Northern Wild Sheep and Goat Council

Proceedings of the Twentieth Biennial Symposium

May 9-12, 2016
Moscow, Idaho
Pullman, Washington

Northern Wild Sheep and Goat Council

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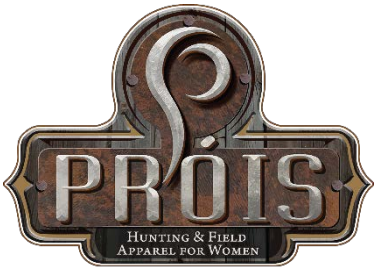

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Northern Wild Sheep and Goat Council Symposia

Date	Symposium	Location	Symposium Coordinator/Chair	Proceedings Editor(s)	NWSGC Executive Director
May 26-28, 1970	NWSC 1	Williams Lake, BC	Harold Mitchell		
April 14-15, 1971	NAWSC 1	Fort Collins, CO	Eugene Decker/Wayne Sandfort	Eugene Decker	
April 11-13, 1972	NWSC 2	Hinton, AB	E.G. Scheffler		
April 23-25, 1974	NWSC 3	Great Falls, MT	Kerry Constan/James Mitchell		
Feb. 10-12, 1976	NWSC 4	Jackson, WY	E. Tom Thorne		
April 2-4, 1978	NWSGC 1	Penticton, BC	Daryll Hebert/M. Nation	Daryll Hebert/M. Nation	
April 23-25, 1980	NWSGC 2	Salmon, ID	Bill Hickey		
March 17-19, 1982	NWSGC 3	Fort Collins, CO	Gene Schoonveld	James Bailey/Gene Schooneveld	
Apr. 30-May 3, 1984	NWSGC 4	Whitehorse, YK	Manfred Hoefs	Manfred Hoefs	Wayne Heimer
April 14-17, 1986	NWSGC 5	Missoula, MT	Jerry Brown	Gayle Joslin	Wayne Heimer
April 11-15, 1988	NWSGC 6	Banff, AB	Bill Wishart	Bill Samuel	Wayne Heimer
May 14-18, 1990	NWSGC 7	Clarkston, WA	Lloyd Oldenburg	James Bailey	Wayne Heimer
Apr. 27-May 1, 1992	NWSGC 8	Cody, WY	Kevin Hurley	John Emmerich/Bill Hepworth	Wayne Heimer
May 2-6, 1994	NWSGC 9	Cranbrook, BC	Anna Fontana	Margo Pybus/Bill Wishart	Kevin Hurley
Apr. 30-May 3, 1996	NWSGC 10	Silverthorne, CO	Dale Reed	Kevin Hurley/Dale Reed/Nancy Wild (compilers)	Kevin Hurley
April 16-20, 1998	NWSGC 11	Whitefish, MT	John McCarthy	John McCarthy/Richard Harris/Fay Moore (compilers)	Kevin Hurley
May 31-June 4, 2000	NWSGC 12	Whitehorse, YK	Jean Carey	Jean Carey	Kevin Hurley
April 23-27, 2002	NWSGC 13	Rapid City, SD	Ted Benzon	Gary Brundige	Kevin Hurley
May 15-22, 2004	NWSGC 14	Coastal Alaska	Wayne Heimer	Wayne Heimer/Dale Toweill/Kevin Hurley	Kevin Hurley
April 2-6, 2006	NWSGC 15	Kananaskis, AB	Jon Jorgenson	Margo Pybus/Bill Wishart	Kevin Hurley
April 27-May 1, 2008	NWSGC 16	Midway, UT	Anis Aoude	Tom Smith	Kevin Hurley
June 7-11, 2010	NWSGC 17	Hood River, OR	Craig Foster	Vern Bleich	Kevin Hurley
March 12-15, 2012	NWSGC 18	Kamloops, BC	Steve Gordon/Steve Wilson/Mari Wood	Steve Wilson/Mari Wood	Kevin Hurley
June 2-5, 2014	NWSGC 19	Fort Collins, CO	Janet George	Bruce Watkins, Ricki Watkins	Kevin Hurley
May 9-12, 2016	NWSGC 20	Moscow, ID Pullman, WA	Hollie Miyasaki,/Rich Harris/David Smith	Rich Harris	Kevin Hurley

Northern Wild Sheep and Goat Council

GUIDELINES OF THE NORTHERN WILD SHEEP AND GOAT COUNCIL

The purpose of the Northern Wild Sheep and Goat Council is to foster wise management and conservation of northern wild sheep and goat populations and their habitats.

This purpose will be achieved by:

1) Providing for timely exchange of research and management information;

2) Promoting high standards in research and management; and

3) Providing professional advice on issues involving wild sheep and goat conservation and management

I The membership shall include professional research and management biologists and others active in the conservation of wild sheep and goats. Membership in the Council will be achieved either by registering at, or purchasing proceedings of, the biennial conference. Only members may vote at the biennial meeting.

II The affairs of the Council will be conducted by an Executive Committee consisting of: three elected members from Canada; three elected members from the United States; one ad hoc member from the state, province, or territory hosting the biennial meeting; and the past chairperson of the Executive Committee. The Executive Committee elects it's chairperson.

III Members of the Council will be nominated and elected to the executive committee at the biennial meeting. Executive Committee members, excluding the ad hoc member, will serve for four years, with alternating election of two persons and one person of each country, respectively. The ad hoc member will only serve for two years.

The biennial meeting of members of the Council shall include a symposium and business meeting. The location of the biennial meeting shall rotate among the members' provinces, territories and states. Members in the host state, province or territory will plan, publicize and conduct the symposium and meeting; will handle its financial matters; and will prepare and distribute the proceedings of the symposium.

The symposium may include presentations, panel discussions, poster sessions, and field trips related to research and management of wild sheep, mountain goats, and related species. Should any member's proposal for presenting a paper at the symposium be rejected by members of the host province, territory or state, the rejected member may appeal to the Council's executive committee. Subsequently, the committee will make its recommendations to the members of the host state, territory or province for a final decision.

The symposium proceedings shall be numbered with 1978 being No. 1, 1980 being No. 2, etc. The members in the province, territory or state hosting the biennial meeting shall select the editor(s) of the proceedings. Responsibility for quality of the proceedings shall rest with the editor(s). The editors shall strive for uniformity of manuscript style and printing, both within and among proceedings.

The proceedings shall include edited papers from presentations, panel discussions or posters given at the symposium. Full papers will be emphasized in the proceedings. The editor will set a deadline for submission of manuscripts.

Members of the host province, territory, or state shall distribute copies of the proceedings to members and other purchasers. In addition, funds will be solicited for distributing a copy to each major wildlife library within the Council’s states, provinces, and territories.

IV Resolutions on issues involving conservation and management of wild sheep and goats will be received by the chairperson of the Executive Committee before the biennial meeting. The Executive Committee will review all resolutions, and present them with recommendations at the business meeting. Resolutions will be adopted by a plurality vote. The Executive Committee may also adopt resolutions on behalf of the Council between biennial meetings.

V Changes in these guidelines may be accomplished by plurality vote at the biennial meeting.

Northern Wild Sheep and Goat Council

FOREWORD

The papers or abstracts included in these proceedings were presented during the 20th Biennial Symposium of the Northern Wild Sheep and Goat Council, held May 9-12, 2016 in Moscow, Idaho and Pullman, Washington, USA.

Heart-felt thanks are extended to the sponsors of, and all those participating in, this highly successful 20th biennial symposium. Special thanks to Hollie Miyasaki and Rich Harris (Symposium Co-Chairs) for leading the dedicated Idaho and Washington organizing committee, and delivering another in a long series of first-class symposia. David Smith deserves special recognition for handling logistics, registration, and symposium minutiae. Thanks to all of the session and poster presenters for assembling and sharing relevant new science on wild sheep and goat ecology and management.

These Proceedings were edited by Rich Harris and volunteer NWSGC members prior to publication. Peer-reviewers included E. Almberg, E. F. Cassirer, D. Coltman, S. Gordon, R. Milner, K. Hurley, H. Miyasaki, R.A. J. Nicholas, K. White, and P. L. Wolff. Suggested editorial comments were provided to each senior author; senior authors had opportunity(ies) to accept or reject suggested edits, prior to submission of their final manuscripts. Formatted page proofs were forwarded to respective senior authors prior to inclusion into the final proceedings. Final content, particularly verification of literature citations, is the responsibility of the authors.

While NWSGC strives for professional, scientific presentations at our symposia, followed up with quality manuscripts for our proceedings, NWSGC Guidelines do not rigidly specify format, minimum data requirements, or thresholds of statistical analysis for subsequently-included manuscripts. Thus, NWSGC Proceedings may contain manuscripts that are more opinion-based than data- or fact-based; critical evaluation of information presented in these proceedings is the responsibility of subsequent readers.

Kevin Hurley
NWSGC Executive Director
May 15, 2017

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Dynamics of a Recolonizing Dall's Sheep Population in Southwest Yukon

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ABSTRACT Dall's sheep (*Ovis dalli* spp.) are distributed throughout Yukon. In the Coast Mountains south of Whitehorse, Dall's sheep (*O. d. dalli*) occur at relatively high density, with regional numbers estimated at roughly 2,500 individuals. Sheep are distributed in a number of discrete populations within this broader regional context. One such population is found on Caribou/Nares Mountains (Caribou/Nares), adjacent to the community of Carcross and roughly 20 km north of the Yukon-British Columbia border (Figure 1). This area is located along the route used by people travelling north during the Klondike gold rush in the late 1800s. The influx of people into this area is believed to have had a significant effect on wildlife populations from both commercial hunting and increased access. Up until the 1960s, small numbers of sheep were observed on Caribou/Nares, but disappeared in the early 1970s for unknown reasons. During the mid-1970s and 1980s, small numbers (~10) of transient groups were occasionally present. In 1990, a larger group of 23 animals recolonized the area from either the Gray Ridge population to the west or the Montana Mountain population to the south (Figure 1). Sheep have remained on Caribou/Nares to the present time. This recolonization event provided a unique opportunity to examine the dynamics of an establishing Dall's sheep population over a 25-year timeframe. From 1990 to 2015, Yukon Department of Environment conducted seven aerial surveys (helicopter-based) in mid-June to mid-July. Information collected included minimum population counts, productivity in the form of lamb:nursery sheep (i.e., ewe-like) ratios, sex ratios, and ram age structure. I fitted non-lamb counts to a logistic population model to estimate both carrying capacity (K) and intrinsic growth rate (r_{max}). K and r_{max} were estimated as 64.5 (SE = 1.3) and 0.44 (SE = 0.02), respectively (Figure 2). During this time there was no detectable trend in lamb productivity in the population from a linear regression of productivity over time (β_{Trend} = -0.11, SE = 0.63). A survey in the summer of 2016, subsequent to this analysis, observed a non-lamb count of 59 animals on Caribou/Nares. Further investigation into the demographic mechanisms governing this population's apparent regulation around K will provide greater insight regarding Dall's sheep carrying capacity.

Biennial Symposium of the Northern Wild Sheep and Goat Council 20:1-3.

KEYWORDS Dall's sheep, intrinsic growth rate, productivity, *Ovis dalli*, recolonization, Yukon

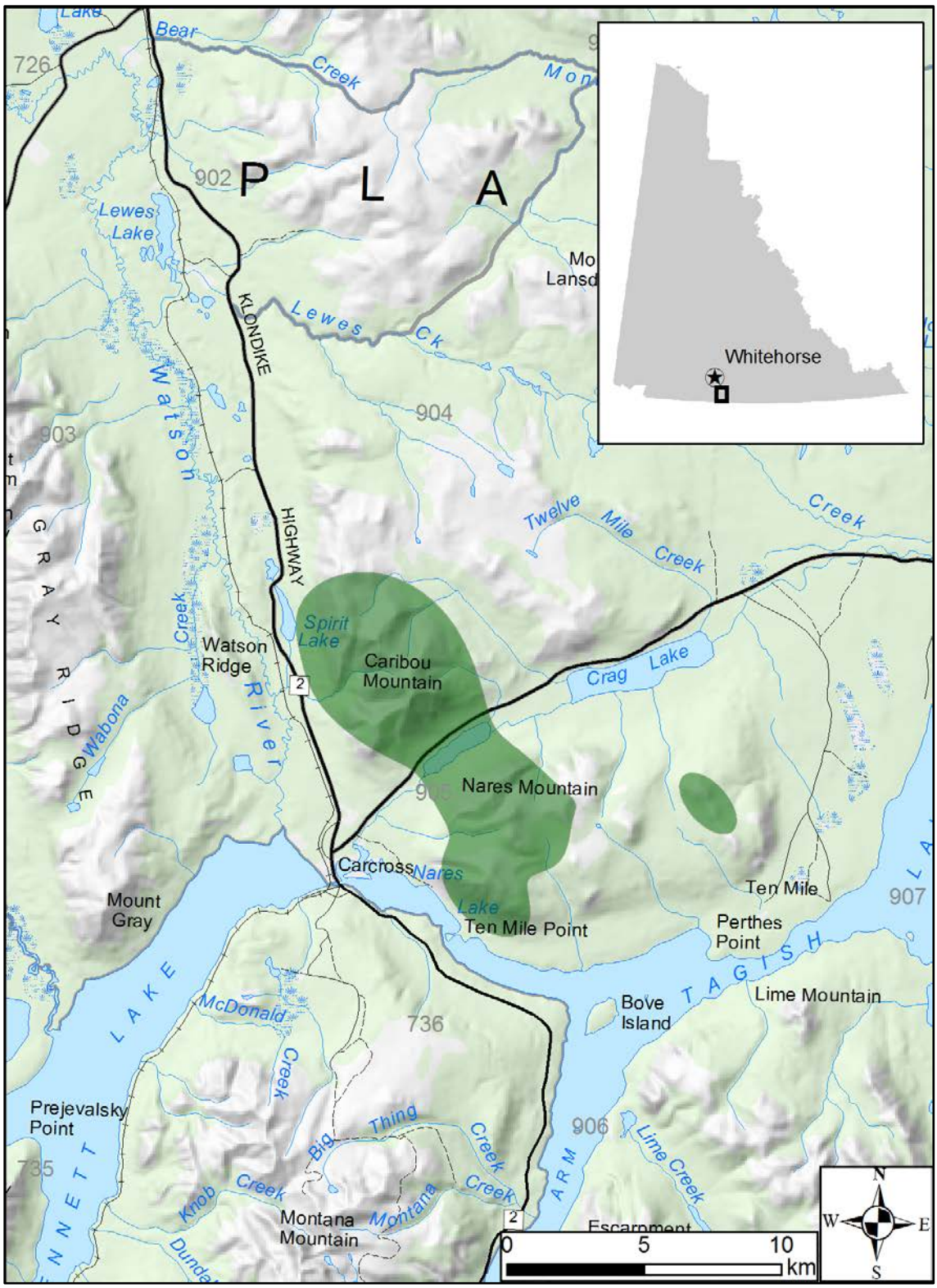

Figure 1. Distribution of Dall's sheep on Caribou and Nares Mountains in southwest Yukon.

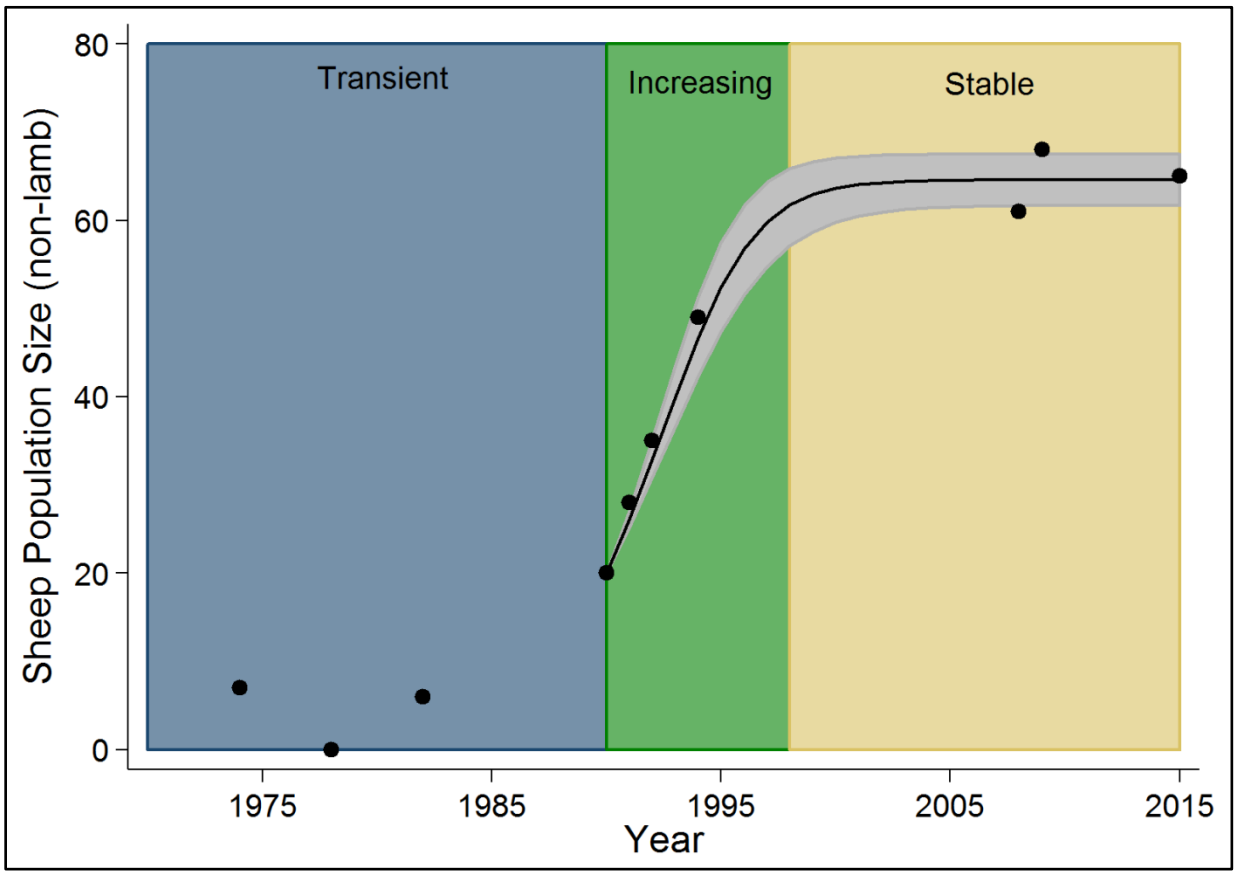

Figure 2. Dall's sheep counts (black circles) and logistic growth model predictions (solid line) on Caribou and Nares Mountains (1974-2015). The time series is roughly categorized by the population's transient, increasing, and stable states. The gray area represents the 95% confidence interval of the predictions.

Behavioral Connectivity among Bighorn Sheep Suggests Potential for Disease Spread

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ABSTRACT Connectivity is important for population persistence and can reduce the potential for inbreeding depression. Connectivity between populations can also facilitate disease transmission; respiratory diseases are one of the most important factors affecting populations of bighorn sheep (*Ovis canadensis*). The mechanisms of connectivity in populations of bighorn sheep likely have implications for spread of disease, but the behaviors leading to connectivity between bighorn sheep groups are not well understood. From 2007–2012, we radio-collared and monitored 56 bighorn sheep in the Salmon River canyon in central Idaho. We used cluster analysis to define social groups of bighorn sheep and then estimated connectivity between these groups using a multi-state mark-recapture model. Social groups of bighorn sheep were spatially segregated and linearly distributed along the Salmon River canyon. Monthly probabilities of movement between adjacent male and female groups ranged from 0.08 (0.004 SE) to 0.76 (0.068) for males and 0.05 (0.132) to 0.24 (0.034) for females. Movements of males were extensive and probabilities of movement were considerably higher during the rut. Probabilities of movement for females were typically smaller than those of males and did not change seasonally. Whereas adjacent groups of bighorn sheep along the Salmon River canyon were well connected, connectivity between groups north and south of the Salmon River was limited. The novel application of a multi-state model to a population of bighorn sheep allowed us to estimate the probability of movement between adjacent social groups and approximate the level of connectivity across the population. Our results suggest high movement rates of males during the rut are the most likely to result in transmission of pathogens among both male and female groups. Potential for disease spread among female groups was smaller but non-trivial. Land managers can plan grazing of domestic sheep for spring and summer months when males are relatively inactive. Removal or quarantine of social groups may reduce probability of disease transmission in populations of bighorn sheep consisting of linearly distributed social groups.

Biennial Symposium of the Northern Wild Sheep and Goat Council 20:4.

KEY WORDS behavioral connectivity, bighorn sheep, disease, Idaho, multi-state mark-recapture, *Ovis canadensis*, Salmon River, social groups.

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Movements of a Localized Mountain Goat Herd: Implications for Harvest

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ABSTRACT Mountain goat (*Oreamnos americanus*) numbers in the White River watershed of western Washington State, USA, near the Muckleshoot Indian Tribe's reservation have declined, and as a consequence sustainable harvest opportunity for all user groups in this area has ended. The Tribe conducts annual helicopter surveys of mountain goats in areas of the Cascade Mountain range near the reservation to document trends in goat numbers. Our objective was to assess whether goats found in one area of the White River are migratory and part of a larger east Cascades sub-population that could be large enough to allow harvest, or are a localized, isolated western Cascades herd. We radio-marked 1 female and 4 male goats out of an estimated 10 to 15 animals present to document movements and migrations. We used two types of GPS collars that transmitted locations to satellite at 9 and 23 hour intervals, and relayed them to us. Both collar types collected adequate data to reveal that goats in this small area are west-side animals and represent a small group that would not be sustained if harvest occurred.

