Notch. There have been several pneumonia-related mortalities documented in this sub-herd over the past 12 years, with the most recent occurring in September 2020. Tracking data from 2008-2017 suggests lamb production >50 lambs: 100 ewes while lamb recruitment has shown a decline from >40 lambs: 100 ewes during 2008-2012 to <20 lambs: 100 ewes currently. During the 2016 health assessment, six bighorn sheep were captured and tested from the Notch Bottom sub-herd (4 ewes, 1 yearling ewe, 1 female lamb). *M. ovi* was detected in this sub-herd by serology but not by PCR; nasal mucus was observed on one of the ewes.

<u>Sheep Mountain and La Marche sub-herds</u>– These sub-herds were formed in 2015 after nine ewes from the Missouri Breaks/Winnifred area were captured and released at the Maiden Rock Fishing Access Site in the East Pioneers in December 2014. All transplanted animals were radio-collared, making it easy to track their establishment. In the first summer, two of the nine ewes died of unknown causes. The remaining seven ewes (all of which had lambs) separated into two sub-herds. One group of four ewes and their lambs took up residence in La Marche Gulch while the other three collared ewes, along with some resident bighorn sheep, established territory on Sheep Mountain five miles to the south. Both sub-herds display non-migratory behavior, and both are highly dependent on irrigated hay fields during the lamb-rearing months. Ground-tracking data from 2016-2017 and anecdotal observations since then indicate lamb recruitment within both sub-herds to be >30 lambs: 100 ewes. During the 2016 health assessment three ewes from each sub-herd were captured and tested. *M. ovi* was not detected in either sub-herd, either serologically or from PCR.

<u>Red Mountain sub-herd</u> – This sub-herd is the only group within the Highlands metapopulation that is comprised mostly of “resident” bighorn sheep, i.e. animals that are descendants of the survivors of the 1990’s pneumonia die-off. As such, they have retained migration patterns that existed prior to the die-off, spending summers at the uppermost peaks of the Highlands (East Peak, Red and Table Mountains) and wintering in the foothills east of Melrose and south of the Foothills sub-herd range. Their range is primarily on public land. Because this sub-herd originates from a transplant back in 1967, there have only been 1-2 collared ewes in this group. Neither lived long enough to get substantial movement or vital rate data, so most of what is known about this sub-herd has been gathered opportunistically. Summer and winter observations

over the past decade suggest that lamb production is on par with the other sub-herds and recruitment is >30 lambs: 100 ewes. Because we did not have collared animals in this sub-herd at the time of the health assessment in 2016, none of these bighorn sheep were captured and tested for *M. ovi*.

Project Overview

The objective of this project is to evaluate the efficacy of management actions to increase lamb survival and population performance, and ultimately inform management directed at restoring bighorn sheep populations. One of the major factors limiting bighorn sheep populations is respiratory disease (Cassirer et al. 2018). The gregarious nature of bighorn sheep results in high contact rates among individuals, particularly in ewe-lamb groups, allowing a few infected animals to spread respiratory pathogens throughout the herd. The resulting disease effects ultimately impact herd health and can result in stagnant or decreasing populations. The removal of *M.ovi.* chronically shedding animals aims to short circuit this epidemiological process, and has been used successfully in some herds to improve population performance (Garwood et al. 2020). One of the challenges in applying this management approach is identifying chronically shedding animals because there may be multiple individuals shedding at any given time, and given the uncertainty in disease testing efficacy (Butler et al. 2017, Paterson et al. 2020), repeat testing may or may not accurately identify chronically shedding individuals. Although we predict some sub-herds within the Highland Mountains are limited by disease-related lamb mortality, attributes of the environment such as access to minerals may also be related to disease expression and limiting population performance. This may be especially true for herds that do not migrate.

We designed our research experiment to evaluate the effectiveness of two management actions on lamb survival and population performance using a before-after-control-treatment design. All 5 sub-herds within the Highland Mountains will be monitored for 2 years prior to management treatments. This monitoring period is required to determine sub-herd disease exposure, identify chronically shedding individuals, monitor baseline lamb survival, and identify potential interchange among sub-herds. Assuming baseline data justify the proposed design, management treatments will be implemented in year 3 and 4 of the project. The Foothills and Notch Bottom sub-herds will be treated with two years of test and removal actions, and the Sheep Mountain and La Marche sub-herds will be treated with two years of mineral supplementation. The remaining Red Mountain sub-herd will receive no treatment. All five sub-herds will be monitored for two years after treatments are implemented. This monitoring period is required to determine post-treatment disease exposure and lamb survival. Following two years of treatment, a fifth and final pathogen assessment will be conducted to determine if *M. ovi* infection has been eliminated and/or *M. ovi* exposure levels have decreased within the population. At the conclusion of this study, based on results of each treatment, management actions may be adaptively continued or modified, as needed. If no post-treatment improvement in lamb survival and population performance is observed and disease is confirmed within the treated sub-herds, the sub-herds with persistent disease exposure may be proposed for removal to protect the other sub-herds from disease exposure.

In this project, we propose to evaluate the following predictions during the two-years pre-treatment.

1. There are five sub-herds within the Highlands metapopulation. We predict they are distinct from one another and are not connecting through shared seasonal ranges where mixing of animals and pathogens occurs regularly. If true, interchange of animals (male and female) between sub-herds is rare and lamb exposure to pathogens is likely the result of pathogen presence within their sub-herd unit. If true, management treatments aimed at increasing lamb survival should be applied at the sub-herd level.
2. We predict the Foothills and Notch Bottom sub-herds may have different pathogen communities than the Sheep Mountain, La Marche and Red Mountain sub-herds as evidenced by the observations of poor lamb recruitment. If true, we predict that we will be able to identify individuals infected with *M. ovi* within these two sub-herds and we predict summer lamb survival will be lower in these sub-herds due to pneumonia related summer mortality.
3. We predict trace minerals, specifically Selenium levels, may be low in the Sheep Mountain and La Marche sub-herds during the lamb rearing period because these sub-herds are closely tied to an agricultural monocrop and use a very restricted seasonal range that may or may not provide for mineral requirements. These mineral deficiencies may be associated with disease expression and/or low reproductive performance. If true, mineral supplementation may be an effective strategy for increasing resistance to disease expression and reproductive performance.

If these predictions are supported by baseline information collected in the first 2 years of population monitoring, pathogen infection in the Foothills and Notch Bottom sub-herds and mineral access in the Sheep Mountain and LaMarche sub-herds may be limiting lamb survival and population performance. With these findings we propose 2 management experiments aimed at increasing lamb survival and population performance.

1. The Foothills and Notch Bottom sub-herds will be treated for 2-years with a test and removal program. Infected animals will be removed from the population in year 3 and 4, either through lethal removal or removal to a captive animal facility. We predict this action will result in reduced ewe-lamb disease transmission and increased lamb survival in the Foothills and Notch Bottom sub-herds. This action may also decrease ewe survival. We will monitor disease exposure and lamb survival before and after implementation of the test and remove program in these sub-herds and an untreated control sub-herd (Red Mountain). Decreases in disease exposure and increases in lamb survival will indicate the test and remove program achieved management objectives of increasing lamb survival. Increases in population growth rate will indicate the test and remove program achieved the management objective of increasing population performance.
2. The Sheep Mountain and La Marche sub-herds will be treated for 2 years with mineral supplementation. We will administer Selenium injections and make salt supplemented with Selenium available to animals in year 3 and 4. We predict this action will result in improved physiological condition, reduced disease expression, and increased lamb survival in the Sheep Mountain and La Marche sub-herds. We will monitor trace mineral

levels, disease exposure, and lamb survival before and after implementation of the mineral supplementation program. Decreases in disease exposure and expression and increases in trace minerals and lamb survival will indicate the mineral supplementation program achieved management objectives. Increases in population growth rate will indicate the mineral supplementation achieved management objectives of increasing population performance.

If these predictions are not supported by baseline information, the management experiment will be adjusted. For example, if frequent interchange between sub-herds is observed the test and remove experiment may be applied to the entire population. If the Sheep Mountain, La Marche and Red Mountain sub-herds test positive for *M. ovi*, they may be treated with test and removal, and the overall allocation of treatment and control groups may be modified.

Project Objectives

Our project objectives during this 5-year grant are to:

1. Collect movement data and pathogen profiles on all ewes and a sample of rams in each of the five sub-herds in the Highland Mountains for 2 years pre-treatment and 2 years post-treatment. A final pathogen assessment in all sub-herds will be collected in the fifth year to assess the effectiveness of management experiments to reduce disease exposure.
2. Monitor lamb survival in each of the 5 sub-herds for 2 years pre-treatment and 2 years post-treatment.
3. Monitor trace mineral profiles in each of the 5 sub-herds for 2 years pre-treatment and 2 years post-treatment.
4. Evaluate the effects of 2 management treatments, mineral supplementation and test and removal, on lamb survival and population performance.
5. Develop recommendations for application of test-and-remove and mineral supplementation management treatments to facilitate recovery of other struggling bighorn sheep populations.