Biennial Symposium of the Northern Wild Sheep and Goat Council 20:5-15.

KEY WORDS GPS, management, mountain goat, *Oreamnos americanus*, survey, sustainable harvest, tribal hunting

Mountain goats (*Oreamnos americanus*) exist in small, somewhat isolated groups and are sensitive to harvest due to demographic and environmental stochasticity (White et al. 2011), low recruitment rate, advanced age of female sexual maturity (Festa-Bianchet et al. 1994), and misidentification of female goats where males are hunted. Hamel et al. (2006) found that small populations of goats <50 had high extinction risk even in the absence of hunting and that nonselective harvest rate >1% was not sustainable short term. Gonzalez-Voyer et al. (2003) concluded that factors other than hunting contributed to the decline of some herds, and that a herd of 100 individuals could sustain a harvest of only 1 or 2 males. Rice and Gay (2010) concluded that past harvest on goats in several herds in Washington State, USA led to declines and recommended that there be no harvest on populations <50 individuals. Their modeling and conclusions were consistent with Côté et al. (2001), Gonzalez-Voyer et al. (2003), and Hamel et al. (2006), who all concluded that harvest in small goat herds was destabilizing and could lead to localized decline or extirpation.

Indian tribes in Western Washington who have recognized treaty hunting rights establish their own harvest regulations. Although tribal members are not bound by regulations promulgated by the Washington Department of Fish and Wildlife (WDFW), many tribes consider themselves as co-

managers with WDFW, and are concerned about long-term sustainable game populations and hunting opportunities. Most, if not all, tribes that are federally recognized as part of the 1855 Treaty of Point Elliott limit mountain goat hunting by issuing only limited permits, but the demand for animals exceeds availability. WDFW harvest guidelines as of 2016 specified that population estimates must be >100 goats within an identified hunting area before that group of mountain goats can be subject to recreational harvest, female harvest must be limited, and harvest must be 4% or less of the estimated local population aged one year-old and above (WDFW 2014). Because the spatial scale and definition of a mountain goat population in this area remains an issue under consideration, an objective of our work was to shed light on these questions. Tribal members of the Muckleshoot Indian Tribe (MIT) have a long history of harvesting mountain goats and using their hair, horns, hooves, bones, meat, and other parts for a variety of necessary cultural purposes. When hunting opportunity for a culturally important species is limited, tribal members find it difficult to carry on their traditions. As managers of wildlife resources for the MIT, we are frequently asked to find harvest opportunities for goats but have found this difficult in recent years due to the low numbers of goats seen.

The Muckleshoot Tribe has been surveying goats since 2003 covering an area near the MIT's reservation, south of Interstate 90 Highway (I-90), north of Mount Rainier National Park (MRNP), and west of the Cascade Crest including the Cedar, Green, and White River watersheds. The MIT began coordinating Cascade Crest surveys with WDFW in 2009 to improve data collection and sharing. Most of the MIT survey area lacks specifically delineated survey blocks because habitat patches are relatively small and isolated, and because mountain goat group sizes are small. Along the Cascade Crest, however, the WDFW has delineated blocks used for conducting sightability surveys based on potential habitat, elevation, time to survey the block, and local expert knowledge (Rice et al. 2009). We have seen large groups of goats in some survey blocks along the crest but we have never seen groups >10 in the Mutton Mountain survey block, located 4 km west of the crest. Because the Mutton Mountain block is only about 1 km from the Castle Mountain survey block where we had recently observed more goats, we hypothesized exchange between these blocks, as well as movement between east and west of the crest. If exchange occurred, this could provide support for including Mutton Mountain animals as part of a larger population, and offer a very limited opportunity for MIT goat hunters.

East of the Cascade Crest and south of I-90, mountain goats are abundant enough to allow WDFW to authorize permit-only hunting in 3 delineated hunt areas (named Naches Pass, Blazed Ridge, and Bumping River). Within the area west of the Cascade Crest, south of I-90, and north of Mount Rainier National Park, available information suggests that goats are not sufficiently abundant to support hunts permitted by either WDFW or tribal authorities. Mountain goats are more abundant in certain areas north of I-90 to the Canadian border west of the crest within the Point Elliott Treaty area, and the WDFW manages permit-only hunts for goats in these areas. Some of the Point Elliott Treaty tribes issue goat permits in these areas we well. Some MIT hunters, however, prefer to stay near home in familiar landscapes, and to reduce travel expense.

Our objective was to document movements of mountain goats captured west of the Cascade Crest (in the Mutton Mountain survey block) to assess whether they form part of a larger population of goats that reside mostly east of the Cascade Crest, and thus if these animals could provide harvest opportunity for members of the MIT. We also

compared movements of these goats to a study animal captured nearby as part of an earlier WDFW study (Rice 2006, 2008).

Study area

The 80 km^2 study area was west of the Cascade Crest within the Mount Baker-Snoqualmie National Forest (MBSNF), approximately 3 km east of MRNP in Washington State. It encompassed lands within the Treaty of Point Elliott as well as the WDFW White River Game Management Unit (GMU) 653 (Fig. 1). The study area contained all of the Mutton Mountain goat survey block (9.1 km^2) and most of the Castle Mountain goat survey block (7.6 km^2), but did not extend south as far as the Norse Peak mountain goat survey block (Fig. 1). The study area included the WDFW Corral Pass goat hunt unit west of the Cascade Crest which had been open for WDFW hunting until 2010. We refer to the Castle Mountain survey block as Corral Pass for ease of understanding because the 2 roughly overlap (Fig. 1). The area to the east of the Cascade Crest and adjacent to the study area is in GMU 346, contained the WDFW Naches Pass mountain goat hunt unit, and was outside of the Treaty of Point Elliott boundary. The Forest Service Corral Pass Road provided access to a campground and trails within 2 km of Mutton Mountain at Corral Pass (Fig. 1), making goats in this area particularly vulnerable to hunting, poaching, and human disturbance. Elevations within the study area vary between 800 and 1,900 m. Potential natural vegetation zones include Pacific silver fir (*Abies amabilis*), subalpine fir (*Abies lasiocarpa*), mountain hemlock (*Tsuga mertensiana*), and parkland (Franklin and Dyrness 1988, Henderson et al. 1992).

Methods

We darted mountain goats from a helicopter using 1 mL Pneu-Dart transmitter darts with 32 mm double-barbed needles containing 3.0 mg carfentanil and 30 mg xylazine. The helicopter hazed animals to a safe area and remained in the air to guide a ground crew who hiked to the site. Once goats were immobilized, the ground crew applied a blindfold and secured the them in safe positions. Goats were injected with2.0 mL 8-way clostridium vaccine, 2.0 mL MuSe, 8.0 mL vitamin B complex, and 8.0 mL penicillin G procaine. Age was estimated based on horn growth annuli. Goats were collared and finally, anesthesia antagonized by injecting them with 200 mg naltrexone subcutaneously, and 200 mg naltrexone with 30 mg yohimbine intravenously. Capture and handling followed the American Society of Mammalogists guidelines for the use of wild mammals in research (Sikes et al. 2011) adopted by the Muckleshoot Wildlife Program. We used 2 types of Vectronic Aerospace collars, a GPS Plus 2010 Globalstar 1D collar programmed to acquire locations at 9-hour intervals, and a Vertex Survey Globalstar 1D collar programmed to acquire locations at 23- hour intervals. The GPS Plus collars had integrated drop-offs with a life expectancy of 3 years; the Vertex Survey collar did not have a drop-off and had a life expectancy of 4 years. Additionally, we incorporated into our analysis GPS locations for a female goat that was studied by Rice (2006, 2008) from August 2004 through June 2006.

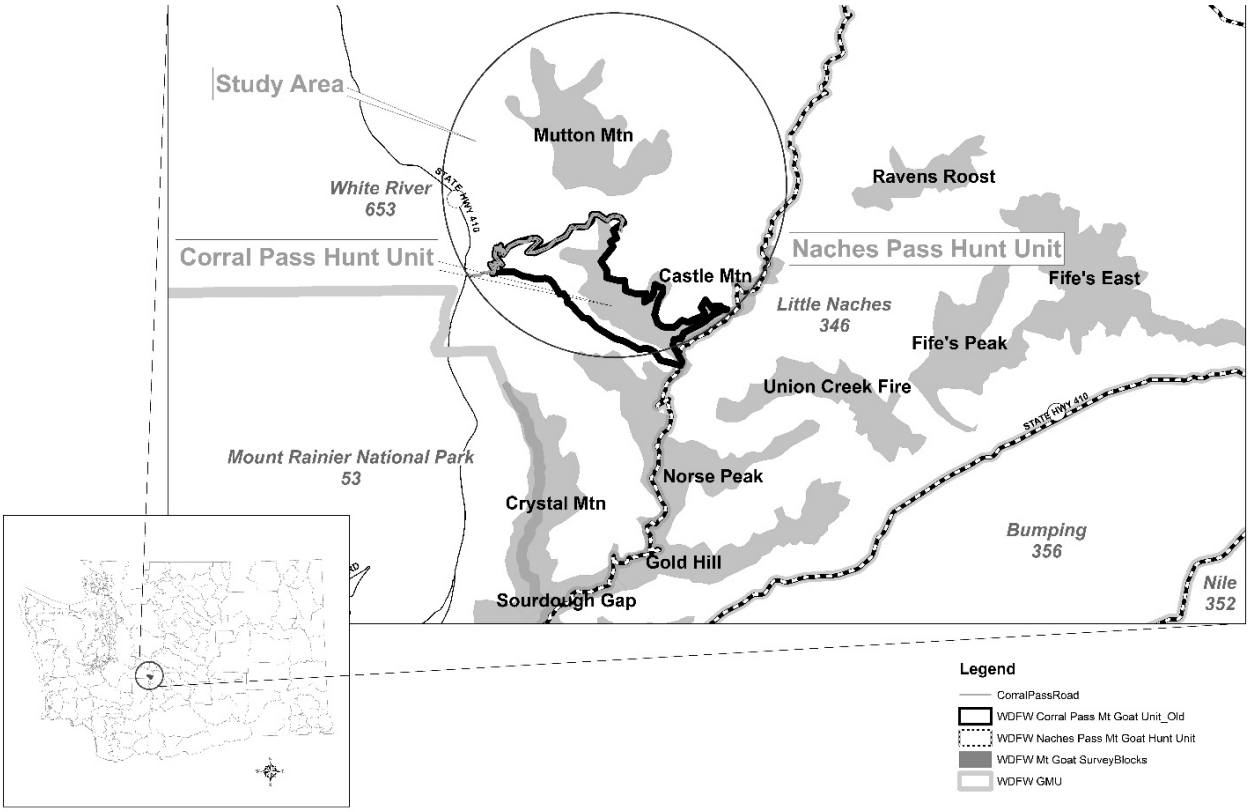

Figure 1. Study area within Washington State (diagonal hatching) showing location ofnamed survey blocks, Corral Pass and Naches Pass hunt units, Mount Rainier National Park, and WDFW Game Management Units.

We also conducted surveys to understand the broader distribution of mountain goats relative to the marked animals, and identify where there may be opportunity for harvest. MIT staff conducted goat surveys by helicopter in late August beginning in 2003 using a BellJet Ranger and 3 observers. These surveys included the WDFW goat survey blocks described above, as well as potential goat habitat throughout the area west of the Cascade Crest, south of I-90, and north of MRNP. Starting in 2009 MIT staff have coordinated surveys on the crest with WDFW, and all data have been shared between the two management entities.

We summarized goat harvest data from WDFW game harvest reports dating back to the 1994 hunting season. The harvest data are complimentary to survey data showing distribution of harvest relative to marked animals, and where there may be harvest opportunity provided populations meet minimum number criteria. They may also reveal overharvest, and explain the low abundance of mountain goats found in some areas.

Results

GPS location and movement data

We captured and collared 5 mountain goats between 2012 and 2014. We equipped 2 adult male goats captured in late July 2012 with equipped with GPS Plus collars. In late July 2013, we placed Vertex Survey collars on 1 adult male and 1 adult female. In August 2014, we collared 1 adult male with a GPS Plus collar retrieved from a mortality. We monitored goats for 109 to >1,033 days each, fewer than the expected collar life, due to goat mortality or early collar failure. We acquired

from 262 to 2,079 valid locations per animal (Table 1). The GPS Plus collars had higher fix success rates and higher satellite upload success rates than the Vertex Survey collars,

Four of the 5 marked goats confined their movements to GMU 653; goats M11078-1 (♂) and F12960 (♀) had 100% of their locations, and male goats M11078-2 and M12907 had 99.1% and 99.6% of their locations within GMU 653, respectively. These movements are consistent with the results of the female earlier collared by WDFW, 040CPF, whose locations remained in GMU 653 99.4% of the time from 2004 – 2006 (Fig. 2). The maximum distance from the GMU 653 boundary for these 3 goats was 1 km. One goat, M11077 (♂), moved east during rut in 2 consecutive years, with 87.3% of its locations in GMU 653 and the remainder within GMU 346. His locations east of the Cascade Crest occurred during early November through mid-January, coinciding with rut and return to winter range. He returned to the study area in winter despite snow depths >75 cm in early January 2014 and >90 cm in December 2014-January 2015 (Corral Pass site 418 SNOTEL).

Although all goats generally remained in GMU 653, some goats made smaller-scale movements between the survey blocks. For goat M12907, 22% of it locations were in the Mutton survey block and 28% were in the Corral Pass survey block. Male goats M11077 and M11078-1, however, had 44% and 40% of their locations in Mutton, with less than 5% in Corral Pass. Female goat F12960 had 30% of her locations in Mutton and none in other survey blocks, whereas 040CPF goat had 72% in Corral Pass, 21% in Norse Peak, and none in Mutton. Locations outside of survey areas were associated with winter range, small isolated patches of habitat outside survey blocks, or movements between areas (Fig. 2). During the 2 winters we had her marked, F12960 made a horizontal movement to winter range below 1,400 m (Fig. 2). Male M11077 also moved to the same winter range for part of the first winter (January 30-May 4, 2013) but only for 2 weeks (January 12-26, 2014) during the second. He then minimized his movements, and used an area only 0.12 km^2 from January 27 to May 4, 2014. Male M11078-2 used the same separate winter range area as did female F12960 but only from December 2, 2014 to January 9, 2015, and then moved up to summer range area where the Corral Pass SNOTEL snow depth reported 75 to 120 cm during January through April. The male goats generally spent winter in various parts of their summer range below 1,400 m, and rarely moved among portions of their range through the winter, but generally did not exhibit the distinct horizontal movement to winter range that F12960 did.

Mortality and collar longevity

Three goats died and one prematurely went off air during the study period (Table 1). Goat M11078-1 died November 8, 2012 during the rut but we were not able to get to the animal early enough to examine the carcass. Goat F12960 died May 18, 2015 from breached birth complications and had fallen off a cliff. Goat M11078-2 died July 14, 2015 and was consumed by the time we investigated it on August 3. We did not receive an immediate mortality message due to animal position and location of the carcass not having a clear sky view. No evidence suggesting the cause of mortality was found due to extensive scavenging. We acquired locations from M11077 for 841 days through November 22, 2014 when the collar began emitting a double-beep recovery mode VHF signal. We continued intermittent VHF tracking and located this animal by helicopter east of the Cascade Crest on December 17 and 30, 2014, and west of the crest on March 18 and July 7, 2015 when it was last heard. As of January 31, 2017 goat M12907 was alive and being tracked.

Table 1. Animals captured including animal ID, collar type, GPS fix interval, number of locations (*n*) received via satellite relayand offloaded from collar, number of valid locations collected, amount of time monitored, and fate of animal.

ID	Type	Interval	*n*[3]	*n*[4]	*n*[5]	%[6]	Fix Success[7]	Age at capture	Sex	Days monitored	Fate
M1107 7	GPS Plus	9 h	2,1 24	est 2,242	2,079[1]	95%[2]	98%[1]	7-10	M	841	Premature failure, collar not retrieved
M1107 8-1	GPS Plus	9 h	229	288	262	80%	91%	7-10	M	109	Died -unknown, suspect rut injury
M1107 8-2	GPS Plus	9 h	827	918	887	90%	97%	5+	M	344	Died - unknown
M1290 7	Survey	23 h	680	est 1,078	516[1]	63%[2]	76%[1]	4-5	M	1033+	Ongoing as of January 2017
F1296 0	Survey	23 h	514	687	579	75%	84%	4-5	F	658	Died – suspect parturition related

[1]Based on data received via satellite only for % fix success
[2]Estimated from time monitored and expected number of locations
[3]*n* = number of locations received relayed through satellite
[4] *n* = number of locations downloaded from retrieved collar
[5] *n* = number of attempted locations that had a valid GPS location
[6]% = percent of location attempts that were relayed through satellite and received via email
[7]Fix success = percent of location attempts that resulted in a valid location

Surveys

From 2003 through 2015, larger numbers of goats were counted in the Corral Pass and Norse Peak blocks than the Mutton Mountain block (Table 2). The highest count for the Corral Pass block was 57 goats in 2011, with the largest group containing 42 individuals. The highest count for Norse Peak block was 84 goats in 2008, with a large group of 53 individuals. Counts in Corral Pass were variable, and were likely related to goats moving between Norse Peak and Corral Pass in a given year during the survey window. In 11 annual surveys of Mutton Mountain the largest group was only 8 and the maximum total was only 16 (Table 2).

Historical harvest

The total State of Washington goat harvest for the Corral Pass hunt unit during 1994–2003 was 20 (i.e., $\bar{x}$ = 2/yr) from 30 permits issued. This hunt unit excluded the Mutton Mountain survey block and was separate from the Naches Pass hunt unit. The Naches Pass hunt unit harvest was 30 (i.e., $\bar{x}$ = 3/yr) with 37 permits issued during 1994–2003. The Corral Pass unit was merged with the larger east side Naches Pass unit in 2004 through 2009, and had a total harvest of 9 from11 permits issued. The Corral Pass hunt unit was removed from the Naches Pass unit in 2010 and closed by WDFW to State goat hunting. Goats living in the Corral Pass survey block, however, are likely susceptible to harvest when travelling to the east side of the block or to the east side of the Norse Peak block, which are both inside the Naches Pass hunt unit, and still open to permit hunting. Data on tribal harvest from this area were not available because they were reported at the coarser GMU scale, but it was likely very low because the total reported harvest in GMU 653 was only 3 during the 16-year reporting period 2000—2015 (Northwest Indian Fisheries Commission Big Game Harvest Reports http://nwifc.org/publications/big-game-harvest-reports/ accessed August 1, 2016).

Table 2. Helicopter aerial survey counts of total number of mountain goats observed in 3 survey blocks, 2003-2015, western Washington State, USA. Data for 2003-2008, 2014, and Mutton Mountain data collected by the Muckleshoot Indian Tribe. Italicized data for Corral Pass and Norse Peak 2009-2013, and 2015 are from WDFW.

Year	Mutton Mtn.	Corral Pass	Norse Peak
2003	13	1	26
2004	7	12	11
2005		1	14
2006			
2007	9	2	52
2008	10		84
2009	16	*3*	*74*
2010	4	*1*	*49*
2011	4	*57*	*1*
2012	10	*13*	*30*
2013	9	*26*	*48*
2014	10		50
2015	*4*	*8*	*65*

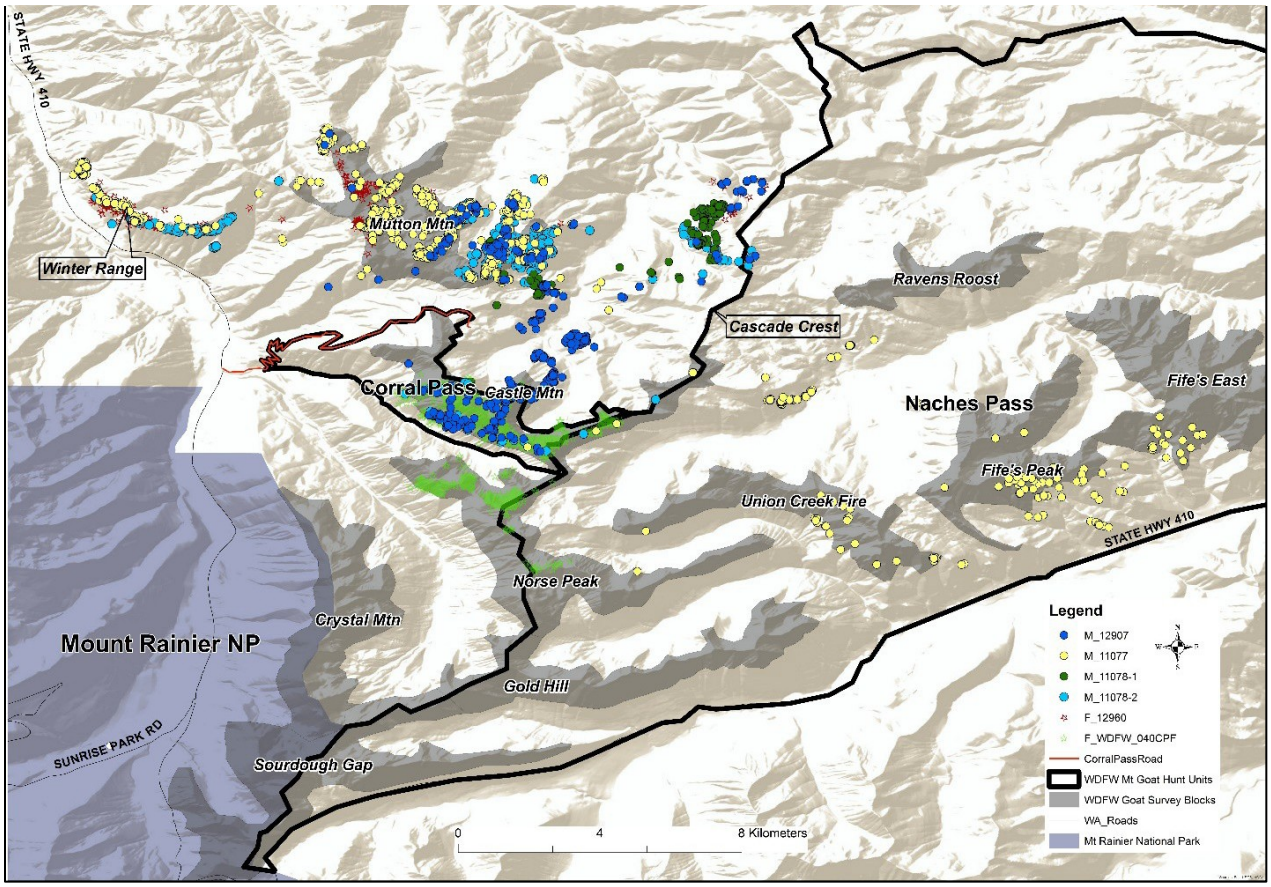

Figure 2. GPS locations for 4 collared males, and 1 collared female mountain goats and one WDFW collared goat relative to survey blocks (grey shading) and goat hunt units (black boundary lines).