Our objectives during this annual reporting period were to:

1. Initiate collection of bighorn sheep pathogen profiles and movement data.
2. Initiate collection of lamb survival data.
3. Initiate monitoring of trace mineral profiles.

Location

The Highland Mountains study area (Hunting District 340) is located just south of Butte, MT in Silver Bow County (Figure 1). The district includes the Highland Mountains and the northern portion of the East Pioneer Mountains. The area is comprised of shrub grasslands (sagebrush, mountain mahogany, bluebunch wheatgrass, Idaho fescue), coniferous forests, and agricultural lands.

Objective 1: Initiate collection of bighorn sheep pathogen profiles and movement data.

We used helicopter net-gunning to capture all age-sex classes of bighorn sheep. We outfitted sheep with GPS collars and conducted pathogen sampling. We sampled all age-sex classes for pathogens. The pathogen sampling protocol included: 1) collecting blood to test for *M. ovi* exposure using ELISA, 2) collecting fecal pellets to screen for lungworm and intestinal parasites, 3) collecting replicate nasal swabs to test for *M. ovi* shedding using PCR, and 4) collecting replicate tonsil swabs for aerobic culture and using PCR test for *Pasturellaceae* species and in particular those producing Leukotoxin A. In total, 5 nasal swabs and 4 throat swabs were collected. For each animal, we submitted a single swab for *M. ovi* testing using PCR to Washington Animal Diagnostic Laboratory (WADDL). If *M. ovi* was not detected, we submitted a second swab to confirm results. The final *M. ovi* status for each individual was based on the combined results of the first and second test. We considered animals that tested PCR positive for *M. ovi* infected. Whole blood was fixed on a gene card and the ear punch biopsy saved for genetic archiving. We also tested for pregnancy using blood serum and conducted a body condition assessment.

We equipped each ewe with an Advanced Telemetry Systems brand G5-2D Iridium GPS collar. We assessed pregnancy status using transabdominal ultrasonography, and if pregnant, we also equipped the ewe with a vaginal implant transmitter (VIT) that was linked to her collar. The collars and VITs are able to communicate, and when the VIT experiences a temperature differing from the body temperature or the presence of light, the VIT sends a birth-alert through the ewe's collar. Ewe collars are programmed to collect locations every 4 hours for approximately 3 years and allow location and mortality data to be collected remotely. One juvenile ram was outfitted with a Lotek Lifecycle collar programmed to collect locations every four hours. The Lotek Lifecycle collar allows location and mortality data to be collected and transmitted remotely. Although we intended to deploy 10 juvenile ram collars, the sizing was too snug to safely deploy on most rams.

We captured and sampled a total of 54 bighorn sheep in the Foothills, Notch Bottom, La Marche, and Sheep Mountain sub-herds (34 ewes, 13 rams, 7 6-month old lambs, Figure 2, Table 1). We were unable to locate Red Mountain animals, and this sub-herd was not sampled. PCR testing detected *M. ovi* presence in Foothills, Notch Bottom, La Marche, and Sheep Mountain sub-herds (Table 2), suggesting active infections in these sub-herds. Serology results also suggest *M. ovi* exposure. We detected most other pathogens in most sub-herds sampled (Table 3). Results of the fecal analysis to screen for lungworm and intestinal parasites are pending. A total of 34 adult (age >= 1.5) females were tested for pregnancy status based on pregnancy-specific protein B analysis conducted at the Herd Health Diagnostics lab in Pullman, WA. Thirty-one of 34 females (91%) were pregnant. Two of the three open females were animals transplanted to the Highlands as adult animals in the 2008 translocation, making these females at least 15 years old.

We captured and collared a total of 32 bighorn sheep (31 ewes, 1 ram) in Foothills (n= 5), Notch Bottom (n= 16), Sheep Mountain (n= 9), and La Marche (n= 2). We were not able to locate or capture animals in Red Mountain. We have collected 54,965 locations (Figure 3). Six ewes have died since capture and 1 ewe collar has malfunctioned. The causes of mortality include mountain lion predation (n=1), vehicle strike (n=1), capture related (n=1), pericarditis (n=1), and 2 mortality causes are pending disease sampling results. We are currently monitoring 24 collared ewes and 1 ram. To date, some interchange between the Highland Mountains sub-herds has been documented. A juvenile ram from Sheep Mountain has visited the Foothills and La Marche sub-herd range. Multiple ewes from Sheep Mountain crossed the Big Hole River in October and visited the LaMarche sub-herd range.

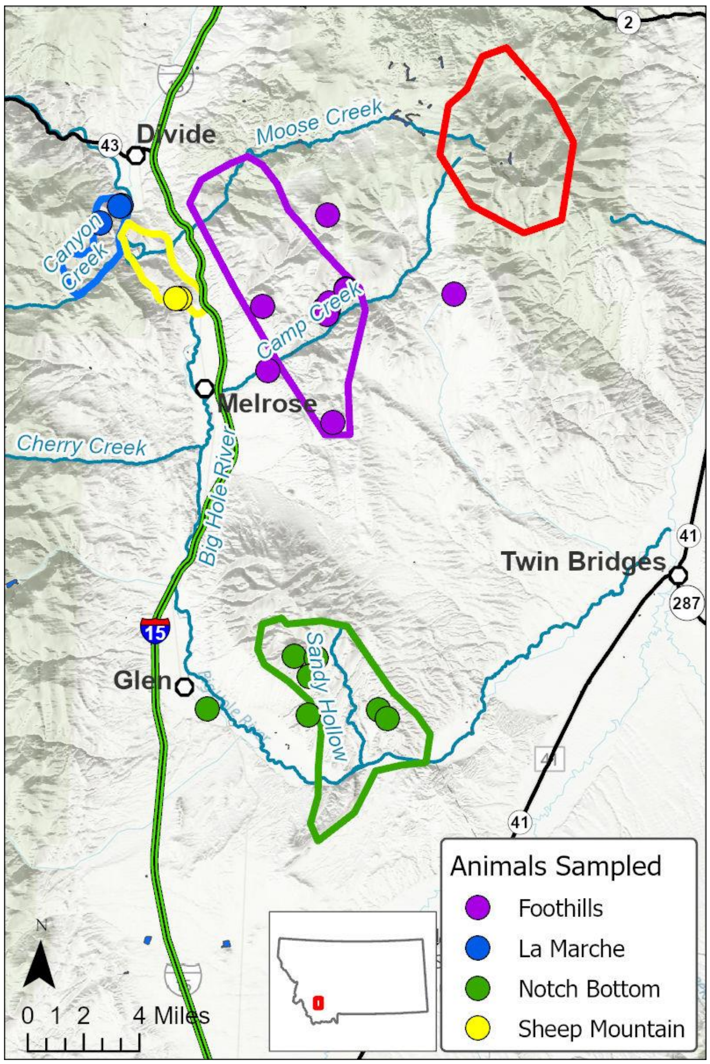

Figure 2. The location of 54 male and female bighorn sheep captured and sampled for pathogens in the Foothills (purple), Notch Bottom (green), Sheep Mountain (yellow), and La Marche (blue) sub-herds. Multiple animals were sampled at some points. No animals were captured or sampled in the Red Mountain (red) sub-herd.

Table 1. The number of animals sampled for each pathogen protocol in each sub-herd. Pathogens tested for include Bibersteinia trehalose (B. treh), Mannheimia haemolytica (M. haem), Trueperella pyogenes(T. pyog), Mannheimia ruminalis (M. rumi), Pasteurella multocida (P.mult), Pasteurella leukotoxin lktA (Leuk), Mannheimia caviae (M. cavi), and Mannheimia glucosida (M. gluc). Protocols included culture of nasal swabs, culture of tonsil swabs, PCR testing, and blood serum testing. The M.ovi2 Nasal PCR represents the second nasal swab that was tested, following a non-detected or indeterminate result on the first swab from a given animal. The number of samples varies for each pathogen-protocol because not all samples yielded determinate results for each protocol; indeterminate samples are censored from reporting.