Discussion and management implications

Our objective was to determine if mountain goats in the Mutton Mountain survey block area constituted a separate demographic unit from the nearby Corral Pass (Castle Mountain) and Norse Peak survey block animals. If Mutton Mountain goats were separate, then hunting should not occur in this area because it was a very small population. In contrast, if goats observed at Mutton Mountain migrated to the east side, or were part of a larger population, then there could be limited harvest of these goats because they would be replaced by animals from other areas where they are already hunted.

Our survey data suggest that Mutton Mountain goats were somewhat isolated based on consistently small groups and low numbers observed in the block during surveys conducted beginning in 2003 (Table 2). Larger groups and more animals were consistently found in the nearby Corral Pass and Norse Peak survey blocks (Table 2). Although sample sizes were small, our marked animal location data supported our assessment that the Mutton Mountain animals were relatively isolated, particularly females. Neither the female goat marked in this study, nor the female in the prior WDFW study (040CPF), showed any movement between the Mutton Mountain and Corral Pass survey blocks, despite two blocks being only 1 km apart at

their closest. Female 040CPF remained largely in Corral Pass, while F12960 stayed in Mutton Mountain. One male exhibited movement between these, but the other 2 that were on the air long enough for analysis had <5% of their locations in the Corral Pass block, and instead spent time in Mutton Mountain and other areas north and east of Corral Pass.

Some goats using Corral Pass may also use Norse Peak as seen with 040CPF and it is possible that goats move readily between these two areas. During annual surveys, large groups were found in either the Corral Pass or Norse Peak blocks, but not in both blocks in the same year (Table 2). Five of the 6 radio-marked animals showed a west side tendency with >99% of their locations in GMU 653; 1 male moved east during the rut. More animals marked over a longer time period might have revealed more interaction, or might strengthen our observations that goats are segregated at Mutton Mountain. However, we feel our sample of 5 goats out of an estimated total herd of 10 to 15 was a fair sample size.

Goats are known to occur in isolated herds but have the ability to immigrate or emigrate 20 km or more although these movements are infrequent and male-biased (Côté and Festa-Bianchet 2003). We did not have any marked goats emigrate, possibly due to the marked goats being mature animals. Our furthest-short-term movement was approximately 15 km for a male who moved during rut and returned soon after. Côté and Festa-Bianchet (2003:1066) stated that "Males could also make extensive movements during the rut in some populations but not in others, depending on the distance between neighboring groups." In our study area, behavioral variation among males may have determined whether they moved long distance during rut or not because all males were not far from neighboring groups and were of mature age.

Our observation of variation among individuals in their use of summer and winter range was similar to that found by Poole and Heard (2003), who documented some goats that moved to distinct winter range while others shifted elevation within their summer range. The horizontal distance of goats that moved to distinct winter ranges was 3-6 km, shorter than the 8 to 13 km for those of Poole and Heard (2003). Our goats had winter altitudinal changes of 400—700 m, similar to that reported by Rice (2008). One of our males moved from summer to distinct winter range in January 2015, when snow was 150-190 cm deep, but in January 2016, with snow only 75–140 cm deep, he spent winter within summer range. Other males also stayed within summer range but used lower elevation and forested habitats during winter. Consistent with our findings, Rice (2008) noted that seasonal altitudinal movements are highly variable, and related to snow depth consistent with our findings. Côté and Festa-Bianchet (2003:1066) wrote "Some populations remain in the same area throughout the year, whereas others have distinct summer and winter ranges." We suspect that many of the Norse Peak and Corral Pass goats move east during winter but lack movement data to support this. Female goat 040CPF stayed within the Corral Pass block during winter, but used lower elevation in 2006 when there was more snow than in 2005.

Our survey data recorded a maximum of 16 goats in the Mutton Mountain unit in 2009, and a largest group size of 9 in 2007. Over time total numbers have been stable at around 10. We have frequently seen 3 kids in late August, barely enough to maintain this herd. The 3 mortalities we experienced were a significant loss to this herd. Parks et al. (2015) reported the goats in the southern Cascades had the lowest genetic diversity of all known goat populations in Washington and were likely separated genetically from the rest of the state by I-90. They hypothesized

"…resistance to landscape-level gene flow may further erode genetic diversity and limit the ability of [Washington] populations to recover." (Parks et al. 2015:1200). Furthermore, Mainguy et al. (2009) have shown that reduced genetic diversity in mountain goats was associated with reduced juvenile survival. If our data represent natural mortality rates for this population, this herd would not be able to support harvest if it is to persist, and would likely benefit from an augmentation to improve genetic diversity.

In 2015 the WDFW observed 161 goats in the Naches Pass hunt unit, a sufficient number to allow a permit-only hunt. During the same flight there were only 4 goats seen in the Mutton Mountain block and 8 in the Corral Pass block. Historically, harvest in the Corral Pass hunt unit averaged 2 per year when open. The harvest may have included Mutton Mountain male goats, as well as Norse Peak animals. The high harvest relative to the number of resident western Cascades animals may have exceeded what was sustainable and has resulted in persistently low numbers west of the Cascade Crest.

The Muckleshoot Tribe and 8 other tribes have reserved hunting rights under the Treaty of Point Elliott which lies mostly west of the Cascade Crest (State v. Buchanan 138 Wash. 2d 186 1999). Tribes who manage a small number of hunters might have tighter control over their hunters and be able to harvest a small number of males in smaller populations by using creative strategies such as alternate or every 3rd-year hunting. Such a strategy might target lone males who have dispersed, but it would rely on replacements from nearby larger populations to sustain opportunity. Hunting opportunity south of I-90, however, is nonexistent to implement a conservative tribal strategy due to too few mountain goats throughout the area, and potential number of treaty tribes hunting.

Because we documented the movement of a female goat between Norse Peak and Corral Pass, the question arises whether the Corral Pass area should be opened for harvest? The marked goat data suggest that some of the animals that use this block are west side animals, which seem to occur in low numbers, certainly less than 50, and as such, should be protected. Hunters in the adjacent Norse Peak unit have a low chance of harvesting a west side goat, and this supports keeping the Naches Pass hunt unit (which includes Norse Peak) open as long as survey data continue to reveal adequate numbers. If additional goats are collared in or near our study area, they should include females from the large groups observed in the Norse Peak or Corral Pass survey blocks. Studying these animals may reveal migrations for those goats and possibly interactions that we did not detect by collaring only Mutton Mountain goats.

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Limiting Factors of Bighorn Sheep Populations in Central and Southwestern Idaho

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ABSTRACT In central and southwestern Idaho, connectivity among populations, dispersal, and habitat preferences of bighorn sheep (*Ovis canadensis*) are poorly understood. In addition, health status, risk of disease transmission, and the influence of habitat quality on disease prevalence have not been identified. We developed a project to provide information on these poorly understood population attributes. Our objectives were to 1) define dispersal patterns and connections between neighboring populations; 2) establish baseline health status and disease prevalence for the herd; 3) estimate productivity and lamb survival; and 4) measure forage quality and quantity. In March 2016, we captured 62 bighorn sheep, 34(11♂, 23 ♀) in the East Fork of the Salmon River, and 28 (11♂, 17♀) in the Owyhee Mountains, fitting them with GPS collars. Data collected from these individuals will be used to determine seasonal movements and range use, assist domestic sheep/goat-wild sheep risk assessments being conducted by the U.S. Forest Service, quantify lamb production and survival, quantify cause-specific mortality, and develop seasonal habitat suitability models. In addition, we collected blood, fecal, pharyngeal, nasal swabs and ear swab samples. Body Condition Scores (BCS) were assigned, and body fat measurements and pregnancy status were collected via portable ultrasound. Blood selenium levels were higher in East Fork Salmon than Owyhee animals, suggesting better immune function. None of the sheep sampled in the Owyhees and 8 of the 34 sheep sampled in the East Fork were positive for *Mycoplasma ovipneumoniae* on PCR. About 5% Owyhee sheep and about 85% of East Fork sheep were positive for *M. ovipneumoniae* antibodies on ELISA serology. All ewes in both study areas were pregnant except for one in the East Fork. Body Condition Scores (BCS) were similar in both populations at about 2.5, or fair condition. Forage quantity and quality will be measured during summer 2016 using a methodology described by Cook et al. (2016). Lambing success of collared ewes will be documented in June, and lamb survival will be documented in early fall. More collars will be deployed in winter 2017 to maintain sample size at around 30 collared sheep for each study area.

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KEYWORDS connectivity, disease, dispersal, forage quality, lamb survival, *Mycoplasma ovipneumoniae, Ovis canadensis,*

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Delineating and Estimating Seasonal Migration patterns of Rocky Mountain Bighorn Sheep in the Mountain Valley Complex of South Central Idaho

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ABSTRACT We monitored 52 Rocky Mountain bighorn sheep from four herds across the Lemhi and Beaverhead Ranges wearing GPS collars from January 2013 to July 2015. This population exhibits spatiotemporal behavior, forays into winter range for short periods (1-3 days) and returning to summer range that complicated our interpretation of seasonal migration movements, as well of our spatial delineation of summer range. Once this behavior is accounted for methodologically, more accurate estimates are calculated and reported. We investigated and modified Net-Squared Displacement (NSD) for the purposes of identifying when and where individual sheep initiated their spring and fall migrations between seasonal ranges. On average, spring migration commenced in late April to mid-May, where an individual will have increased rates of movement for 12 days over a distance of 12.8 km (3 annual migrations). Fall migration began at the end of October to early November, when increased movements occurred over a period of 8 days, returning to winter range over a distance of 11.5 km. Inter-annual and intergroup differences were observed in the data and reported. We also overlaid maps of seasonal movement patterns onto to domestic sheep allotments, as well as intergroup movement patterns.

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KEYWORDS bighorn sheep, Beaverhead Range, Idaho, Lemhi Range, migration

Modeling Management Strategies for the Control of Bighorn Sheep Respiratory Disease

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ABSTRACT Infectious pneumonia has plagued bighorn sheep populations and stymied recovery efforts across the western United States for decades. Management efforts aimed at controlling the disease have met limited success. Here we present a simple, non-spatial, stochastic, discrete-time model that captures basic bighorn sheep demographics and in which we simulate the dynamics of *Mycoplasma ovipneumoniae,* the suspected primary causative agent in bighorn sheep respiratory disease, based on our current working knowledge of pathogen transmission and impacts. We then use the model to explore the impacts of management approaches, including augmentation, depopulation followed by reintroduction, density reduction, and test and cull, aimed at reducing or eliminating the pathogen, its transmission, or associated infection costs. Preliminary results, pending a full sensitivity analysis, suggest that test and cull (testing 95% of a herd for 1 or 2 consecutive years and removing any individuals that test PCR positive) and depopulation and reintroduction (assuming ability to only depopulate 95% of the herd) offer the best probability of eliminating the pathogen, although neither are expected to be 100% successful. Augmentation (adding 30 adult ewes), whether we assume the ewes are susceptible or immune to *M. ovipneumoniae,* does not increase the probability of pathogen extinction or herd recovery, and in some cases may prolong pathogen persistence and diminish herd recovery. Density reduction (randomly removing 25-50% of the herd) modestly increases the probability of stochastic pathogen extinction and herd recovery, but only if ≥50% of the herd is removed. Stochastic pathogen extinction and herd recovery is predicted to occur on occasion without any management intervention. Ultimately, decisions to manage respiratory disease in wild sheep must weigh the predicted success of the management tool against financial, logistical, ethical, and value-based considerations. Here, we aim to supply mechanistic-based predictions of the relative efficacy of currently employed or proposed tools, as well as characterize the sensitivity of these predictions to our assumptions about how the disease process works.

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KEY WORDS augmentation, bighorn sheep, depopulation, disease dynamics, disease management, pathogen persistence, pneumonia, respiratory disease, simulation model, test and cull.

Status of Western Montana Bighorn Sheep Herds and Discussion of Control Efforts After All-Age Die-Offs

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ABSTRACT A western Montana bighorn sheep metapopulation is made up of 9 herds: Bonner, Petty Creek, Lower Rock Creek, Upper Rock Creek, Skalkaho, East Fork of the Bitterroot, Painted Rocks, Garrison, and Anaconda. During the winter of 2009-2010, the East Fork of the Bitterroot, Bonner, Upper Rock Creek, and Lower Rock Creek herds experienced pneumonia related all age die-offs. Montana Fish, Wildlife, and Parks personnel attempted to prevent the spread of the disease to healthy herd segments and neighboring populations by aggressively culling bighorn sheep in two of the herds. In the East Fork of the Bitterroot herd any bighorn sheep showing clinical signs of disease was culled. In the Bonner herd the agency attempted to cull all bighorn sheep within a containment zone. Samples were collected from all culled bighorn sheep for respiratory pathogen testing. The pneumonia outbreaks were allowed to run their course in the other two herds (Lower and Upper Rock Creek), although these herds experienced some lethal sampling for diagnostic purposes. Now, five years later, we report on the fate of these treated and untreated herds, as well as that of the five other herds within the population. Our data suggest that culling did little to affect the 5-year post-outbreak population trends or pathogen communities for the affected herds. Die-offs, followed by chronically reduced lamb recruitment were detected in the Skalkaho and Anaconda herds in 2011.

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KEY WORDS bighorn sheep, disease control, containment zone, culling, die-off, Montana, pneumonia, respiratory disease

Epizootic pneumonia is the most important challenge faced by bighorn sheep and wildlife staff tasked with managing them (Sells et al. 2015). These disease events can have devastating effects on bighorn herds because they often result in all-age die-offs and poor lamb recruitment for several to many years following an outbreak (Onderka and Wishart 1984, Coggins and Matthews 1992, Ryder et al. 1994, Semmens 1996, Aune et al. 1998). State management agencies have long struggled to find effective tools for managing these outbreaks and subsequent poor lamb recruitment in the face of uncertainty over the primary causative agents of the disease. Here, we report on the results of Montana Fish,

Wildlife, and Parks' efforts to use culling to manage a large-scale pneumonia epizootic in 2009-2010 that spanned a sizable portion of a 9-herd metapopulation.

During winter 2009-2010, all-age die-offs, defined as a loss of an unusual proportion of the population attributable to an infectious disease (WAFWA Wildlife Health Committee 2014), were detected in 4 bighorn sheep herds (Bonner, East Fork Bitterroot, Lower Rock Creek, and Upper Rock Creek) in western Montana. The Montana Department of Fish, Wildlife and Parks (MFWP) implemented three different culling strategies based on the specific circumstances for each population and the manifestation of disease in each herd. At the time of the outbreaks, uncertainty remained over the etiology and transmission ecology of the primary causative agents involved in bighorn sheep epidemic pneumonia. Management options were to either allow the outbreak to run its course or to cull individual animals with the goal of reducing within-herd transmission, protecting neighboring herds and possibly improving post-die-off lamb recruitment.

Strategies implemented were:

1) Selective culling (SC) only. This strategy involved culling any bighorn sheep seen displaying clinical signs of pneumonia, and was applied to the East Fork (EF) herd.

2) Containment zone (CZ) plus selective culling. This strategy involved culling all bighorn sheep within a delineated area (the containment zone, CZ), combined with culling of symptomatic bighorn sheep outside the CZ, and was applied to the Bonner herd.

3) Limited culling (LC). This strategy involved limited lethal removal of symptomatic animals only for the purpose of diagnostic sampling, and was applied to both the Upper Rock Creek (URC) and Lower Rock Creek (LRC) herds.

The objectives of this paper are to outline the status of these treated herds 5 years after they experienced pneumonia-associated all-age die-offs, report the status of neighboring herds, and discuss lessons learned by MWFP from these efforts. We address our hypotheses that culling infected or exposed bighorn sheep decreases the spread of pneumonia to healthy animals; that two or more pneumonia outbreaks were related; and that lamb recruitment in subsequent years can be improved by removing symptomatic animals during a pneumonia outbreak.

STUDY AREA

The bighorn sheep metapopulation discussed in this paper is in western Montana, MFWP Administrative Region 2. The metapopulation consists of 9 herds: East Fork of the Bitterroot, Bonner, Lower Rock Creek, Upper Rock Creek, Anaconda, Skalkaho, Garrison, Petty Creek, and Painted Rocks (Fig. 1). Four of these herds (Bonner, East Fork of the Bitterroot, Lower Rock Creek, Upper Rock Creek) experienced pneumonia-related all age die-offs during winter 2009-2010. At the time that these die-offs occurred, Petty Creek and Skalkaho herd counts were stable to increasing, Painted Rocks and Anaconda appeared to be stable, and the Garrison herd had been gradually declining for at least 5 years for unknown reasons (Fig. 2). The EF herd is located 5 miles southeast of Darby, Montana, in Hunting District (HD) 270 (Fig. 1). This herd was established in 1972 with the reintroduction of 54 bighorn sheep from the Sun River herd. The population grew quickly and MFWP allowed hunting in 1976. The population objective was to manage for 200 sheep +/- 20%, and population counts reached a record high of 246 bighorn sheep in 2006 (MFWP 2010:166). Lamb:ewe ratios had been relatively steady in the EF herd prior to the pneumonia outbreak (35 to 40 lambs:100 ewes in good years, and 18 to 25 lambs:100 ewes in poorer years; Fig. 3). This herd had been used as a source herd for translocations in 2002, 2004, and 2007 (Fig.

2). During spring trend surveys in April 2009, MFWP obtained a count of only 187 bighorn sheep, substantially lower than the record high count of 246 in March 2006 (Fig. 2). Lamb:ewe ratio in April 2009 was 39 lambs:100 ewes (Fig. 3).

The Bonner herd is located northeast of Missoula, Montana, in HD 283 (Fig. 1). In 1987 MFWP released 14 bighorn sheep from URC to historic bighorn habitat on Woody Mountain, and in 1990 added 30 bighorn sheep from Montana's Sun River herd (MFWP 2010). Bighorn sheep soon became well established in suitable habitat near the community of Bonner. The population objective for the Bonner herd was 100 bighorn sheep (+/- 10%) prior to the pneumonia outbreak of 2009-10 (MFWP 2010:171). Survey counts were as low as 35 animals in 1991 but steadily increased to 128 in 2007 (Edwards et al. 2010; Fig. 2). After 2007 the population trend and lamb:ewe ratio declined (Fig. 3). In the May 2009 survey, 94 bighorn sheep were counted, and the recruitment ratio was 30 lambs:100 ewes. At that time, the low lamb:ewe ratio was suspected to be the result of survey difficulty. This was supported by the fact that lamb:ewe ratio was high again in spring 2010 (Fig. 3). The Bonner herd had been used as a source herd four times since 1997, with the most recent capture and transplant of apparently healthy animals out of the herd in 2007. Another transplant was being considered just prior to the detection of the pneumonia outbreak in 2010 (Edwards et al. 2010).

The LRC herd is located about 20 miles southeast of Missoula, in HD 210 (Fig. 1). MFWP introduced 25 bighorn sheep to LRC from Wild Horse Island in 1979 and 28 bighorn sheep from Lost Creek (near Anaconda, Montana) in 1987 (MFWP 2010). The herd grew quickly, and was used as a source herd in 1997. The population objective for the LRC herd was 200 (+/- 20%) prior to detection of the 2009-10 pneumonia outbreak (MFWP 2010). In 2004, the lamb:ewe ratio reached a recorded high of 65 lambs:100 ewes, but declined to less than half that in 2005-2006 (Fig. 3). A low herd count of 103 bighorn sheep in 2009 was believed at the time to be an anomaly due to late survey date and poor visibility of animals (Edwards et al. 2010); however, we cannot be sure that this was not the result of disease introduction to the herd. LRC was used as a source herd again in 2007, after which the lamb:ewe ratio increased to over 50 lambs:100 ewes by 2009 (Fig. 3).