	B. treh Nasal-Culture	*B. treh* Tonsil-Culture	*M. cavi* Tonsil-Culture	*M. gluc* Tonsil-Culture	*M. haem* Nasal-Culture	*M. haem* Tonsil-Culture	*M. ovi* Nasal PCR	*M. ovi* 2 Nasal PCR	*M. ovi* Serum-ELISA	*M. rumi* Nasal Culture	*M. rumi* Tonsil-Culture	Leuk PCR	*P. mult* Nasal-Culture	*T. pyog* Nasal-Culture	*T. pyog* Tonsil-Culture
All age-sex classes															
Foothills	14	6	6	6	14	6	14	13	11	14	6	4	14	14	6
Red Mountain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notch Bottom	22	8	8	8	22	8	22	16	20	22	8	2	22	22	8
Sheep Mountain	13	10	10	10	13	10	12	11	11	13	10	4	13	13	10
La Marche	5	4	4	4	5	4	5	4	5	5	4	1	5	5	4
Adults only															
Foothills	13	6	6	6	13	6	13	12	10	13	6	4	13	13	6
Red Mountain	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notch Bottom	21	8	8	8	21	8	21	16	19	21	8	2	21	21	8
Sheep Mountain	9	8	8	8	9	8	8	8	7	9	8	3	9	9	8
La Marche	3	3	3	3	3	3	3	3	3	3	3	1	3	3	3

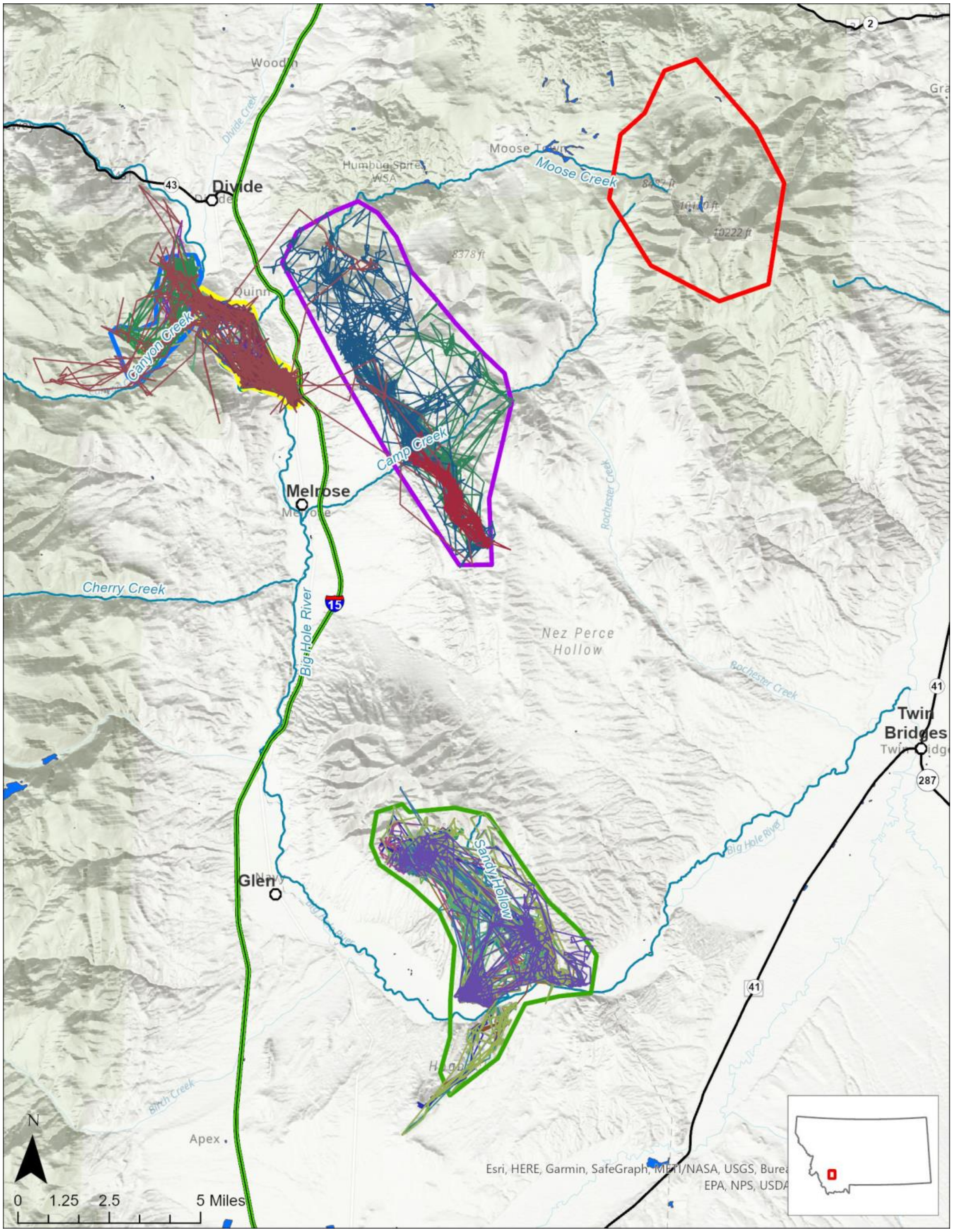

Figure 3. The movements of collared bighorn sheep (colored lines) in the Highland Mountains study area and the approximate range for the Foothills (purple), Red Mountain (red), Notch Bottom (green), Sheep Mountain (yellow), and La Marche (blue) sub-herds. No animals are collared in the Red Mountain sub-herd.

Table 2. Number of animals (n) sampled and prevalence of Mycoplasma ovipneumoniae (M. ovi) based based on PCR testing of nasal swabs and serum ELISA assays. The first five rows show the results from sampling all age-sex classes including lambs and the last five rows show the results from only adult males and females.

	M.ovi PCR		*M.ovi* serum ELISA	
	n	Prevalence	n	Prevalence
All age-sex classes				
Foothills	14	0.14	11	0.82
Red Mountain	0	-	0	-
Notch Bottom	18	0.17	20	0.50
Sheep Mountain	12	0.17	11	0.82
La Marche	5	0.40	5	0.80
Adults only				
Foothills	13	0.15	10	0.90
Red Mountain	0	-	0	-
Notch Bottom	18	0.17	20	0.50
Sheep Mountain	8	0.13	7	1.00
La Marche	3	0.33	3	1.00

Table 3. Number of samples (n) and prevalence of various respiratory pathogens in each sub-herd. An individual was considered to host the pathogen if any sampling protocol detected the pathogen. Pathogens tested for include Bibersteinia trehalose, Mannheimia haemolytica, Trueperella pyogenes, Mannheimia ruminalis, Pasteurella multocida, Pasteurella leukotoxin lktA, Mannheimia caviae, and Mannheimia glucosida. The number of samples varies for each pathogen because not all samples yielded determinate results for each protocol; indeterminate samples are not included in reporting.

	B. trehalosi		*M. haemolytica*		*T. pyogenes*		*M. ruminalis*		*P. multocida*		*leukotoxin*		*M. caviae*		*M. glucosida*	
	n	Prev	n	Prev	n	Prev	n	Prev	n	Prev	n	Prev	n	Prev	n	Prev
Foothills	6	0.50	6	0.17	7	0.29	7	0.29	14	0.36	4	0.25	6	0.17	6	0.17
Red Mountain	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Notch Bottom	8	0.25	8	0.00	11	0.55	8	0.00	22	0.55	2	0.00	8	0.00	8	0.00
Sheep Mountain	10	0.40	10	0.20	10	0.30	10	0.10	13	0.54	4	0.25	10	0.00	10	0.10
La Marche	4	0.25	4	0.00	4	0.25	4	0.25	5	0.60	1	0.00	4	0.00	4	0.00

Objective 2: Initiate collection of lamb survival data.

From April – July 2022, we captured and collared neonatal bighorn sheep lambs (Figure 4). We used alerts from VITs and daily movements of ewes to monitor and identify birth events. All lambs were blind-folded, weighed, ear tagged, and collared. We collared lambs of collared ewes with an ATS Neolink expandable collar, if available, that could communicate with the ewe's collar. Otherwise, we fit lambs with an ATS VHF expandable collar. If more than 4 hours passed without communication between the Neolink lamb collar and ewe collar, the ewe collar transmitted an absence alert through the Iridium system. After 8 hours of no communication between the lamb and ewe, a mortality alert was sent and we investigated as a potential mortality.

We collared a total of 24 neonatal lambs (15 females and 9 males, 19 Neolink, 5 VHF collars). VHF collared lambs were monitored daily to determine live/dead status. We promptly investigated all mortality events and conducted a necropsy to determine cause of death. To date, 5 lambs have died, 5 collars have fallen off, and 0 collars have malfunctioned. The causes of mortalities for lambs include captured related (n =1), mountain lion (n=1), natural (n=1), unknown cause of death (n=2). One lamb has an unknown fate.