The URC herd is located about 10 miles west of Philipsburg, Montana, in HD 216 (Fig. 1). It is a native population that suffered a die-off in 1967 which caused a decline from approximately 200 sheep to only 15. Lamb recruitment was very low for years afterward (Berwick 1968, Edwards et al. 2010). In 1975 the population was augmented with 31 bighorn sheep from the Sun River herd. The population gradually increased over the subsequent 3 decades to reach a high of 347 sheep in 2007 (MWFP 2010:151). The URC herd was used as a source herd twice since 1985. The population objective was 300 bighorn sheep (+/- 20%), and the herd numbered approximately 350 animals in 2008 and 2009 (MWFP 2010:152). Lamb:ewe ratios averaged 43:100 from 1990-2009, but declined from 2007 to 2009 (Fig. 3). In 2009 during the last survey prior to detection of the pneumonia outbreak, the lamb:ewe ratio had declined to 32 lambs:100 ewes (Edwards et al. 2010).

Neighboring herds

The Anaconda herd (HD 213) is located northeast of EF and southeast of URC (Figs. 1, 4), and immediately west of the town of Anaconda, Montana. The herd was established with a transplant of 25 bighorn sheep from the Sun River herd (MFWP 2010). The herd grew, and was used as a source herd for transplants until a pneumonia outbreak occurred in 1991. The population rebounded

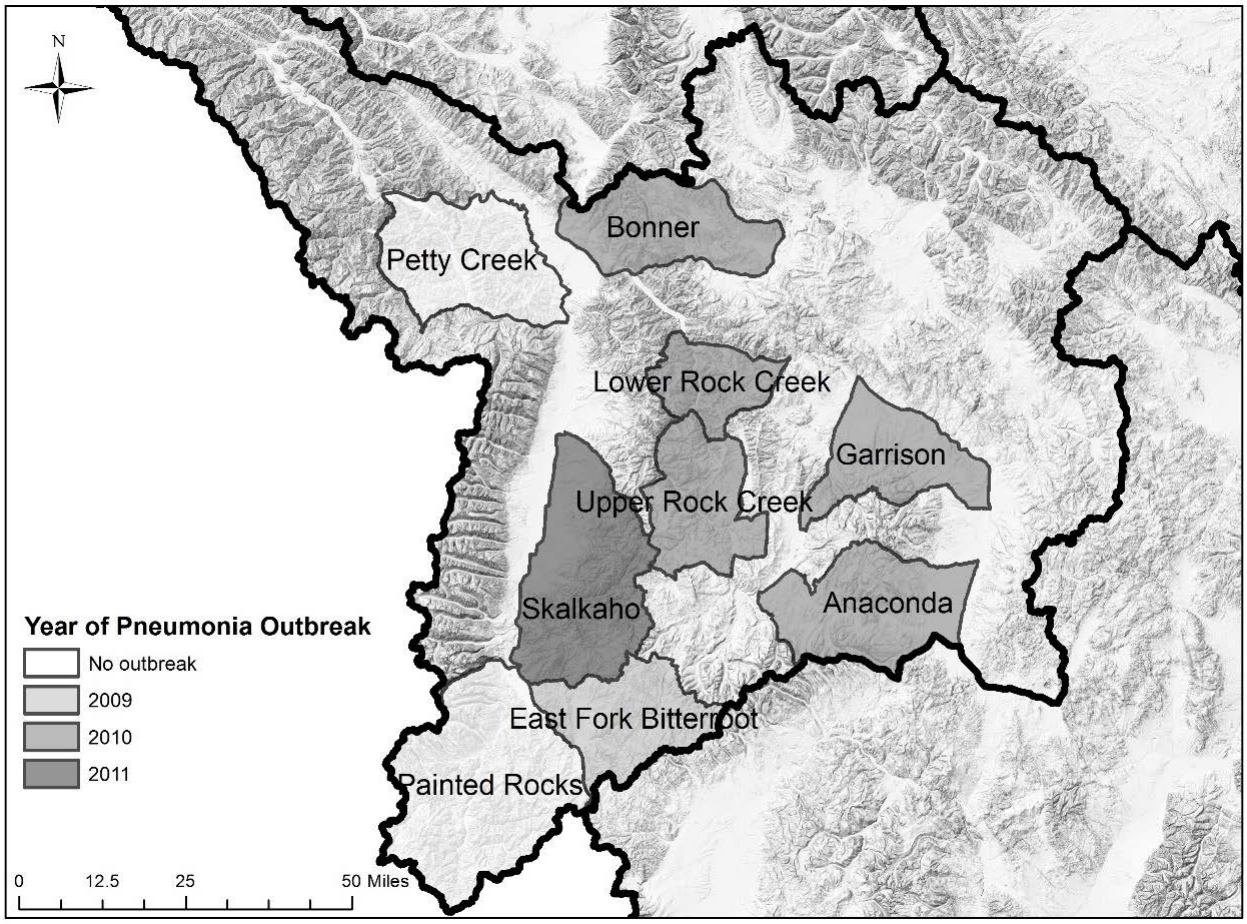

Figure 1. Map of bighorn sheep herds with pneumonia outbreaks during winter 2009-2011 and neighboring populations in Montana Fish, Wildlife and Parks, Administrative Region 2, western Montana.

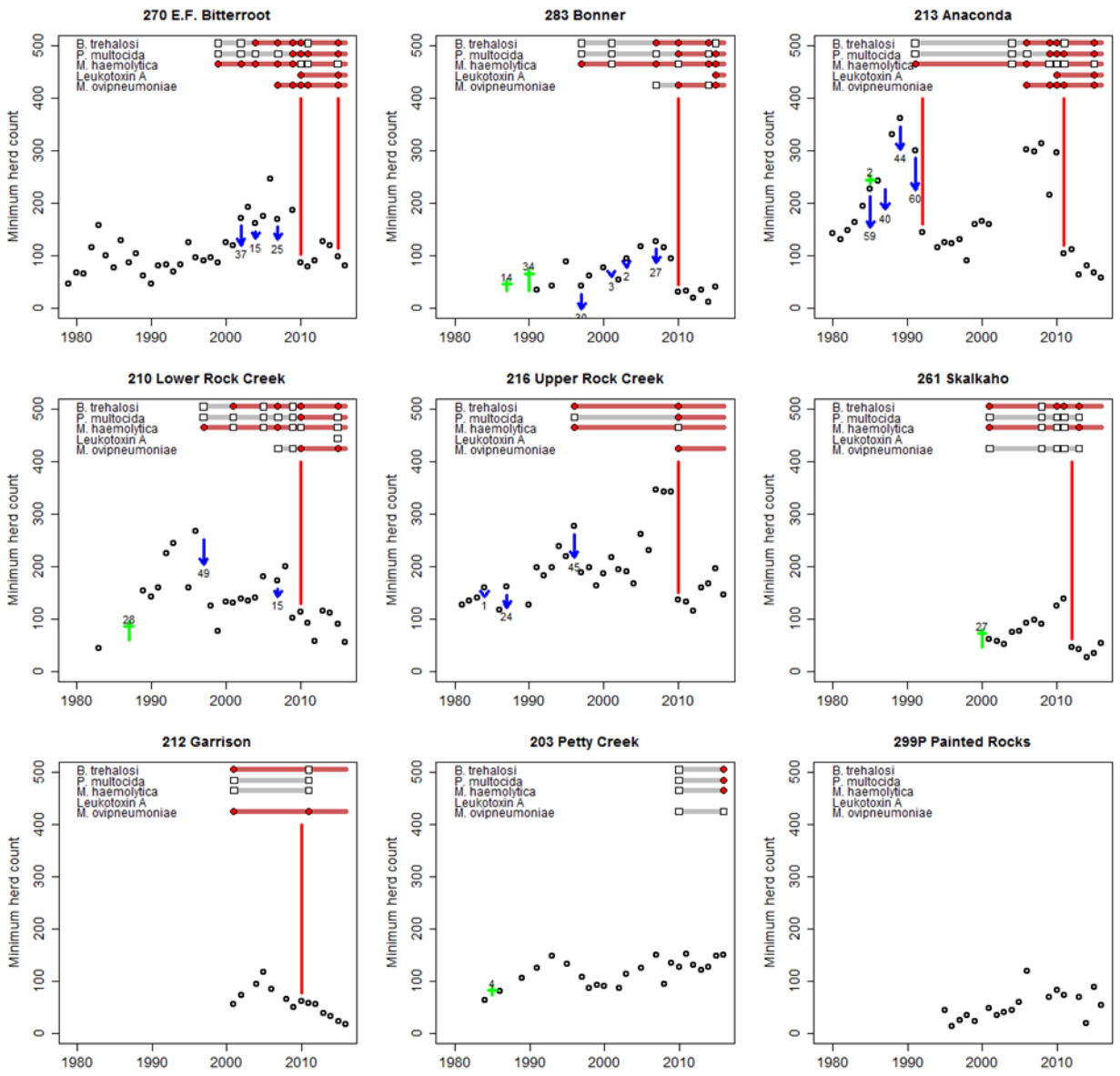

Figure 2. Plots of population size over time for 9 bighorn sheep herds within Montana Fish, Wildlife and Parks, Administrative Region 2, western Montana. Red vertical lines indicate the year of an observed all-age pneumonia die-off. Blue arrows indicate translocations out of the herd with the number of animals moved noted. Green vertical lines with a plus sign at the top indicate translocations into the herd, with the number of animals noted. Pathogen presence data is displayed at the top for *Bibersteinia trehalosi*, *Pasteurella multocida*, *Mannheimia haemolytica*, Leukotoxin A, and *Mycoplasma ovipneumoniae*. Red circles and white squares indicate detections or lack thereof, respectively, for each pathogen.

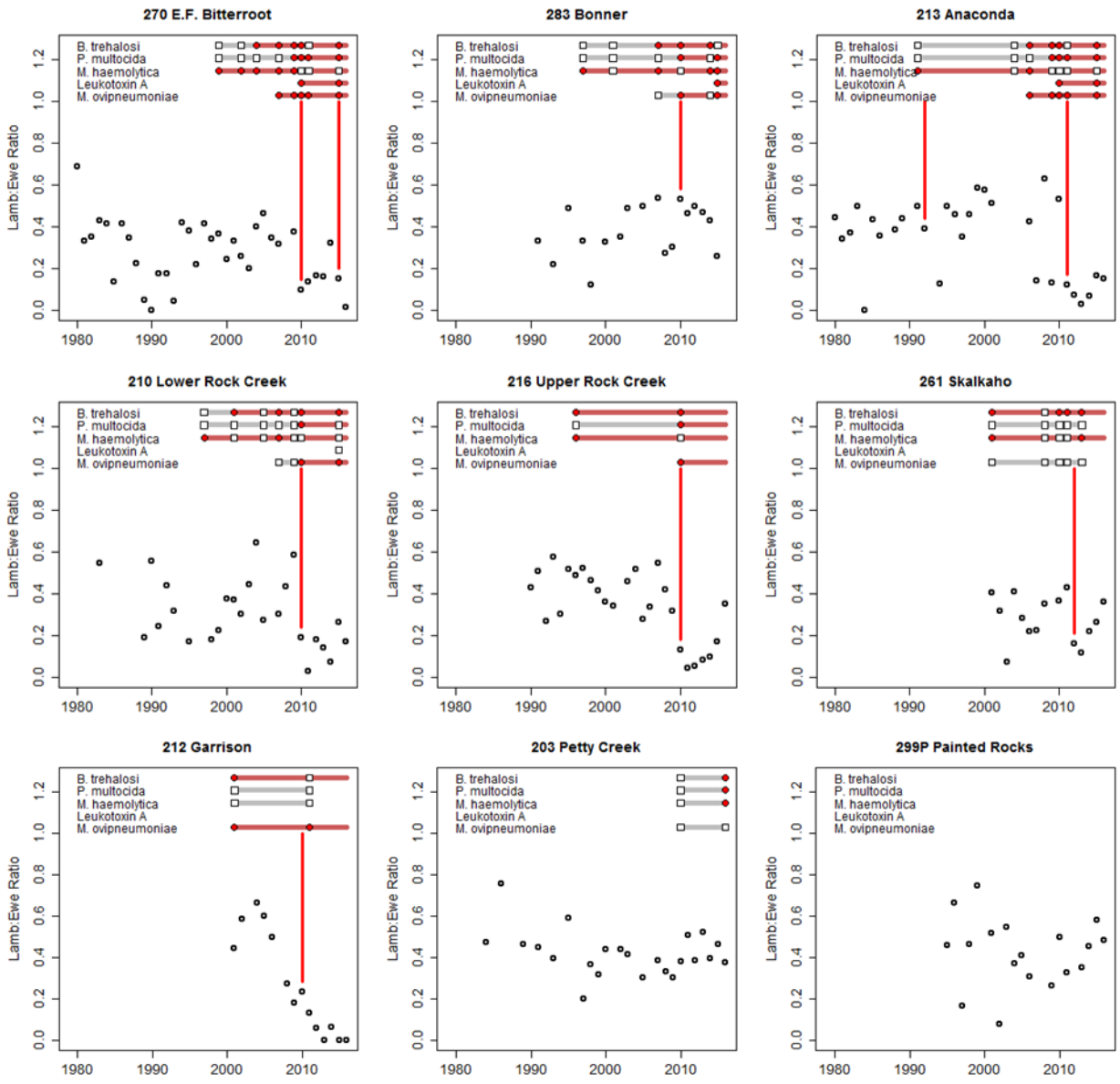

Figure 3. Plots of lambs per 100 ewes over time for 9 bighorn sheep herds within Montana Fish, Wildlife and Parks, Administrative Region 2, western Montana. Red vertical lines indicate the year of an observed all-age pneumonia die-off. Pathogen presence data is displayed at the top for *Bibersteinia trehalosi*, *Pasteurella multocida*, *Mannheimia haemolytica*, Leukotoxin A, and *Mycoplasma ovipneumoniae*. Red circles and white squares indicate detections or lack thereof, respectively, for each pathogen.

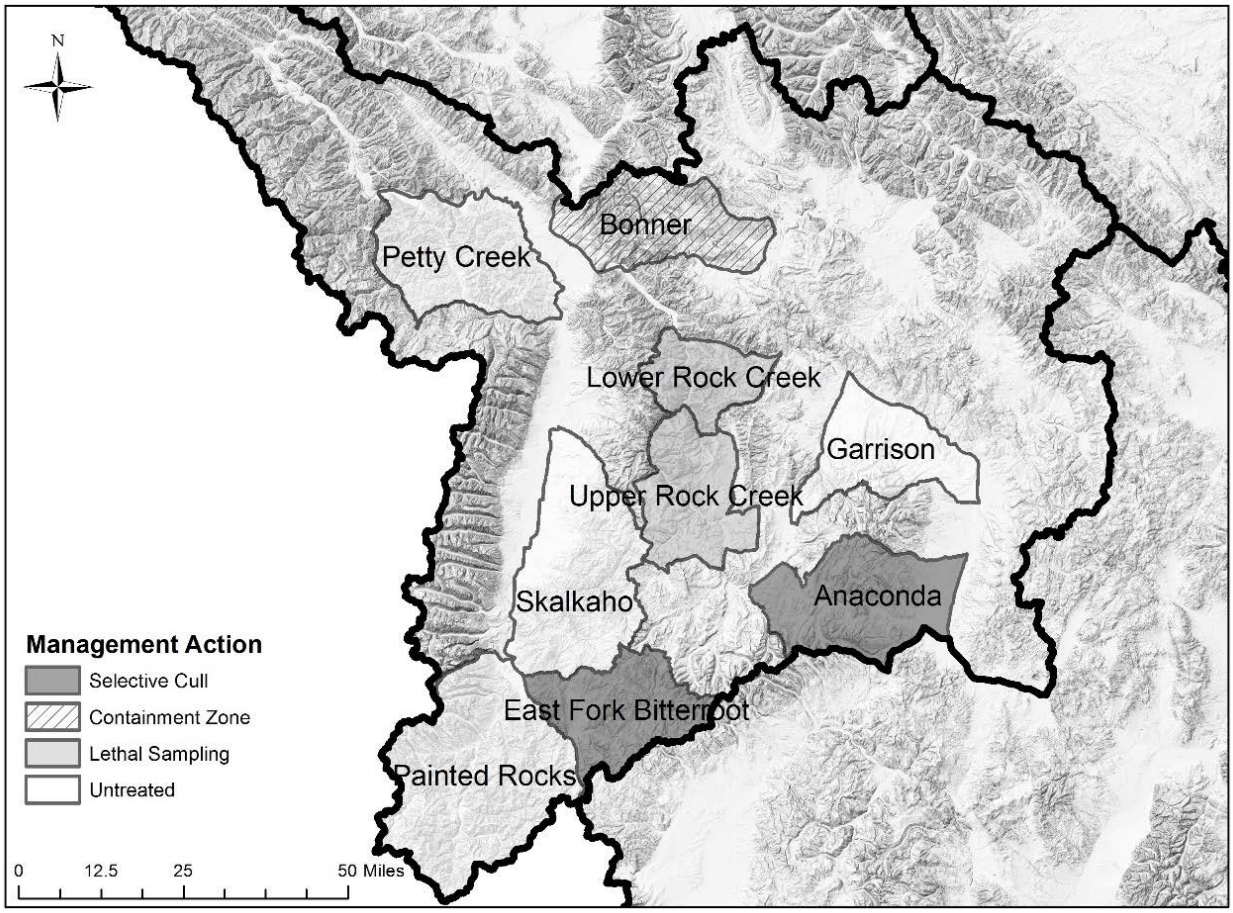

Figure 4. Map of bighorn sheep herds and the corresponding management action applied during the 2009-2010 pneumonia outbreak in Montana Fish, Wildlife and Parks, Administrative Region 2, western Montana. A selective culling effort was initiated in Anaconda, but was abandoned because the pneumonia outbreak was deemed too pervasive for the effort to result in an improved outcome.

and appeared to be stable in winter 2010 when the pneumonia outbreaks discussed in this paper were detected.

The Skalkaho herd (HD 261) is located directly north of EF and west of URC. Although the area is historic bighorn sheep habitat, no bighorn sheep had been documented there in recent years until two ewes were observed there in 1973, one year after a reintroduction of bighorn sheep into the EF (MFWP 2010). By 1999, approximately 36 bighorn sheep were present in the Skalkaho, and the herd was augmented with 27 bighorn sheep from the Sun River herd (MFWP 2010). Herd counts were increasing when the pneumonia outbreaks were detected in herds discussed in this paper (Fig. 2).

Garrison (HD 212) is a neighboring herd located directly east of URC and LRC (Fig. 1). Bighorn sheep were first documented in Garrison in the early 1980's. The herd is presumed to have been established by bighorn sheep from the Anaconda herd colonizing new habitat (MFWP 2010). Surveys of this herd have revealed declining numbers of bighorn sheep since 2005 (Fig. 2). The decline in observed bighorn sheep by nearly 50% in the Garrison herd from 2005 to 2008 was attributed to lack of detection during aerial surveys rather than to a true population decline by MFWP (2010). The Garrison herd is difficult to access and survey due to terrain with extensive cover and difficult land ownership issues.

The Petty Creek herd (HD 203) is the nearest herd to Bonner. The herd was established in 1968 with 16 bighorn sheep from the Sun River herd, and was augmented in 1985 with four rams from the National Bison Range (MFWP 2010). A population decline in 1997 was attributed to a severe winter. The herd recovered within 8 years and was stable at the time of detection of the pneumonia outbreaks discussed in this paper (Fig. 2).

The Painted Rocks herd (HD 299) is located south of the EF herd (Fig. 1). Thirty-eight bighorn sheep from the Sun River herd were transplanted to Painted Rocks in 1990, and 28 from Anaconda in 1991 (MFWP 2010). After several years of poor lamb survival, the herd was augmented with 10 bighorn sheep from Sun River in 2004. The Painted Rocks herd trajectory appeared to be stable when the 2009-2010 pneumonia outbreaks discussed herein were detected (Fig. 2).

Detailed descriptions of each study area and neighboring herd, as well as additional details of earlier disease outbreaks and translocations, are provided by MFWP (2010).

METHODS

The critical components of the SC efforts were to remove all bighorn sheep showing clinical symptoms of infection and obtain quality diagnostic samples from culled sheep (Edwards et al. 2010). We implemented this strategy under the assumption that infectiousness and clinical symptoms of disease were linked factors, and that pathogen transmission might be slowed by removing symptomatic animals from the herd, thereby minimizing the potential for contact and disease transmission between infected and healthy animals. We culled a small number of asymptomatic bighorn sheep to determine whether all infected animals displayed obvious clinical symptoms. Bighorn sheep that were not obviously symptomatic were often watched for extended periods of time and sometimes pushed uphill in an attempt to produce visible symptoms such as coughing or lagging behind the group (Edwards et al. 2010).

In Bonner, where the CZ selective culling outside the CZ approach was adopted, bighorn sheep within the CZ were not watched for extended periods of time or stressed to try to demonstrate symptoms prior to culling. MFWP personnel selectively culled symptomatic bighorn sheep outside the CZ (Edwards et al. 2010) to contain the pneumonia outbreak to a small portion of the hunting district. The CZ was a dynamic boundary based on topography, known bighorn movement, and flight data, and was designed to encompass the core affected area plus the area over which dispersing bighorn sheep from the core area might contact healthy bighorn sheep (Edwards et al. 2010). Our underlying assumption was that by creating a containment zone and testing the boundary through selective culling, we would eliminate all infectious animals as well as prevent transmission to neighboring herds.

Our primary goals for LC were to confirm that a pneumonia outbreak was taking place and to collect quality biological samples for respiratory disease sampling. In LRC, the terrain is very steep and was treacherous at the time of the outbreak due to snow conditions. MFWP made the decision to allow the disease outbreak to run its course (Edwards et al. 2010). We removed LRC bighorn sheep from private and public land primarily in the Babcock Mountain area where animals were most visible and accessible. The high percentage of symptomatic animals in URC, the continuous distribution of the herd, and landowner opposition to culling resulted in the decision to apply only limited culling to collect biological samples for testing in that herd (Edwards et al. 2010).