During spring and summer 2022, we also conducted visual observations of adults and lambs in all five sub-herds to monitor for signs of disease, including coughing, droopy ears, lethargy, or fluids from their mouth or noses. We observed signs of disease in the Foothills, Notch Bottom and Sheep Mountain sub-herds but not in the Red Mountain or La Marche sub-herds. In the Foothills sub-herd, we first observed lethargic lambs in late June, and in early August we observed lethargy and coughing in several lambs and a yearling ewe. We also found an uncollared lamb on July 26 that died from pneumonia. In the Notch Bottom sub-herd, we first observed a single coughing lamb on July 10. By early August, we routinely observed multiple coughing lambs and signs of disease are still present within the sub-herd; however, we have not documented any pneumonia related mortality in Notch Bottom. In the Sheep Mountain sub-herd, we observed a weak looking yearling shaking it head on multiple occasions in early May; however, we had no additional observations suggested disease in this individual or other individuals after May.

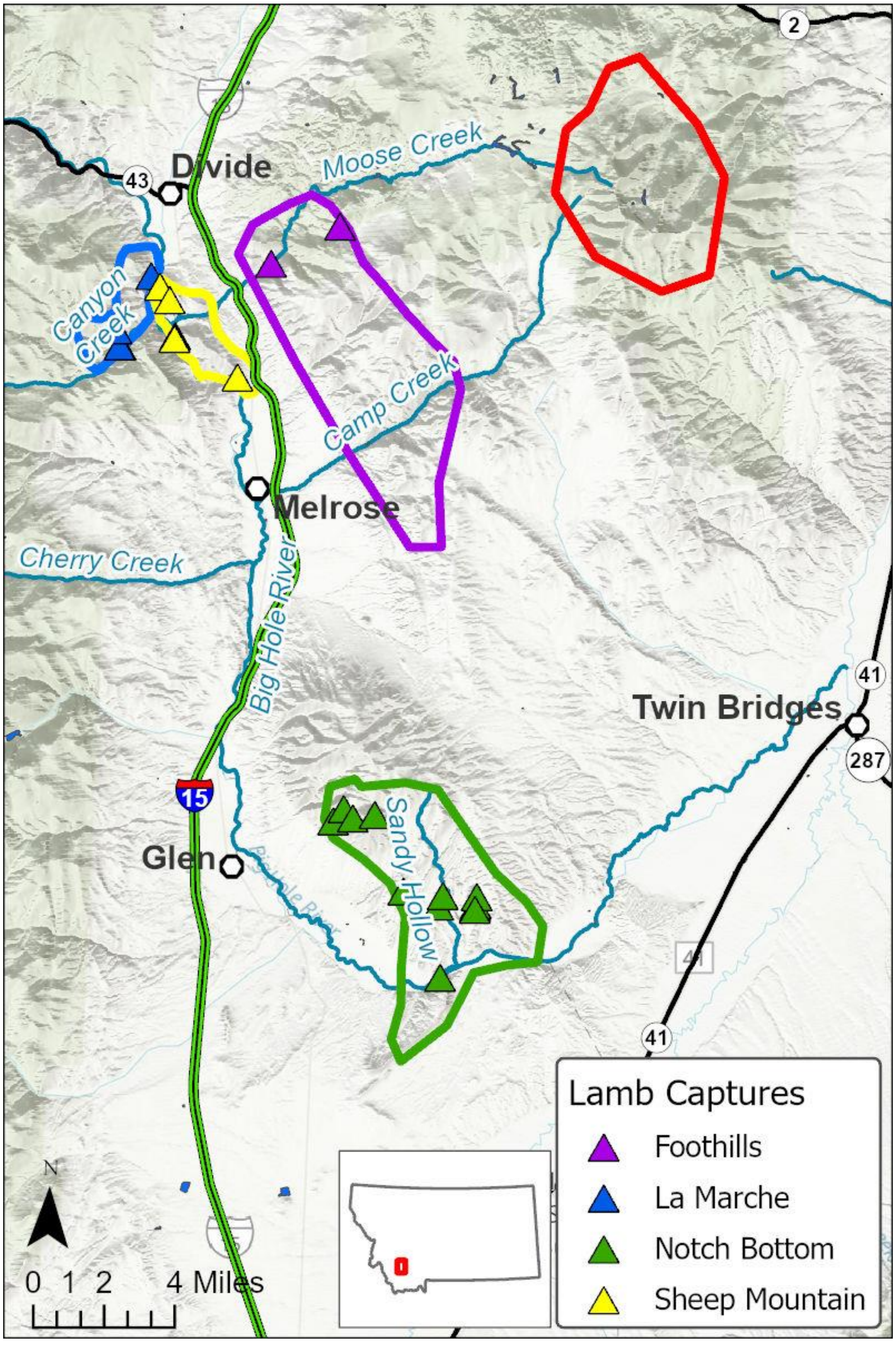

Figure 4. Location of neonatal lambs captured and collared during the 2022 lambing season in the Foothills (purple), Notch Bottom (green), Sheep Mountain (yellow), and La Marche (blue) sub-herds. No lambs were collared in the Red Mountain (red) sub-herd.

Objective 3: Initiate monitoring of trace mineral profiles.

Mineral deficiencies may lead to immunological deficiencies and increase susceptibility to bacterial infections. In particular, selenium (Se) deficiencies may result in poor reproductive success and reduced immune responses that may affect susceptibility to pneumonia in domestic sheep (*Ovis aries*) (Rooke et al. 2004). However, the limited case studies that attempted to improve bighorn sheep herd performance through mineral supplementation have documented mixed results. In Nevada, one study that treated bighorn herds with mineral supplements found that while treated herds had a significant increase in mean liver selenium values, there was no evidence the result was due to mineral supplements or that herd performance increased during the study (Cox 2006). In Oregon, there is correlative evidence that mineral supplementation was associated with higher blood selenium levels, but there was no association between selenium levels and population performance (Coggins 2006).