MFWP biologists conducted herd composition surveys annually or biennially to estimate population numbers. Due to scheduling and other difficulties, surveys were not always conducted at the same time of year for each herd. We surveyed the EFB, URC, LRC, Garrison and Anaconda herds primarily from the air via helicopter or fixed-wing aircraft in early spring, with most surveys occurring in March and April (MFWP 2010). The Skalkaho herd was surveyed via helicopter twice per year, once in late December or early January, and again in April (MFWP 2010). The Bonner and Petty Creek herds were surveyed biennially via helicopter (MFWP 2010). At Bonner and Petty Creek, bighorn sheep were counted and classified by age and sex, and rams were assigned by horn development to yearling, sub-adult (1/2 to ¾-curl), or adult (greater than ¾-curl) classifications (MFWP 2010). For all other surveyed herds, bighorn sheep were classified by age and sex, and rams were classified based on horn development as Class I, II, III, or IV (Geist 1971; MFWP 2010). For the purposes of this paper, we refer to the year in which elevated disease-induced mortality was first detected as the year of the outbreak, although in some cases we present evidence that suggests the outbreak may have begun prior to the year it was detected.

Prior to the die-offs in winter 2009-2010, biological samples were primarily collected from live sheep during capture efforts associated with translocation. Live animal samples included blood, nasal swabs, pharyngeal or tonsil swabs, and fecal samples. During culling efforts, most carcasses were brought to a central processing site for necropsy and sample collection shortly after death. Bighorn sheep that were found dead were either brought to the MFWP Wildlife Lab in Bozeman for necropsy and sample collection, or were necropsied and sampled in the field by trained wildlife biologists. Samples from carcasses included blood, lung, liver, nasal swabs, fecal samples, and parasites.

We focus on bacteria that have previously been identified as playing a role in bighorn sheep pneumonia: *Mycoplasma ovipneumoniae*, *Mannheimia haemolytica*, *Bibersteinia trehalosi*, *Pasteurella multocida*,

and *Pasteurellaceae* containing the Leukotoxin A gene. During the outbreaks, culture of nasal swabs and lung tissue for *M. ovipneumoniae* and aerobic cultures of lung tissue were conducted at the Washington Animal Disease Diagnostic Laboratory (WADDL). Polymerase Chain Reaction (PCR) and serological testing for *M. ovipneumoniae* were not commercially available at that time. In 2009, banked serum samples from the EF, Bonner, and LRC outbreaks were submitted to WADDL for cELISA serological testing (Edwards et al. 2010). Beginning in November 2009, lung samples from bighorn sheep found dead or euthanized in Montana have been submitted to WADDL for *M. ovipneumoniae* PCR testing. An experimental PCR test for *M. haemolytica* was performed on a subset of samples from the EF herd during the outbreak by Dr. Srikumaran Subramaniam's lab at Washington State University (Shanthalingam et al. 2014). A detailed summary of parasitology, trace minerals, and respiratory pathogen testing during the die-offs is presented in Edwards et al. (2010).

RESULTS

East Fork of the Bitterroot (EF) — selective culling (SC)

The outbreak in the EF herd was detected in November 2009 when 2 rams found dead near U.S. Highway 93 were confirmed to have pneumonia (Edwards et al. 2010). Herd counts in EF increased in 2012 and 2013 following the SC effort (Fig. 2), and lamb:ewe ratio had been stable to slightly increasing in those years (Fig. 3).

Respiratory pathogen presence over time is displayed in Figs 2 and 3. *M. haemolytica* and *B. trehalosi* had been detected by culture in the EF herd years before the pneumonia outbreak occurred (Fig. 2, Tables 2 and 4). Leukotoxin testing and *M. ovipneumoniae* PCR had not been available when early captures occurred, and the presence of these agents was unknown. The last capture and sampling of apparently healthy animals for transplant from EF occurred in 2007. *M. ovipneumoniae* was not detected by culture from animals captured in EF in 2007; however, 19 of 25 banked serum samples that had been collected in 2007 were later determined to be seropositive for *M. ovipneumoniae*.

During the die-off, *M. ovipneumoniae* was detected by culture and PCR in 60% of lung samples from the EF herd. We also detected *M. haemolytica*, *B. trehalosi*, and *P. multocida* in lung samples from EF bighorn sheep during the outbreak (Tables 1, 2, 4, and 5). The experimental PCR test detected *M. haemolytica* in 30/31 bighorn sheep lung samples from EF during the die-off (Shanthalingam et al. 2014). One of these *M. haemolytica* positive lung samples was tested for the presence of the leukotoxin gene and was positive. The experimental test also detected the leukotoxin gene from *M. haemolytica* isolates that were obtained by culture from lungs of 3 additional EF bighorn sheep that were euthanized during the outbreak.

We detected a subsequent pneumonia outbreak in EF in 2015. A spring 2016 survey detected 81 bighorn sheep in EF and only one lamb (Fig. 2), although MFWP received a few anecdotal reports from residents of 2-3 lambs in the population. *M. ovipneumoniae*, Leukotoxin A, *B. trehalosi*, and *P. multocida* were all detected in pneumonic EF bighorn sheep in 2015 (Tables 1, 3, 4, and 5); however, sample size in 2015 was too small to estimate prevalence of any pathogens.

Bonner — Containment zone and selective culling (CZ)

The pneumonia outbreak in Bonner was detected in January 2010 when a coughing bighorn sheep was reported by a resident in the West Riverside community. The outbreak appeared to be localized to the highly visible segment of the population in

this densely-developed area (Edwards et al. 2010). Thirty bighorn sheep were counted in April 2010 trend surveys. By June 2010, we counted only 12 bighorn sheep in Bonner. Two surviving lambs were documented in August 2010 (Edwards et al. 2010). The Bonner herd count has remained low since the outbreak, ranging from 12 to 40 animals, with small fluctuations from year to year (Fig. 2). Lamb: ewe ratios appear to be decreasing since the outbreak (Fig. 3), but lamb numbers have been low (3-8 lambs), and trend is difficult to evaluate with such a small herd size.

We detected both *M. haemolytica* and *B. trehalosi* in Bonner prior to the pneumonia outbreak in 2010 (Tables 2 and 4). *M. ovipneumoniae* was not detected by culture from animals captured in Bonner in 2007; however, detection of *M. ovipneumoniae* by culture has been shown to be problematic (Yang et al. 2014). We detected no evidence of exposure to *M. ovipneumoniae* in banked serum samples from animals captured in 2007 that were tested by cELISA at WADDL in 2009. *M. ovipneumoniae* was detected by PCR in 92% of lung samples during the die- off (Table 1). *B. trehalosi* and *P. multocida* were also detected by culture from lung samples during the outbreak (Tables 4 and 5). Lung samples have been collected from a few animals that have been hit by automobiles or found dead since the outbreak, and *M. ovipneumoniae*, *M. haemolytica*, *B. trehalosi*, and *P. multocida* have remained present in this herd.

Lower Rock Creek (LRC) — limited culling (LC)

The first confirmed case of pneumonia in the LRC herd was detected on January 22, 2010 when a coughing ewe was euthanized and necropsied. MFWP biologists conducted spring trend counts on March 23, 2010. At that time, the lamb: ewe ratio was 19 lambs:100 ewes, and a population decline of 43% was estimated (Edwards et al. 2010). Culled animals represented 21.8% of the observed population decline (Edwards et al. 2010). By May 2010, MFWP biologists documented 29 lambs:100 ewes (4:14). No lambs were detected in August 2010 during ground surveys (Edwards et al. 2010), although a public report of 4 lambs in late August suggests that a few lambs may have gone undetected in prior surveys (Edwards et al. 2010). Herd counts declined through spring 2012 (Fig. 2), but the herd did not die out. Just over 100 bighorn sheep were observed in 2013 and 2014 (Fig. 2), which is approximately the number of animals present in the herd at the time the disease outbreak was detected. Lamb: ewe ratios have been variable since the outbreak, varying from 3 lambs:100 ewes (2:67 in 2011) to 26 lambs:100 ewes (15:57 in 2015; Fig. 3).

We did not detect *M. ovipneumoniae* by culture in healthy bighorn captured in the LRC herd in 2007, and exposure to *M. ovipneumoniae* was not detected by cELISA when banked serum from was tested in 2009 (Table 1). *M. haemolytica* and *B. trehalosi* were both detected by culture of pharyngeal swabs in 2007 (Tables 2 and 4).

We detected *M. ovipneumoniae* by PCR on lung tissue during the outbreak in the LRC herd, and *B. trehalosi* and *P. multocida* were detected by aerobic culture (Tables 1, 4, and 5). Samples have been collected from only 3 animals in the LRC herd since the pneumonia outbreak. *M. ovipneumoniae and B. trehalosi* were detected (Tables 1 and 4). *M. haemolytica* was detected in both of the 2 samples in the experimental PRC test (Shanthalingam et al. 2014).

Upper Rock Creek (URC) — limited culling (LC)

The pneumonia outbreak was detected in the URC herd on January 29, 2010 when 3 bighorn sheep were euthanized and necropsied the day after a coughing bighorn sheep was reported to MFWP (Edwards et al. 2010). In February 2010, 174 bighorn sheep were observed during aerial survey, and 45% of

those appeared to be showing signs of respiratory impairment or distress. During aerial survey on March 23, 2010 we observed only 136 bighorn sheep and 13 lambs:100 ewes (Edwards et al. 2010; Fig. 3). Removal of 28 bighorn sheep from the URC herd accounted for 13.6% of the 60% decline in population detected during 2010 aerial survey. Adult animals continued dying in spring and summer, and no surviving lambs were documented in August 2010 (Edwards et al. 2010). Although bighorn sheep numbers remained lower than numbers prior to the pneumonia outbreak (the highest count was 197 in 2015), the URC herd has experienced a gradual increase in both population counts and lamb: ewe ratios since 2010 (Figs. 2 and 3).

Baseline health data was not collected for the URC herd prior to the pneumonia outbreak. We detected *M. ovipneumoniae* by PCR in 100% of lung samples and 93% of pharyngeal swabs collected from URC bighorn sheep during the outbreak (Table 1). *B. trehalosi* was detected by aerobic culture in 18% of samples from the URC herd, and *P. multocida* was detected in 96% of samples (Tables 4 and 5). No samples have been collected from bighorn sheep in the URC herd since the pneumonia outbreak.

Neighboring herds- Anaconda, Garrison, Skalkaho, Petty Creek, Painted Rocks

A pneumonia outbreak was confirmed in Anaconda in August 2010. An aggressive effort to cull symptomatic animals began on August 27, 2010 and proceeded through November 3, 2010, when MFWP concluded that the outbreak was already too pervasive for our effort to result in an improved outcome, and decided to let it continue to run its course. The Anaconda herd has declined since 2010 (Fig. 2). Lamb:ewe ratios have been very low (< 3:100) in some years, and have remained below 16 lambs:100 ewes (Fig. 3).

We detected *M. ovipneumoniae, M. haemolytica, B. trehalosi,* and *P. multocida* in Anaconda prior to 2010 detection of the pneumonia outbreak in this herd (Tables 1, 2, 4, and 5). Testing for Leukotoxin A was not routinely being done at that time. However, leukotoxin A was detected from bighorn sheep that were culled in Anaconda during the pneumonia outbreak (Table 3). *M. ovipneumoniae, M. haemolytica, B. trehalosi,* and *P. multocida* were also detected during the outbreak (Tables 1, 2, 4, and 5). All of these pathogens except *M. haemolytica* have been detected in samples from the small number of bighorn sheep that have been sampled from Anaconda since the outbreak.

A pneumonia outbreak was detected in the Skalkaho herd in 2011. We decided to allow the outbreak to run its course because the disease appeared to be prevalent within the herd and both neighboring herds had already experienced a die-off. Surveys of the Skalkaho herd in 2012 revealed a sharp decline in herd count and lamb:ewe ratio with no evidence of recovery (Figs. 2 and 3). The herd declined from 139 animals in 2011 to only 54 counted in 2016 (Fig. 2).

M. haemolytica and *B. trehalosi* were detected in the Skalkaho herd prior to the pneumonia outbreak (Tables 2 and 4). Samples have only been collected from two animals from the Skalkaho herd since the outbreak was first detected. Test results confirm that *M. haemolytica* and *B. trehalosi* remain present in the herd, but because of the small samples size and the low probability of detection of Pasteurellaceae by aerobic culture (Walsh et al. 2012, Butler 2017), we are not certain of the *M. ovipneumoniae* status of the herd or the prevalence of other pathogens of concern.

The Garrison herd experienced a decline in numbers from 2005-2009 (Fig. 2). Another decline was noticed in 2011, after a slight increase in herd count was observed in 2010 (Fig. 2). In January 2011, the MFWP biologist observed several bighorn sheep coughing in Garrison. One coughing yearling

Table 1. Prevalence and sample size (in parentheses) for *Mycoplasma ovipneumoniae* prior to, during, and after the detected outbreak. Animals were considered positive if *M. ovipneumoniae* was detected by either culture or PCR, or if exposure to *M. ovipneumoniae* was detected by cELISA on at least one sample.

			***M. ovipneumoniae* Culture, PCR, and Serology**		
Herd	**Herd Name**	**Outbreak Year**	**Pre**	**Outbreak**	**Post**
270	EF	2009 & 2010	0.76 (25)	0.61 (70)	0.88 (8)
283	Bonner	2010	0 (27)	0.92 (91)	0.33 (3)
213	Anaconda	2010	0.43 (21)	0.71 (45)	0.42 (12)
210	LRC	2010	0 (16)	0.61 (18)	0.67 (3)
216	URC	2010	-	1.00 (29)	-
261	Skalkaho	2011	0 (18)	0 (1)	0 (1)
212	Garrison	2010	0.8 (5)	-	0.5 (2)
203	Petty Creek	NA	0 (18)	NA	NA
299	Painted Rocks	NA	-	NA	NA

Table 2. Prevalence and sample size (in parentheses) for *Mannheimia haemolytica* prior to, during, and after the detected outbreak. Animals were considered positive if *M. haemolytica* was isolated by culture on at least one sample.

			***Mannheimia haemolytica* Culture**		
Herd	**Herd Name**	**Outbreak Year**	**Pre**	**Outbreak**	**Post**
270	EFB	2009 & 2010	0.29 (98)	0.11 (65)	0 (9)
283	Bonner	2010	0.3 (61)	0 (35)	0.67 (3)
210	LRC	2010	0.16 (55)	0 (16)	0 (3)
216	URC	2010	0.09 (45)	0 (28)	-
213	Anaconda	2010	0.22 (45)	0 (46)	0 (8)
261	Skalkaho	2011	0.05 (20)	0 (1)	1 (1)
212	Garrison	2010	0 (8)	-	0 (2)
203	Petty Creek	NA	0 (18)	NA	NA
299	Painted Rocks	NA	-	NA	NA

Table 3. Prevalence and sample size (in parentheses) for Leukotoxin A prior to, during, and after the detected outbreak. Animals were considered positive if Leukotoxin A was detected by PCR on at least one sample.

			Leukotoxin A PCR		
Herd	**Herd Name**	**Outbreak Year**	**Pre**	**Outbreak**	**Post**
270	EFB	2009 & 2010	-	1 (1)	1 (3)
283	Bonner	2010	-	-	1 (1)
210	LRC	2010	-	-	0 (3)
216	URC	2010	-	-	-
213	Anaconda	2010	-	1 (12)	0.33 (6)
261	Skalkaho	2011	-	-	-
212	Garrison	2010	-	-	-
203	Petty Creek	NA	1 (4)	NA	NA
299	Painted Rocks	NA	-	NA	NA

Table 4. Prevalence and sample size (in parentheses) for *Bibersteinia trehalosi* prior to, during, and after the detected outbreak. Animals were considered positive if *B. trehalosi* was isolated by culture on at least one sample.

			***Bibersteinia trehalosi* Culture**		
Herd	**Herd Name**	**Outbreak Year**	**Pre**	**Outbreak**	**Post**
270	EFB	2009 & 2010	0.3 (98)	0.55 (65)	0.56 (9)
283	Bonner	2010	0.41 (61)	0.4 (35)	0.33 (3)
210	LRC	2010	0.33 (55)	0.13 (16)	0.67 (3)
216	URC	2010	0.02 (45)	0.18 (28)	-
213	Anaconda	2010	0.44 (45)	0.13 (46)	0.5 (8)
261	Skalkaho	2011	0.4 (20)	1 (1)	1 (1)
212	Garrison	2010	0.88 (8)	-	0 (2)
203	Petty Creek	NA	0.94 (18)	NA	NA
299	Painted Rocks	NA	-	NA	NA

Table 5. Prevalence and sample size (in parentheses) for *Pasteurella multocida* prior to, during, and after the detected outbreak. Animals were considered positive if *P. multocida* was isolated by culture on at least one sample.

			***Pasteurella multocida* Culture**		
Herd	**Herd Name**	**Outbreak Year**	**Pre**	**Outbreak**	**Post**
270	EFB	2009 & 2010	0 (98)	0.63 (65)	0.78 (9)
283	Bonner	2010	0 (61)	0.06 (35)	0.33 (3)
210	LRC	2010	0 (55)	0.88 (16)	0 (3)
216	URC	2010	0 (45)	0.96 (28)	-
213	Anaconda	2010	0.02 (45)	0.22 (46)	0.25 (8)
261	Skalkaho	2011	0 (20)	0 (1)	0 (1)
212	Garrison	2010	0 (8)	-	0 (2)
203	Petty Creek	NA	0 (18)	NA	NA
299	Painted Rocks	NA	-	NA	NA

ewe was euthanized, and pneumonia was confirmed at necropsy. The herd has continued to decline, dropping from 62 bighorn sheep in 2010 to only 18 bighorn sheep counted in 2016 (Fig. 2). Lamb:ewe ratio has also declined. One lamb was observed in 2014, but none were found on surveys in 2013, 2015, and 2016 (Fig. 3). We detected *M. ovipneumoniae* by PCR of a lung sample from the ewe that was euthanized in January 2011, but not from the one other bighorn sheep that was tested from the herd that year (Table 1). We do not know whether *Pasteurellaceae* or Leukotoxin A are present in this herd. Sample sizes are very small, and most samples were collected in 2001 prior to availability of PCR and *M. ovipneumoniae* cELISA.

The Petty Creek herd appears to have been unaffected by the 2009-2010 pneumonia outbreak. The herd continues to have steady population counts since 2010 (average 137 bighorn sheep) and lamb:ewe ratios ranging from 37-52 lambs:100 ewes (Figs 2 and 3). The Petty Creek herd is believed to be relatively isolated from Bonner and the other herds that suffered pneumonia outbreaks in 2009-10. We have never detected *M. ovipneumoniae*, *M. haemolytica*, or *P. multocida* in Petty Creek (Table 1). *B. trehalosi* was detected by culture in 1 of 17 tonsil swabs collected during capture in 2016. Current survey and testing data are not available for the Painted Rocks herd. Parasitology, trace minerals, and respiratory pathogen test results from the EF, Bonner, URC and LRC herds during the pneumonia outbreaks in 2009-2010 are provided by Edwards et al. (2010)

DISCUSSION

During the pneumonia outbreaks of 2009-2010, MFWP hypothesized that culling infected or exposed bighorn sheep would decrease the spread of pneumonia to healthy animals within the herd or to healthy neighboring herds. We do not know whether the treatments we applied slowed the spread of pneumonia to healthy animals within the herd. We learned from this experience that the culling strategies we employed were unlikely to stop within-herd transmission because individuals may be infectious even when they are not clearly symptomatic. In the SC effort in EF, some animals that appeared healthy were culled in an effort to determine whether infected animals could be identified. Lung lesions and the full suite of potential respiratory pathogens were detected in samples from some of these asymptomatic animals, while one animal that was euthanized due to coughing had lungs that appeared normal on necropsy and none of the bacteria cultured from the lung were those commonly associated with respiratory disease. Similarly, in the Bonner CZ culling effort, lung lesions consistent with pneumonia were detected in animals that did not show obvious symptoms of disease.

Regardless of which treatment was applied upon detection of a pneumonia event, each of the four herds proceeded to experience an all age die-off. The Bonner and LRC herds have shown no evidence of rebounding since the outbreak and culling efforts. In the EF herd, population trajectory and lamb:ewe ratios seemed to improve in the first 3 years following the outbreak and SC; however, the population trajectory began decreasing in 2014, and in May 2015, another pneumonia outbreak was detected. A small number of symptomatic animals were euthanized and sampled, confirming the continued presence of respiratory pathogens.

Occurrence of a pneumonia outbreak in EF 5 years after the initial event suggests that infected animals remained on the landscape even with very aggressive culling. URC, which was not aggressively culled, experienced increasing lamb:ewe ratios during 2010-2016, and an increasing population trajectory. We do not know whether this is evidence of a true population rebound, and, if so, whether the treatment applied to the herd during the outbreak may have contributed to a better outcome. The population dynamics of URC over the next 5 years will be of great interest to see whether this upward trend continues.

MFWP suspected that two or more of the pneumonia outbreaks that occurred in MFWP Region 2 were related. Prior to detection of pneumonia, we were aware of connectivity between the EF and Skalkaho herds because bighorn sheep showed up in Skalkaho one year after sheep were transplanted into EF in 1972 (MFWP 2010). Bighorn sheep had not previously been present in Skalkaho (Edwards et al. 2010:34). The fact that the Skalkaho herd was increasing through the 2011 spring survey but declined drastically due to a pneumonia outbreak one year after the outbreak was detected in EF likely indicates that SC in the EF herd did not prevent spread of disease to this neighboring herd.

A connection between the LRC and the Bonner herd had been suspected because bighorn sheep were found in the Bearmouth area (situated between LRC and Bonner) one year following a transplant of bighorn sheep into LRC. Connectivity among other herds within the metapopulation were less clearly understood. Unfortunately, we are unable to evaluate the effect of rapid density reduction on transmission between herds, likely due to high connectivity among herds and detection of the outbreak after it had become well established.

The declining population trajectory in Garrison prior to the 2009-2010 pneumonia outbreaks, along with presence of *M. ovipneumoniae*, suggests that a significant disease event may have already occurred in that herd. If there is connectivity between Garrison and some of the herds that

experienced a pneumonia outbreak, it may be possible that pneumonia was present in the Garrison herd for several years prior to detection of the outbreaks in EF, Bonner, URC or LRC, and Garrison may have ultimately served as a source of infection to at least one of these herds.

The die-off across neighboring populations, synchronized in time, suggests that there may be seasonal or occasional connectivity among the herds. The detection of identical IGS strains of *M. ovipneumoniae* from a small number of animals in LRC and Anaconda suggests that this strain was transmitted among these populations by at least one infected bighorn sheep (Besser et al. 2012). We do not know the source of the initial disease outbreak, but we believe that once the outbreak ensued, it was spread among at least some herds by movement of bighorn sheep. We cannot rule out the possibility that the herds experienced a similar external trigger for disease or exposure to a source of infection from domestic sheep/goats, although these explanations seem less plausible.

We also hypothesized in 2010 that treatments we applied during the pneumonia outbreak would increase lamb recruitment in subsequent years. This hypothesis was not supported by the lamb: ewe ratios in the EF herd five years after treatment. The aggressively culled herd did not appear to have higher lamb: ewe ratios than Anaconda, URC, LRC, or Skalkaho, where a smaller number of symptomatic bighorn sheep were removed for testing purposes (Fig. 2). In the Bonner herd, which was also aggressively culled, annual lamb: ewe ratios are difficult to interpret due to the very small numbers of animals that remained in the herd; however, the trajectory for the lamb: ewe ratio since the outbreak suggested a continued decline (Fig. 2). The trajectory of the lamb: ewe ratio has been more promising for URC (Fig. 3), which was subject to only limited culling. Failure to see an increasing trend in lamb: ewe ratio for the most aggressively culled herds suggests that a small number of infectious ewes can trigger widespread recruitment failure.

Historical *M. ovipneumoniae* test results for some of these herds are unreliable because culture was the only available test in earlier years. Similarly, aerobic culture for Pasteurellaceae has been shown to have a low probability of detecting these bacteria (Butler 2017). Unfortunately, with poor detection probability and very small sample sizes from these herds since the outbreaks, we lack power to detect changes in the prevalence of *M. ovipneumoniae*, *M. haemolytica*, or other Pasteurellaceae. Testing performed since the outbreak has confirmed that these bacteria were at least still present in the four herds regardless of which response strategy was used (Tables 1-5).

CONCLUSIONS

Five years after these response strategies were applied to a pneumonia epizootic that impacted several bighorn sheep herds in western Montana, the effort is viewed by the agency as a learning experience. None of the culling response strategies that were applied seem to have successfully achieved the goals of preventing spread of pathogens and disease transmission to healthy animals within the herd or to neighboring herds, or of improving lamb recruitment and population recovery after the die-off event. A similar conclusion was reached by Bernatowicz et al. (2017) after conducting culling response efforts during bighorn sheep die-offs in Washington. There likely is a combination of reasons for failure of these culling efforts to have the desired outcome. Some of these may include late detection of disease outbreaks, difficulty in survey of animals in rugged terrain and isolated areas, inability to determine which animals are exposed/infected, presence of asymptomatic chronic carriers, and considerable connectivity of bighorn sheep herds within the metapopulation. Similar problems were faced in Washington where the

Yakima Canyon herd experienced a pneumonia outbreak the same winter as those in Montana (Bernatowicz et al. 2017).

Early detection of a disease outbreak is generally a key factor in successful response. The pneumonia outbreak in the EF herd was detected upon necropsy of 2 bighorn rams found dead along U.S. Highway 93 (Edwards et al. 2010). This finding heightened awareness of both the agency and the public to the issue of bighorn sheep pneumonia, and resulted in closer monitoring of herds and increased reporting of sick or dead bighorn sheep by the public. Based on the high proportion of infected animals found in each of the affected herds, it does not appear that the outbreak was in its early stages when detected. In fact, the population trajectories for the LRC and Garrison herds suggested that these populations were declining prior to the detection of pneumonia. Whether these declines were due to pneumonia or some other cause is unknown.

Surveying bighorn sheep in many of these areas is challenging due to steep terrain and heavy cover. Timing of surveys is not always ideal as biologists often rely on shared aircraft that are not always available at the preferred time. The increased variability that results from challenging surveys may allow some population declines to go undetected for a longer time, and lead to difficulty in interpreting survey results.

We have learned from these efforts that symptoms (or lack thereof) are not a good indicator of infection status, as was found to be the case by Bernatowicz et al. (2017) in Washington. Lungs from several apparently healthy bighorn sheep had obvious lesions consistent with pneumonia upon necropsy, and a small number of bighorn sheep that were euthanized because they were seen coughing had normal lungs. Some bighorn sheep would not display symptoms of pneumonia for extended periods of time, and some would only display symptoms when being stressed or exerted. The inability to accurately identify infected animals clearly impedes a response strategy that relies on removal of only infected animals, with a goal of allowing healthy animals to remain.

A good understanding of connectivity among neighboring herds is essential for a culling strategy to be successful at preventing spread of disease to neighboring herds. The pneumonia outbreaks that occurred in 2009-2010 highlighted the extent of connectivity among herds in this region. A highly contagious infectious agent may be transmitted quite efficiently among and within connected herds. The only hope for disease eradication from segments of the population may be very early detection, which is often unrealistic in wild populations.

Given the status of these bighorn sheep herds five years after they experienced epizootic pneumonia and response strategies were carried out, MFWP would be reluctant to employ the selective culling and containment zone culling strategies in the future. Although significant resources were directed to these culling efforts, the agency does not believe that these strategies resulted in a significantly improved outcome for these herds.

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Management Responses to Pneumonia Outbreaks in three Washington State Bighorn Herds: Lessons Learned and Questions yet Unanswered

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ABSTRACT Pneumonia induced die-offs and subsequent periods of low lamb survival are the greatest impediments to restoration of historic bighorn sheep abundance in North America, and developing effective responses to disease outbreaks in bighorn sheep has been frustrating for wildlife managers. A difficulty in understanding the phenomena is that no 2 situations seem identical. Thus, careful documentation of individual events is needed to understand common patterns and processes. We provide an update on 3 bighorn herds in Washington State that recently experienced pneumonia-related declines (Yakima Canyon, Asotin, and Tieton); management responses and outcomes differed in each case. The Yakima Canyon herd experienced an all-age die-off during winter 2009-2010, during which we culled animals showing signs of respiratory disease. In 2011 and 2012, the herd briefly rebounded, but then suffered 2 consecutive years of recruitment less than 10 lambs:100 ewes, accompanied by pneumonia (cohorts born in 2013-2014). The Yakima Canyon herd is characterized by considerable spatial structuring that was reflected in intra-herd patterns of disease. The Asotin herd suffered an all-age die-off in 2012, and we took no management actions during the outbreak. Similar to Yakima Canyon, after 1 year of high lamb mortality, survival returned to normal and we failed to detect evidence of disease. In 2015 we removed 3 ewes (~10% of survivors) that tested positive for *Mycoplasma ovipneumoniae* and recruitment has been 31– 54 lambs:100 ewes with no pneumonia or *M. ovipneumoniae* detected through the 2015 cohort. The Tieton herd suffered a catastrophic all-age die-off in winter 2013. Due to its proximity to the adjacent Cleman Mountain herd, we lethally removed every Tieton individual that did not die of pneumonia during the outbreak. To date, the Cleman Mountain herd has shown no evidence of pneumonia. While we do not assert that our management response was the only reason for the differing outcomes, we hope that follow-up monitoring and replication will help us identify which actions are effective at controlling the impacts of disease in bighorn sheep.

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KEY WORDS bighorn sheep, culling, disease, mortality, *Mycoplasma ovipneumoniae*, pneumonia, Washington

Bighorn sheep (*Ovis canadensis*) currently occupy a small percentage of their historic distribution in western North America. Although habitat degradation, hunting, disturbance, and other factors have contributed, mortality resulting from epizootic pneumonia is now thought to be the primary cause of the historic decline and remains the primary factor limiting bighorn sheep recovery (TWS 2010, 2014; Wehausen et al. 2011). Our understanding of the etiology and mechanisms of disease transmission has recently undergone considerable advancement and refinement (Dassanayake et al. 2010, Lawrence et al. 2010, Besser et al. 2012). There is now clear evidence that polymicrobial pneumonia epizootics are typically triggered by spillover of *Mycoplasma ovipneumoniae*, a bacterium commonly carried by domestic sheep and goats that can also suffer pneumonia (Da Massa et al. 1992, Nicholas et al 2008, APHIS 2015), but which is sometimes portrayed as of minor importance to sheep producers (Scott 2017). When introduced into bighorn sheep, *M. ovipneumoniae* binds to respiratory cilia, interfering with mucociliary clearance, thus facilitating invasion of the lungs by other species of bacteria, often resulting in fatal pneumonia (Besser et al. 2008, 2012, 2013, 2014).

After initial transmission, pneumonia events can cause up to 100% mortality in a bighorn sheep herd and typically result in depressed lamb survival for many years afterwards (Foreyt and Jessup 1982, Cassirer and Sinclair 2007, Cassirer et al. 2013). Once introduced, the disease can be spread within and among bighorn sheep herds. Managers have struggled to find ways to cleanse herds of pneumonia once introduced, as vaccines and treatments have generally been ineffective, impractical, or both (Foreyt 1992, Miller et al. 1997, Cassirer et al. 2001, Sirochman et al. 2012). Most commonly, no actions are taken because none can be. At the other extreme is depopulation of the entire herd (McAdoo et al. 2010, McFarlane and Aoude 2010). Between these are interventions such as culling, reducing, or cordoning, conducted to reduce the spread or effect of disease. Managers rarely if ever have the luxury (or logistics) of treating such interventions as formal experiments, which would require the presence of a suitable experimental control. Instead, we generally move forward armed only with hypotheses to link our actions with expected consequences, and must assess our success by comparing multiple case histories, each accompanied by its suite of particular circumstances and particularities.

In this paper, we present case histories of 3 bighorn sheep herds in the state of Washington that were affected by pneumonia outbreaks and die-offs, making reference to relevant, nearby herds when appropriate. Although not designed specifically to do so, these retrospective case studies illustrate an array of management approaches which, together with 3 to 6 years of subsequent monitoring, allow us to draw some general lessons. Although the origins of these disease outbreaks are ultimately of interest, we were unable to pin-point any of them. Instead, we focus in this paper on the temporal and spatial course of the disease events, responses on the part of the Washington Department of Fish and Wildlife (WDFW) and partners to stem them or keep them from spreading, and thus encouraging population recovery, and, finally, on inferences we have been able to draw on pneumonia in bighorns generally. We are fortunate in that, in all 3 cases, WDFW and

partners had, prior to the disease event, developed information on the herd, including approximate population size; movements, range use, and spatial sub-structuring (primarily using VHF telemetry); and had conducted pathogen surveillance (i.e., history of previous infection with, and exposure to relevant bacteria).

Bighorn sheep in Washington were completely extirpated by the early 20th century; all existing herds are the result of agency-implemented reintroductions (Johnson 1983, 1996). However, the current geographic distribution of bighorns, although fragmented and reduced in abundance, resembles closely that estimated from both historical accounts and archeological evidence from Native Americans (Lyman 2009). Areas where bighorns historically occurred but have not been reintroduced are typified by human development and occupation.

Study areas

The Yakima Canyon, Tieton, and Cleman herds are located in Southcentral Washington (Fig. 1). The herds are co-managed with the Yakama Nation and Muckleshoot Indian Tribes (MIT). The Asotin herd is in southeast Washington and is co-managed with the Nez Perce Tribe.

The Yakima Canyon herd occupies both sides of the canyon formed by the Yakima River as it flows northward through the low hills known as Umtanum Ridge, between Ellensburg and Selah, WA (Fig. 1). Most of the core range west of the Yakima River is owned by WDFW; lands east of the river are managed by the Bureau of Land Management, Washington Department of Natural Resources (WDNR), The Nature Conservancy, or private owners. Elevations vary from approximately 400m to 1,380m. Vegetation is predominately bunch-grass and sagebrush steppe, with cottonwoods and riparian along the Yakima River and tributaries (WDFW 1995).

The Tieton herd range is located north of the Tieton River, from its confluence with the Naches River (where US highway 12 intersects Washington state highway 410). Land ownership is a mixture of WDFW wildlife areas and Okanogan-Wenatchee National Forest (OWNF). Elevations vary from approximately 500m to 1,500m. Vegetation consists primarily of bunchgrass steppe on open slopes, and mixed conifer in draws. The adjacent Cleman herd occupies primarily public (WDFW and OWNF) lands north of the Naches River (along state highway 410), dominated by Cleman Mountain and spur ridges associated with Manastash Ridge. Elevations vary from approximately 550m to 1,460m. Vegetation is primarily bunchgrass steppe, with mixed conifer in draws.

The Asotin herd core range is located in the southeast corner of Washington, approximately 24 km southwest of Clarkston, WA, in Asotin and Garfield counties. These steep hills are in the extreme northerly portion of the Blue Mountains. WDFW manages the majority of lands used by the herd, with other major land managers being the Umatilla National Forest and the WDNR. Elevations vary from 425m to 3,629m, with the lower elevations being predominantly in private ownership. Vegetation is primarily perennial bunchgrass (*Pseudoroegenaria spicata*), bare rock, and talus, with north slopes containing shrub and open timber at higher elevations (WDFW 1995).

Methods

Population monitoring

All 3 focal herds were surveyed by helicopter and/or ground surveys annually, although timing of surveys differed. Abundances were derived from minimum counts and mark-resight estimates that we suspect typically tracked true abundance closely. In Asotin we monitored summer survival of lambs born to marked ewes each

year. In Tieton and Umtanum, we estimated lamb:ewe ratios in September (from which we inferred magnitudes of summer lamb mortality). We used lamb:ewe ratios in March or April as an index of recruitment to the yearling age-class. We note that when estimating lamb:ewe ratios, "ewe" includes yearling females, which we rarely could differentiate from older adult females, and which were too young to reproduce in that year. Abundance of the Cleman bighorn herd, which was not affected by pneumonia but is adjacent to the Tieton herd (Fig. 1), was estimated by ground counts in mid-winter at a feeding station.

All 3 herds (as well as the adjacent Cleman herd) had been subject to some level of radio-telemetry monitoring. Radio-collared ewes in the Asotin herd were monitored weekly from the ground during spring through autumn, and biweekly during late autumn and winter. We also conducted weekly fixed-wing telemetry flights to locate animals missed during ground surveys in spring and monthly during the rest of the year. The Yakima Canyon, Cleman, and Tieton herds have been monitored via both VHF and GPS collars.

Health testing

All sheep populations had some level of health monitoring prior to the outbreak. The Asotin herd is part of the Hells Canyon metapopulation, which has been involved in collaborative research by the consortium of states (Idaho, Oregon, Washington) since 1997, and as such, was monitored most intensively. Bighorn sheep were captured for health sampling in all herds, primarily by helicopter net gunning. Some animals were captured via ground darting with immobilization agents in the Asotin herd. Aerial captures occurred during the winter months of November through February. Ground captures occurred September through December. In the Umtanum population we also collected samples (e.g., whole carcass, nasal swab, blood, lungs) for *M. ovipneumoniae* opportunistically from hunters 2010-2015, and from dead, dying, or selectively culled lambs 2013–2015.

Live animal biomedical sampling included collection of oropharyngeal swabs kept cool and submitted within 24 hours for aerobic bacterial culture for Pasteurellaceae and other aerobic bacteria. Nasal swabs were submitted for culture and polymerase chain reaction (PCR) for *M. ovipneumoniae.* Nasal washes of 50 ml buffered saline were collected in the Umtanum population in 2013. Blood serum was collected and submitted for competitive enzyme-linked immunosorbent assay (cELISA) to test for antibodies to *M. ovipneumoniae*. Intact carcasses from animals that were culled in the Tieton and Yakima Canyon populations, or found recently deceased, were additionally subjected to routine necropsy, including histology; as well as sampled from one or more of the following anatomical sites for *M. ovipneumoniae* PCR: nose, nasal sinuses, tonsils, tympanic bullae, trachea, tracheobronchial lymph nodes, and lungs. Samples available from hunter-harvested rams were generally limited to nasal swabs, blood samples, and lung tissue. All diagnostic tests were completed at the Washington Animal Disease Diagnostic Laboratory (WADDL) at Washington State University, Pullman.

Strain-typing of *M. ovipneumoniae* (Cassirer et al. 2016) was conducted in the T. E. Besser laboratory at Washington State University (WSU), as was PCR testing for *M. ovipneumoniae* on nasal washes.

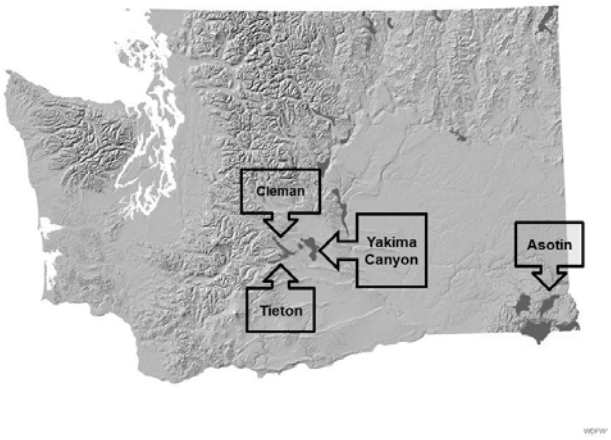

Figure 1. Topographic image of Washington state, showing core ranges of bighorn sheep herds (solid polygons), with locations of the 4 herds discussed in this paper (Yakima Canyon, Asotin, Tieton, Cleman) indicated by arrow boxes.

Results

Yakima Canyon herd

Herd origin and pre-outbreak population performance: The Yakima Canyon herd was established in 1970 with the release of eight animals in what we subsequently began terming Umtanum North (Fig. 2). These 8 founders were translocated from the nearby Quilomene herd in Washington (these animals, in turn, originated from a translocation of animals from Williams Lake, B.C. in 1964). Within 15 years, the Yakima Canyon population had grown to an estimated 200 animals, and some individuals had moved easterly across the Yakima River to form the Selah Butte sub-herd. However, no natural colonization occurred in the southern portions of the canyon. In 1996, WDFW captured 14 individuals in Selah Butte North and Central sub-herds, and moved them to what subsequently grew to become the Umtanum South sub-herd. Only a few of these 14 became resident; thus, in 2001, an additional 11 bighorns were added (in this case, from the distantly-located Lincoln Cliffs herd). This Umtanum South sub-herd grew rapidly and subsequently crossed the Yakima River to form the Selah Butte South sub-herd. By 2006, over 300 animals occupied the canyon, comprising 5 sub-herds on both sides of the Yakima River, and it remained at about approximately this level until 2009 (WDFW 2016). Serology on 11 animals tested in 2007 indicated that the Yakima Canyon herd had not been exposed to *M. ovipneumoniae*, at least within the 2 years prior to the outbreak of 2009-2010 (Fig. 3).

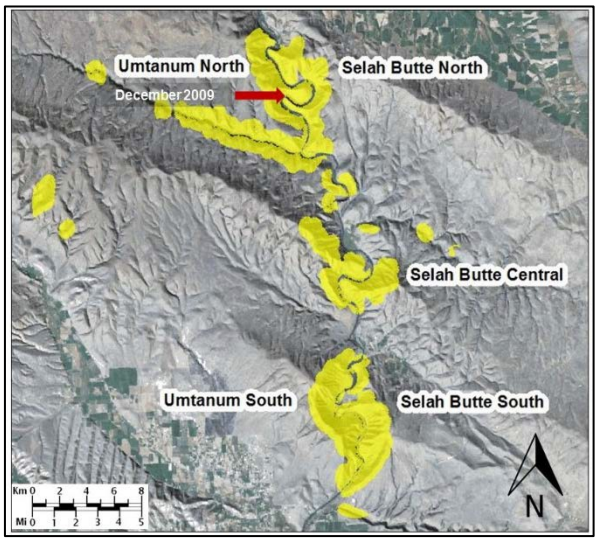

Figure 2. Spatial structure of the Yakima Canyon bighorn herd, Washington, USA (shown in yellow shading), illustrating the sub-herds (from north to south) Umtanum North, Selah Butte North, Selah Butte Central, Umtanum South, and Selah Butte South. The Yakima River runs north to south, separating the Umtanum side (to the west) from the Selah Butte side (to the east). Red arrow shows approximate location of the initial detection of the pneumonia outbreak in December 2009. Despite the presence of mapped habitat across the river directly west of the Selah Butte Central, bighorn ewes used this area sparingly, and thus it is not named as a subherd.