We collected blood samples from all age-sex classes for analysis of six trace minerals: cobalt, copper, manganese, molybdenum, zinc and selenium. Samples were analyzed at the Michigan State University Veterinary Diagnostic Laboratory. A total of 48 samples were collected and sent for analysis. Fifteen samples hemolyzed in transit to the laboratory. When red blood cells rupture, trace minerals may be released into blood serum resulting in higher values. Therefore, we censored hemolyzed samples and included a total of 33 samples in data summaries (Table 4).

We found selenium values were low in all sampled sub-herds relative to domestic sheep standards, but no evidence that selenium values were different between sub-herds. Based on the selenium reference range provided by the laboratory (110-160ng/mL for adults and 80-120 ng/mL for lambs, Friedrichs et al. 2012), all animals except one Sheep Mountain lamb and one Sheep Mountain ewe had low or critically low selenium values.

Table 4. Trace mineral results from all age-sex classes summarized by sub-herd. Cobalt, manganese, molybdenum, and selenium are reported in ng/mL and copper and zinc are reported in ug/mL.

Mineral	Herd	n	Mean	SD	Min	Max
Cobalt	Foothills	6	0.69	0.11	0.53	0.83
Cobalt	La Marche	3	0.98	0.26	0.78	1.27
Cobalt	Notch Bottom	16	0.74	0.22	0.47	1.23
Cobalt	Sheep Mounta	8	0.87	0.4	0.39	1.59
Copper	Foothills	6	1.18	0.37	0.74	1.58
Copper	La Marche	3	0.83	0.02	0.82	0.85
Copper	Notch Bottom	16	0.59	0.2	0.27	1.09
Copper	Sheep Mounta	8	0.75	0.11	0.61	0.92
Manganese	Foothills	6	2.63	1.49	1.5	5.5
Manganese	La Marche	3	2.93	0.95	2	3.9
Manganese	Notch Bottom	16	3.16	0.86	2	4.6
Manganese	Sheep Mounta	8	2.81	0.51	2.1	3.5
Molybdenum	Foothills	6	20.93	33.86	3.6	89.9
Molybdenum	La Marche	3	30.67	13.61	15	39.6
Molybdenum	Notch Bottom	16	18.21	11.15	4.5	50.2
Molybdenum	Sheep Mounta	8	38.15	9.78	26.4	58.6
Selenium	Foothills	6	50.67	22.57	31	84
Selenium	La Marche	3	55.67	1.53	54	57
Selenium	Notch Bottom	16	79.5	9.09	66	99
Selenium	Sheep Mounta	8	89.38	17.02	70	118
Zinc	Foothills	6	1.51	2.3	0.35	6.2
Zinc	La Marche	3	0.72	0.08	0.64	0.79
Zinc	Notch Bottom	16	0.56	0.11	0.39	0.76
Zinc	Sheep Mounta	8	0.58	0.04	0.52	0.64

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