Outbreak and short-term management response: During winter 2009-2010, a poly-microbial pneumonia outbreak caused the loss of approximately 13% of the herd. When

initially detected in November 2009 (and confirmed by WDFW in December 2009), the outbreak was thought to be isolated to the Umtanum sub-herd. Forty-three bighorns were known to have died (presumably from pneumonia), all but 1 in the northern portion of the Umtanum sub-herd. In December 2009, we euthanized 8 animals: 5 on the west side and 3 from the east side, and submitted them to WADDL to document and confirm the cause of the outbreak. Three sheep on the west side were pneumonic, and all were *M. ovipneumoniae* positive. All 3 sheep on the east side were healthy. In February 2010, we began selectively culling sheep on the west side with the most severe clinical signs, with the goal of removing individuals thought likely to be shedding bacteria from the population. The hypotheses undergirding this symptom-based culling were that doing so would stop the infection from crossing the river eastward (from the Umtanum to Selah Butte sub-herds), and increase lamb survival in subsequent cohorts. Criteria for culling were visual evidence of clinical disease, defined as coughing, nasal discharge, droopy ears, and/or lethargy. Most culling was conducted on contract with the U.S. Department of Agriculture, Wildlife Services Division. We lethally removed 52 symptomatic and 6 asymptomatic animals west of the river, and another 3 asymptomatic animals east of the river. In addition, samples were collected from 4 fresh carcasses encountered west of the river during the course of live animal removals (Table 1; an additional 43 carcasses were found west of the river, but were too decomposed to allow sampling).

Serologic testing confirmed exposure to *M. ovipneumoniae* in 98% of 53 symptomatic sheep removed from Umtanum (Fig. 3). Most (97%) sheep sampled in Umtanum were also PCR positive for *M. ovipneumoniae* in lung tissue or on nasal swabs (Fig. 4), and 77% had histological evidence of pneumonia. All 6 non-symptomatic sheep sampled in Umtanum in March 2010 were PCR and seropositive for *M. ovipneumoniae*, but only 3 had histological evidence of bronchopneumonia. A single strain of *M. ovipneumoniae* was identified in all samples. Among the 6 euthanized in Selah Butte during the outbreak in Umtanum, none were seropositive or PCR positive for *M. ovipneumoniae*, but 1 had histological evidence of bronchopneumonia. Culturing of swab and lung samples collected from 53 symptomatic animals during the outbreak revealed presence of *Bibersteinia trehalosi*, *Pasteurella multocida*, and *Mannheimia haemolytica*, (Table 2). *Moraxella* sp. (49% of samples), *Arcanobacterium pyogenes* (40% of samples); a single case of *Staphylococcus aereus* were also cultured.

Post-outbreak. Lamb recruitment (Table 2) was low in 2010 but rebounded to pre-outbreak levels in 2011 and 2012 (Fig. 5), and by spring 2012, the estimated population had increased to near pre-2009 levels. In 2013, initial lamb production was high, but by early September all lambs of marked ewes in Umtanum had died and the overall lamb:ewe ratio was 12:100 (and had declined to 8:100 by March 2014; Table 2). Two lambs collected west of the river had bronchopneumonia and were PCR positive for the same strain of *M. ovipneumoniae* documented in 2009-2010. Lambs east of the river survived longer, but by early winter 2014, most of these had also died. In 2014, overall lamb recruitment was similar, with the lamb:ewe ratio declining to 8:100 by winter 2014-15 and pneumonia was confirmed at WADDL. Over-winter recruitment of the 2015 lamb cohort (to March 2016) recovered somewhat from the low of the previous 2 years, to 23:100 overall (Table 3) but was highly variable by sub-herd (see below).

Table 1. Detection of *Mycoplasma ovipneumoniae* via PCR in asymptomatic, symptomatic and dead bighorn sheep in the Yakima Canyon herd during the 2009-2010 pneumonia outbreak. Rows represent animal condition at the time of sampling.

	Detected	Indeterminate	Not Detected
Symptomatic (culled)	51	1	0
Early Umtanum sampling	5[a]	0	0
Selah Butte visually healthy	0	0	3[b]
Found dead	3	0	1[c]

[a] One was symptomatic when sampled. All animals are from the Umtanum sub-population. Three had pneumonia lesions in lungs at necropsy.

[b] One had histological evidence of pneumonia.

[c] Lung tissue from this animal was not available for testing, PCR on nasal sinus sample was negative.

Table 2. Prevalence of 3 genera of Pasteurellaceae bacteria (% of sampled adults), as detected by culture before, during, and after pneumonia outbreaks in 3 bighorn sheep populations, 2008-2016, Washington State, USA. *P. t. = Bibersteinia trehalosi; P.m. = Pasteurella multocida; M. = Mannheimia spp.*

Sample	Yakima Canyon			Asotin			Tieton		
	B.t.	*P.m.*	*M. h.*	*P. t.*	*P.m.*	*M. h.*	*P. t.*	*P.m.*	*M. h.*
Pre-outbreak				100		17	----77[a] ----		
During outbreak	45[b]	18	4	100	33	50	71[c]	13	13
6 months post outbreak	63[d]	0	25	19		69			
2 years post outbreak				88[e]	0	16			
3 years post outbreak	84[f]	0	32	40	0	0			

[a] *n* = 22, Pasteurellaceae aggregated, none detected in 5 animals.

[b] *n* = 51, includes found dead, culled, and euthanized with no symptoms, none detected in 17 animals.

[c] *n* = 24, includes found dead and culled, none detected in 4 animals.

[d] *n* = 8, hunter sampled animals; none detected in 2 animals.

[e] *n* = 32; 9% undifferentiated *Pasteurella* spp.

[f] Live capture, 25 ewes, 6 rams (*n* = 31), none detected in 2 animals

Table 3. Summary of over-winter lamb recruitment in herds affected by all-age pneumonia die-offs in Washington State, 2009-2016. Years represent spring following disease event (e.g., Year 1 = 2010 for Yakima Canyon, 2013 for Asotin). Shown are sheep:ewe ratios (ewes defined as females older than lamb).

Herd	Yakima Canyon	Asotin
Management response	Removal of symptomatic animals during outbreak	Removal of PCR positive ewes 2 - 3 years after outbreak
	Mean lamb recruitment to March of year following event	
Year 1	10:100	9:100
Year 2	58:100	31:100
Year 3	42:100	52:100
Year 4	8:100	50:100
Year 5	8:100	-
Year 6	23:100	-

Table 4. Attributes of herds affected by pneumonia die-offs, Washington State, 2009-2013. Single quotes denote that we do not necessarily accept the scientific validity of these names as formal sub-species, but rather manage them separately pending clarity about the biological basis for doing so (Wehausen and Ramey 2000).

Herd attributes	Yakima Canyon	Asotin	Tieton
Bighorn Ecotype	'California'	'Rocky Mtn'	'California'
Year of Origin	1970	1973	1998
Subsequent augmentations	1996, 2001	1991, 1993, 1994, 1998	1999, 2000, 2001,2003
Year of outbreak	2009-2010	2012	2013
Estimated abundance at outbreak	> 300	101	200
Estimated herd decline	35%	36%	> 50% prior to complete removal
Management response	Culling symptomatic animals	Removal of putative super-shedders	Complete herd removal
Current status	Declining slowly, but sub-herd performance variable	Evidently slowly increasing	Gone; a few wandering animals from adjacent Cleman herd
Tentative assessment	Failed to stop spread of disease or disease persistence	Population is healthy 4 years after outbreak	Herd extirpated, but adjacent Cleman herd still free of pneumonia

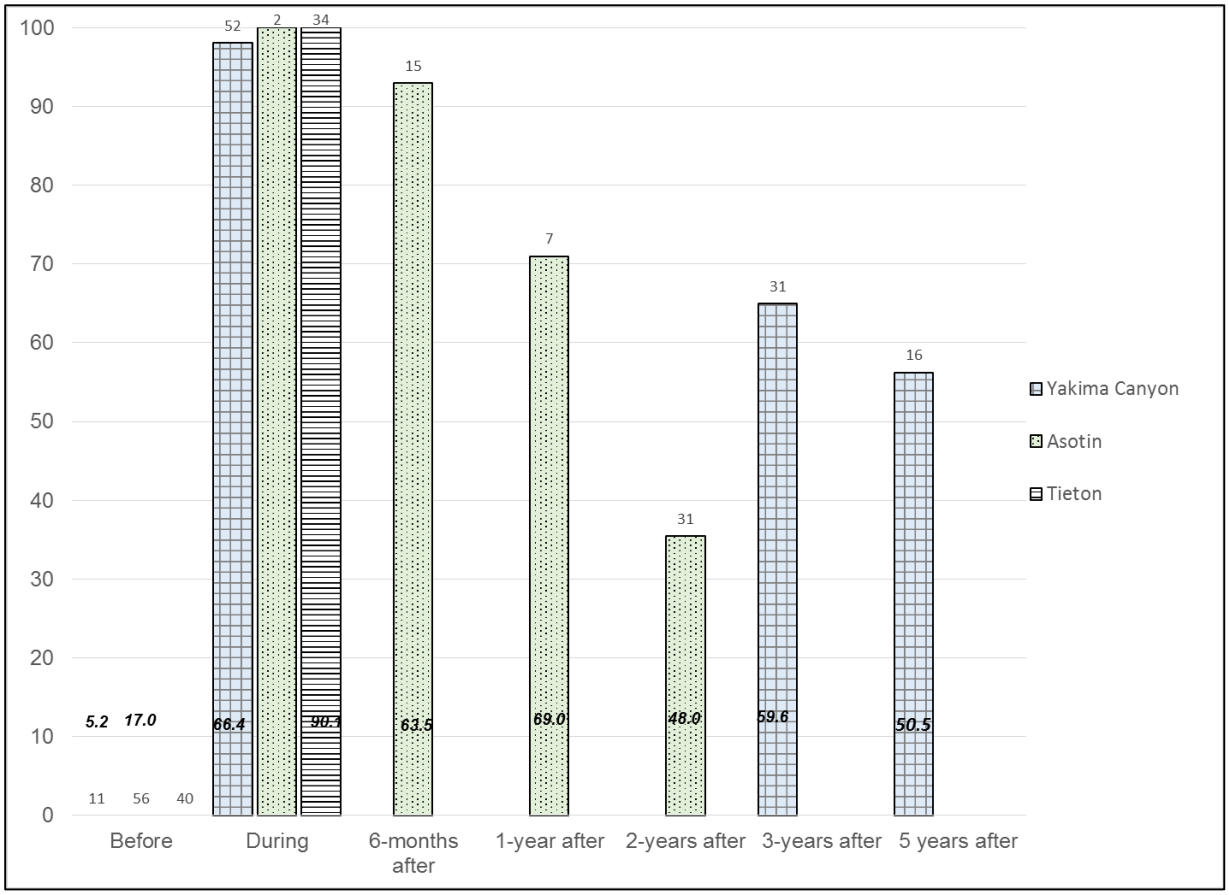

Figure 3. Evidence of exposure to *M. ovipneumoniae* in sampled animals, before, during, and after pneumonia outbreaks in 3 bighorn sheep populations, 2008-2016, Washington State, as indicated by ELISA serology. Shown are percentages of animals testing positive (defined as above the threshold of 40). Where available, mean titers are shown in bars. Sample sizes for each test appear above bars.

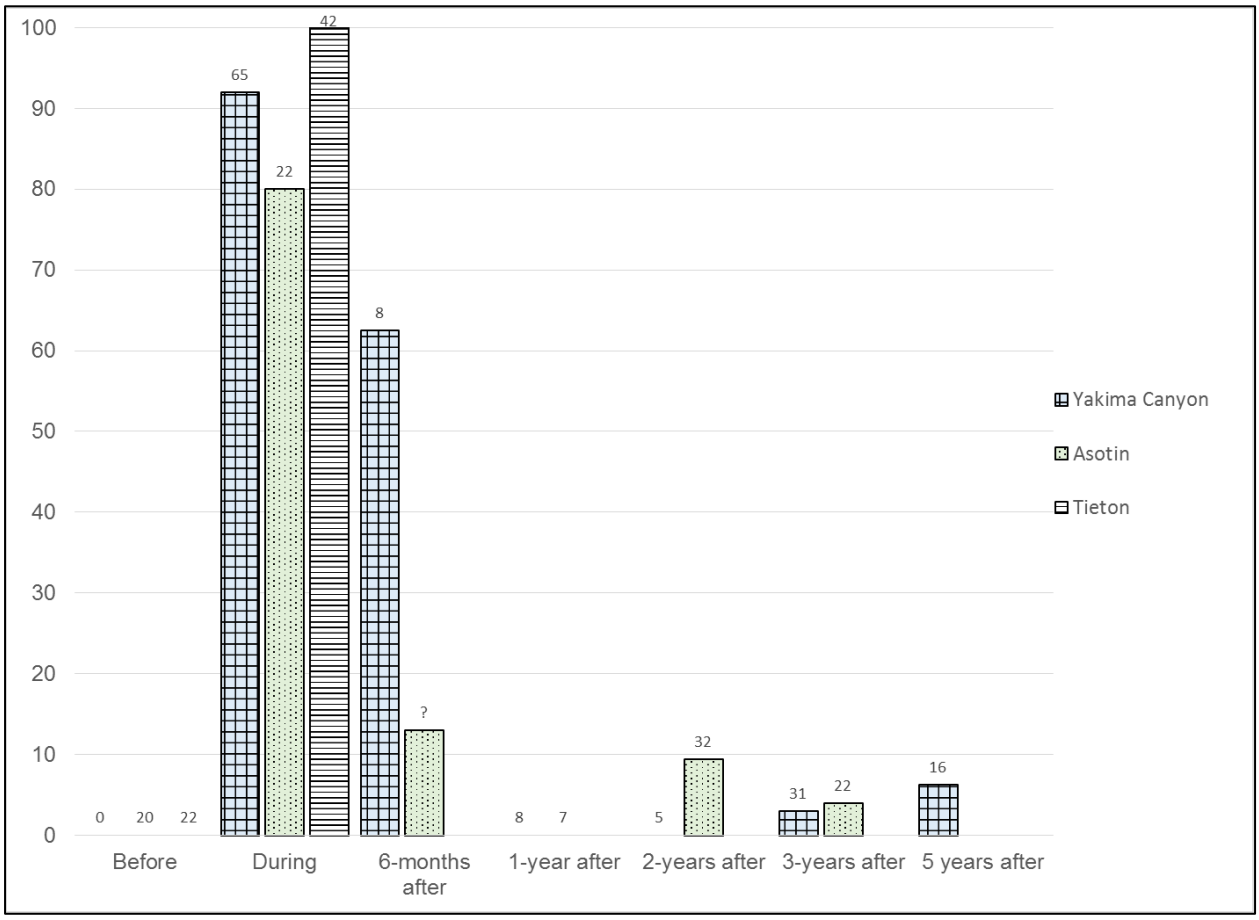

Figure 4. Evidence of *M. ovipneumoniae* infection in sampled animals, before, during, and after pneumonia outbreaks in 3 bighorn sheep populations, 2008-2016, Washington State, as indicated by PCR from various tissues. Shown are percentages of animals testing positive. Sample sizes for each test appear above bars.

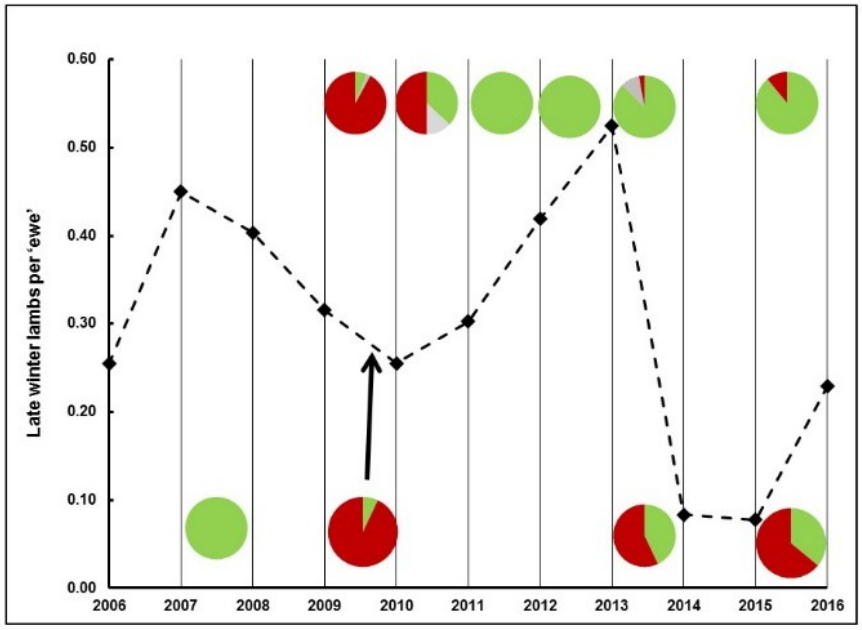

Figure 5. Late winter lamb:ewe ratios of the Yakima Canyon bighorn sheep herd, Washington State, 2006-2016 (dashed line). Pie graphs above show the proportion of individuals tested positive for active *Mycoplasma ovipneumoniae* using PCR from nasal swabs. Pie graphs below show the proportion of individuals with positive ELISA serology titers for *M. ovipneumoniae*. Red = positive; green = negative, grey = indeterminate.

In autumn 2010, 4 animals taken by hunters from Selah Butte were positive for *M. ovipneumoniae* in respiratory tracts on PCR, and all 4 also had moderate to severe bronchopneumonia. Of 4 additional hunter-harvested animals taken from the Umtanum sub-herd, 1 animal was *M. ovipneumoniae*-positive in the respiratory tract, but none of the 4 had bronchopneumonia. However, sampling of bronchial and/or lung tissue from 11 hunter-killed samples in 2011 and 2012 failed to detect *M. ovipneumoniae* (Fig. 4).

In February 2013, 65% of 31 bighorn sheep sampled were positive for serologic antibodies to *M. ovipneumoniae* (Fig. 3). In the Umtanum sub-herd, 71% were positive, whereas 50% were positive in the Selah Butte sub-herd. Nasal swabs from 3 animals were indeterminate on PCR for *M. ovipneumoniae.* A nasal wash sample from one of these individuals, an Umtanum ram, was PCR-positive (Fig. 4).

In February 2015, we captured and tested 17 ewes, including recapture of 15 of the ewes we had sampled in 2013. Nine of 16 (56%) were seropositive for exposure to *M. ovipneumoniae* (3 were indeterminate). Serum antibody percent inhibition (%I) increased in ewes that were seronegative in 2013 ($\bar{x}$ = 23%I in 2013, $\bar{x}$ =46 % I in 2015) and decreased in animals that were seropositive in 2013($\bar{x}$ = 80%I in 2013, $\bar{x}$ 67% I in 2015). Two ewes were PCR-positive: A nasal swab from a Selah Butte sub-herd ewe was positive, and an Umtanum sub-herd ewe that died as a result of capture was PCR-negative on nasal swab but PCR-positive on lung tissue. The ram that had tested PCR positive for *M. ovipneumoniae* in 2013 was no longer living at the time of the 2015 capture. Neither of the 2 animals that tested positive in February 2015 had done so in February 2013, and the 2 ewes that tested indeterminate in 2013 were negative in 2015.

Asotin herd

Herd origin and pre-outbreak population performance: The Asotin Creek bighorn sheep population originated with the release of 4 animals from the nearby Tucannon Herd in 1973. These animals were augmented by animals from the Hall Mountain herd near Sullivan Lake in northeastern Washington (6 in 1991, and another 9 in 1994), and from Spence's Bridge, British Columbia (10 in 1997). From 2004 to 2010, the population grew at a mean annual rate of approximately 18% despite removal by

WDFW due to concerns about contact with domestic sheep and goats (~20 females and ~10 males), for translocation, for use in captive disease experiments primarily at Washington State University, and by licensed hunters. March lamb:ewe ratios averaged 41:100 (approximate 95% confidence interval 34-47:100). The largest recorded population size (in 2011, prior to the outbreak) was approximately 105 sheep. Post-outbreak population estimates in 2012 and 2013 were 70 animals, declining to 60 in 2014. Prior to the outbreak, we had documented no evidence of pneumonia or *M. ovipneumoniae (*health history included blood samples collected in captures for removals and radio-collaring in 2006, 2008, 2009, and 2011, nasal samples collected 2008 – 2011, and necropsies 1997 – 2011).

Outbreak and short-term management response: In January 2012, both contagious ecthyma (orf, not previously documented in the Asotin population), and pneumonia associated with infection with *M. ovipneumoniae* were confirmed by WADDL, and by April 2012, 30% of the ewes had died. Orf was also observed in adjacent bighorn sheep populations in the fall of 2011. One half curl ram captured in the town of Asotin was observed with orf lesions, but no other disease was evident in rams, and ram survival appeared high. Initially, no management actions were taken in response to the outbreak, because other bighorn populations to the south were already known to be infected and the *M. ovipneumoniae* strain in Asotin (IGS 404) matched the predominant strain in the Hells Canyon bighorn sheep metapopulation since 1995 (Cassirer et al. 2016). Four of the 5 mortalities submitted to WADDL during the early 2012 outbreak were positive for *M. ovipneumoniae* on PCR in lung tissue (Fig. 4). Serology conducted on one ewe that had orf but not pneumonia indicated she was negative for antibodies (Fig. 3).

Post-outbreak: In May 2012, at least 14 lambs were observed born to the approximately 25 ewes that survived the outbreak. On June 8, 2012, at approximately 3 weeks of age, 2 lambs were collected by gunshot and necropsied. Both lambs were healthy and uninfected with *M. ovipneumoniae*, but had serologic antibody titers, probably passively-transferred from their dams, indicating the adults had been exposed during the outbreak. As a control, 2 lambs similarly collected in the Black Butte bighorn population approximately 30 km south at Heller Bar on June 1 were pneumonic on necropsy and infected with *M. ovipneumoniae*. Serologic titers were similar in both sets of lambs. However, by March 2013, only 5 lambs were documented from 32 surviving Asotin ewes (Table 3). Lamb recruitment improved in 2013 to just under pre-outbreak levels (9 lambs surviving to spring 2014 from 28 ewes, ratio 31:100; Table 3). In summer 2014, lamb survival was 88%, and subsequent recruitment (to early winter 2015) was 13 lambs from 25 ewes (52:100; Table 3), slightly higher than the pre-outbreak mean. Similarly, the 2015 cohort as of early winter 2016 was 16 lambs from 32 ewes (50:100; WDFW 2016).

By October 2012, PCR detection of *M. ovipneumoniae* infection had fallen to 13% (Fig. 4), although 93% retained antibodies(Fig. 3) including 6 of 7 rams, indicating that they had been exposed although all were PCR negative. One ram taken by a hunter in September 2012 was healthy but PCR positive in the lungs and nose and seropositive. PCR detection of *M. ovipneumoniae* in adults remained between 11 and 20% through 2014.

During November 2013 - February 2014, prevalence of *M. ovipneumoniae* on nasal swabs was 22% in 9 lambs, 0 of 2 yearlings, and 11% in adults (n = 19). Antibody titers to *M. ovipneumoniae* were detected in 33% of lambs and 84% of adults. PCR prevalence for animals captured September 2014 – January 2015

was 20% for adults (n = 15). All lambs and yearlings tested were negative on PCR and ELISA, including a lamb that had been positive in February 2014. Antibodies were detected in 67% of adults (Fortin and Cassirer 2015; Fig. 3).

In 2013, the 3 states involved in the Hells Canyon Initiative agreed to a program of experimental management for the Asotin Herd (as well as the nearby Black Butte herd in Washington, and the Lostine herd in Oregon), in which the "super-shedder" hypothesis would be tested (Lloyd-Smith et al. 2005, Matthews et al. 2006). Briefly, preliminary data gathered from both field and captive settings had suggested that bighorns were individualistic and highly heterogeneous in their propensity to become carriers --and thus transmitters -- of *M. ovipneumoniae* (Cassirer 2014, Fortin and Cassirer 2015). Even in populations in which the pathogen persisted over a number of years, it appeared that a small number of chronic carriers, or "super-shedders" might be responsible for a disproportionate amount of vertical and lateral transmission. If so, and if these "super-shedders" could be identified and removed, it might greatly decrease the time required for the pathogen to become extinguished within the population. Thus, a more intensive program of repeated capture and testing of Asotin female sheep was initiated in autumn 2013. Ewes that tested PCR-positive from nasal swabs for *M. ovipneumoniae* repeatedly, i.e., super-shedders, were captured and transferred to a captive facility at South Dakota State University, where a companion study was being conducted. In January 2015, 3 ewes identified as chronic shedders were removed from the population.

Between 19 February and 7 April, 2015, 4 radio-collared, *M. ovipneumoniae*-negative adult ewes in the Asotin population were killed by cougars. The timing and proximity of the kills suggested predation by a single cougar. Because this represented >15% of the ewes in this heavily studied experimental population, we used a local houndsman to assist in removing the cougar. An adult female cougar was removed after the 4th bighorn sheep mortality on April 8, and no further deaths attributable to predation were detected. However, including the cougar predation, 22 adults died or were removed from the population in 2014 and 2015. Five ewes and 2 rams were transferred to the captive facility at South Dakota State University as part of the super-shedder experiment, and 1ewe died during capture. At least 8 rams were harvested by hunters, a 9th was poached but not recovered, and 1 yearling ram was removed from the town of Asotin. Altogether, these known non-pneumonia-related losses slowed recovery of this small population after the outbreak (WDFW unpubl. data, Fortin and Cassirer 2015).

Tieton herd

Herd origin and pre-outbreak population performance: Bighorn sheep were reintroduced to the Tieton area in 1998–2002 with the translocation of 54 sheep from 4 separate herds. Population growth was rapid, particularly after 2004, and by early 2012 had stabilized at approximately 200 animals despite the legal harvest of 63 animals (during hunting seasons 2004-2012) as well as the removal of 72 for translocation. Based on modeled habitat and home range (O'Brien et al. 2014) from GPS collars, the density estimate was 6.2 sheep per km^2. Twenty-two animals used for translocation were tested for the presence of *M. ovipneumoniae* in nasal mucosae using PCR (Fig. 4), and serology for exposure was examined on 18 (Fig. 3). No evidence of either infection or exposure was documented.

Outbreak and short-term management response: In early mid-March 2013, we became aware of an unusual cluster of mortalities in the Tieton herd. Veterinary testing of samples from 5 carcasses (5 heads, 4 lungs) found during the initial surveillance

showed that all had bronchopneumonia (either on gross inspection of lungs, histological examination of lungs, or gross inspection of sinuses and/or bullae), evidence of upper and/or lower respiratory tract disease typical of *M. ovipneumoniae* infections, and all 5 tested PCR positive for infection with *M. ovipneumoniae*. Ground and aerial surveys on March 26, 2013 documented only 35 live animals, and approximately as many carcasses. We speculate that disease had entered the herd at least 3 months earlier.

Because of the apparently high mortality of this new outbreak (estimated >50% mortality at first detection) and the herd's close proximity to the yet-uninfected Cleman herd, we elected to euthanize all known Tieton herd survivors. We began by using WDFW staff to lethally remove 10 animals closest to the Cleman herd on April 3, 2013 (an additional animal died from the disease that day). This was followed by contracting with USDA Wildlife Services, which removed an additional 31 animals by helicopter shooting during April 9-12, 2013. One of our tribal partners issued permits for tribal hunters in early April, but only 1 animal was harvested. An additional 8 animals were removed by WDFW in May 2013. WDFW consistently documented only 3 remaining animals, mainly via trail cameras baited with salt. The final 3 were removed by skilled citizens operating under contract from with WDFW in early October 2013. No animals were seen or reported for the remainder of the year. We believe we successfully removed every animal in the herd.

During the outbreak, all sampled animals had severe bronchopneumonia (n = 41) and all were *M. ovipneumoniae* positive on serology (Fig. 3) and PCR (Fig. 4). The final 3 animals removed in October (6 months after we confirmed the outbreak) were all *M. ovipneumoniae* positive on serology and 2 were PCR positive in lungs/cranial bronchus. All 37 *M. ovipneumoniae* identified by PCR from Tieton animals were of a single strain (intergenic spacer [IGS] 388). This strain differed from the single strain that earlier affected the Yakima Canyon herd, and it clustered with domestic sheep rather than goats (T. Besser, pers. comm., 2013). *B. trehalosi* was the most commonly cultured of the Pasteurellaceae bacteria; *P. multocida* and *M. haemolytica* were present less frequently (Table 2).

In April 2013, a domestic wether (castrated male sheep) was found wandering about 10 km from core Tieton bighorn range. A USFS lessee had lost 8 animals the previous fall, believed the sheep to be a survivor, and euthanized the animal. A volunteer collected samples a few days later and WDFW submitted them for testing. Although *M. ovipneumoniae* was detected on PCR from nasal mucosae of this sheep, the strain did not match the IGS 388 strain found among all tested Tieton bighorns. The carcass of another domestic sheep was later discovered within the Tieton herd core range, but was too decomposed to provide samples. WDFW also conducted nasal swabbing for *M. ovipneumoniae* PCR on 10 of 11 domestic, mixed-breed goats held on private land near the Tieton herd. Seven goats tested positive, one negative, and 2 indeterminate for *M. ovipneumoniae*. As with the domestic sheep wether however, neither of the strains (IGS 393 and IGS 423) from these goats matched the IGS 388 strain implicated in the Tieton die-off.

Post-outbreak: We have continued to monitor the Tieton area for bighorns, and have identified none believed to be survivors of the die-off or the subsequent lethal removal. We have received occasional reports from the public of bighorns near the Tieton range, but we believe all resulted from short-distance forays of animals associated with the Cleman herd. Because risks of a new infection had not yet been satisfactorily reduced, WDFW made attempts to remove these animals lethally, but

none were successful. All samples (n = 59) from the Cleman herd have been *M. ovipneumoniae* negative via serology and PCR since fall 2013. Lamb recruitment in the Cleman herd has averaged 40 lambs per 100 ewes (n = 95 lambs) in January.

Herd substructure: Yakima Canyon as a case study

Thus far, we have described demographics and epidemiology for each herd considered as single units. However, it has long been recognized, and recently shown relevant for disease transmission by Manlove et al. (2014), that spatial-social structure characterizes many bighorn herds. In some cases structure is geographically obvious; in other cases, it may be latent and require detailed telemetry, genetic, or epidemiological data to discern. Here, we provide additional detail on demographic and pathogen dynamics on the sub-herds we recognized within the Yakima Canyon herd.

Figure 2 shows the spatial configuration of 5 sub-herds within Yakima Canyon, using the definition of summer bighorn habitat developed by the Hells Canyon project and Payette National Forest (O'Brien et al. 2014) to identify geographic areas. The Yakima River, which flows roughly north to south in this stretch, separates the 2 named "Umtanum" to the west, from the 3 named "Selah Butte" to the east. We are unable to confidently quantify the frequency of interaction among individuals associated with each sub-herd. Our radio-collaring was not designed to estimate frequency of forays. Radio-tracking of VHF collared ewes was sporadic. Only 5 rams wore GPS collars and one of those disappeared before the rut. One of the 2 surviving Umtanum rams moved between sub-herds during the rut (Fig. 6), but died in late winter. The 2 Selah Butte rams were collared in different "sub-herds," but showed almost identical home ranges, covering both sub-herds (Fig. 6). No animals were radioed in Selah south. We first confirmed a radio-marked ewe crossing from the Umtanum to Selah sides of the river in late summer 2016. None of the 3 surviving rams crossed the river. Observations, as well as inference from epidemiology (see below) suggest that river crossing occurs on occasion.

The management intervention in winter 2009-10 was designed to test the hypothesis that symptomatic culling would prevent the infection, which evidently began in the Umtanum North sub-herd but quickly spread to the Umtanum South herd, from spreading eastward across the Yakima River to the Selah Butte sub-herds. However, we documented poor lamb recruitment on both sides of the river as early as spring 2010 (August 2010 lamb:ewe ratios of 4:100 west of the Yakima River and 15:100 east of the river), as well as histological evidence of moderate to severe pneumonia in 4 hunter-killed rams east of the river in autumn 2010. Strain typing confirmed that these 4 rams were infected with the same *M. ovipneumoniae* strain that culling had been intended to halt (Tom Besser, WSU, personal communication). Two of the 4 rams harvested on the west (more heavily affected in the outbreak) side of the river in fall 2010 were *M. ovipneumoniae* negative and none had histological evidence of pneumonia, suggesting that acute infection and disease had subsided by September 2010.

In 2011, lamb recruitment was high west of the river (53 lambs:100 ewes), but only 19:100 east of the river. All of the 3 eastern and 4 western hunter-harvested rams were *M. ovipneumoniae* negative from tissue sampling. In autumn 2012, *M. ovipneumoniae* was not detected among 5 harvested rams, but 1 of the 2 eastern rams had histological evidence of pneumonia. These data were consistent with a continued lag in the epidemic on the east side of the river.

Evidence that disease dynamics continued to vary by sub-herd comes from examining the lamb:ewe ratios during years 4

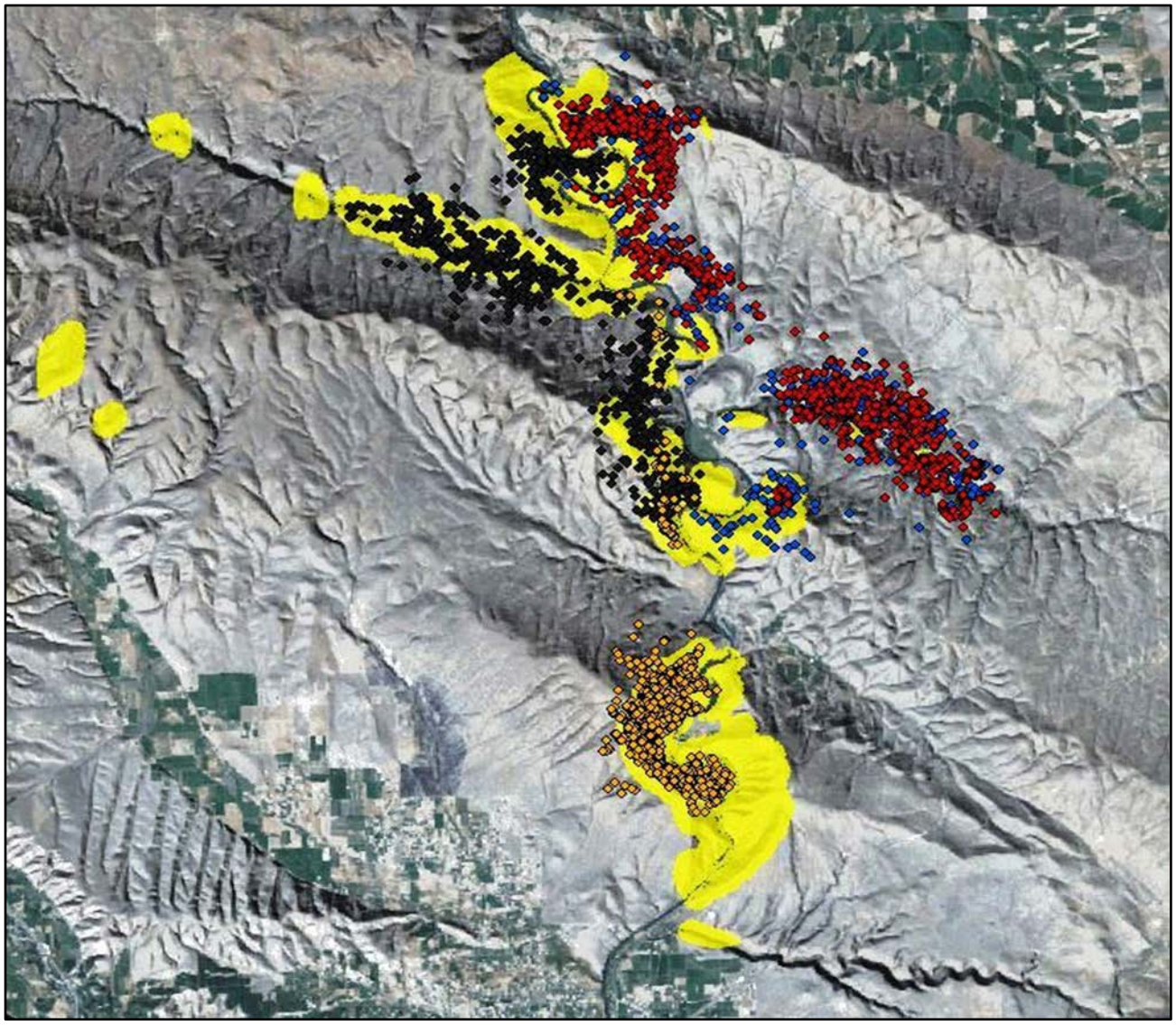

Figure 6. Locations of 4 GPS-radio collared ram bighorn sheep, February 2013-December 2015, within the Yakima Canyon herd, Washington State, USA. Sub-herds (yellow polygons) are as in Figure 2. Each ram is designated with a unique color. The 2 rams on the east (Selah Butte) side travelled among both the Selah Butte North and Selah Butte Central sub-herds; the 2 on the Umtanum side stayed primarily in the sub-herd in which they were collared, but overlapped in habitat between the sub-herd cores. None of the 4 rams was documented to cross the Yakima River, but anecdotal observations indicate that crossings do occur.

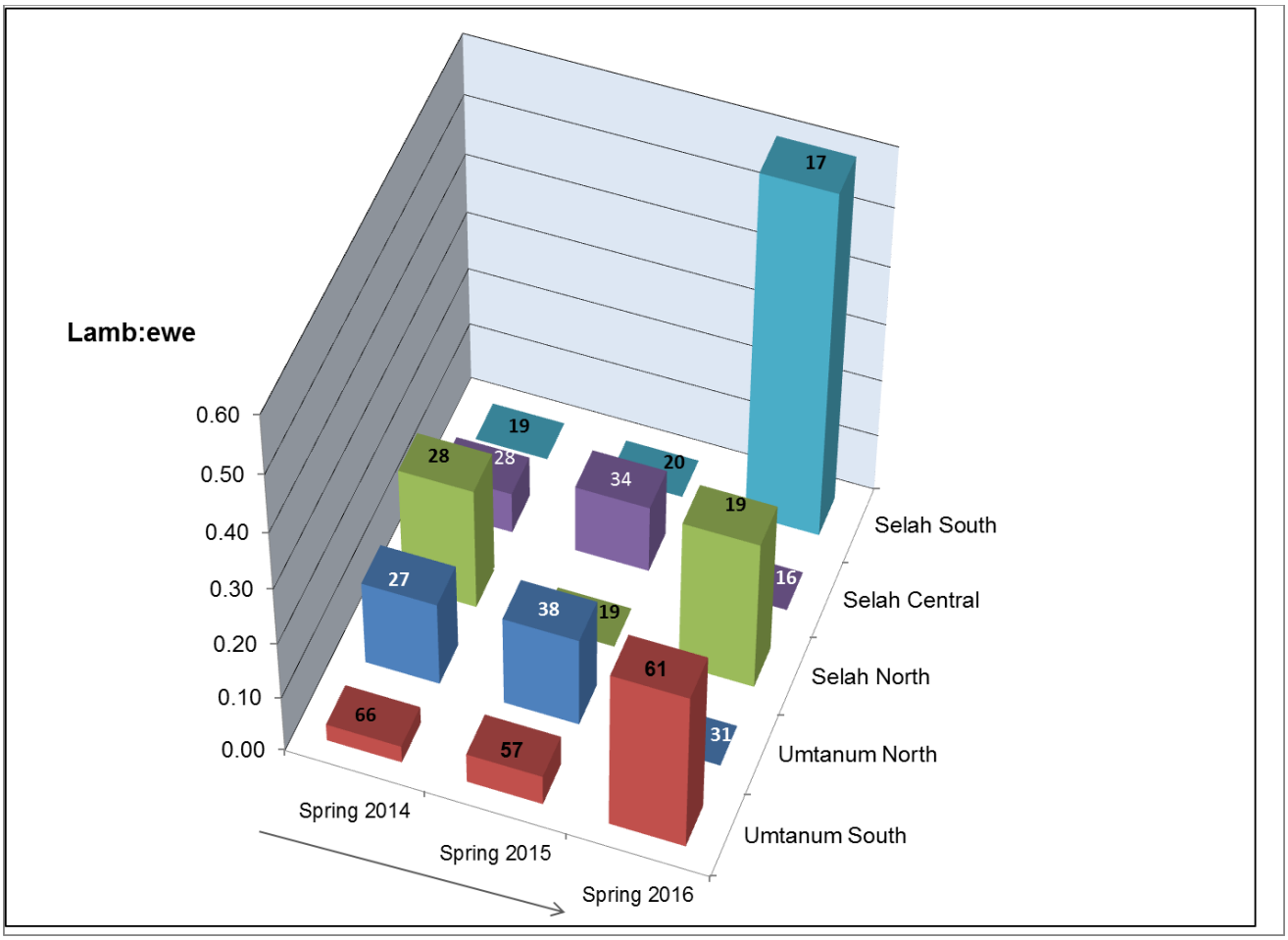

Figure 7. Ratios of lambs to ewes (females older than lamb) recruited to March 2014, 2015, and 2016, for each of the identified sub-herds of the Yakima Canyon bighorn sheep herd, Washington, USA. Numbers atop each bar are number of ewes counted in each sub-herd.

through 6 post-outbreak (cohorts born in 2013 through 2015) in more detail (Fig. 7). The mean Yakima Canyon herd lamb:ewe ratio of 8:100 of March 2014 (i.e., describing recruitment of the spring 2013 lamb cohort) varied from as high as 21:100 (in the Selah North sub-herd) to as low as 0:100 (in in Selah South sub-herd). By March 2015, the overall lamb:ewe ratio was again 8:100, but this similar mean masked a very different geographic pattern: Selah North, with the best recruitment in 2014, had joined Selah South in recruiting no lambs, and most of the limited recruitment came from Selah Central and Umtanum North. In March 2016, overall recruitment had increased somewhat (to 23:100), but had plummeted to zero in the very sub-herds (Selah Central and Umtanum North) providing some recruitment the previous year.

Discussion

Yakima Canyon herd

Based on the movement of pathogens and disease among sub-herds and the continued mortality of lambs after 2010, our management intervention — selectively euthanizing symptomatic individuals — in the Yakima Canyon herd evidently was ineffective in stopping the spread of *M. ovipneumoniae* (Table 4). Based on lamb mortality, symptomatic sheep, and hunter harvested rams, we believe that *M. ovipneumoniae* spread into Selah Butte by June or July, 2010. This would suggest that 1 or more animals moved eastward across the river before that time. Having crossed the Yakima River, subsequent mortality was mostly limited to lambs, and lamb recruitment in 2011 was higher east of the river than where symptomatic animals had been removed.

It is clear to us that visually identifying the animals that were shedding bacteria based on clinical signs was not possible. Even identifying sick sheep was problematic: we found no histological evidence in 20% of the animals euthanized in the South Umtanum sub-herd based on their displaying symptoms, whereas one animal from east of the Yakima River that had pneumonic lungs was *M. ovipneumoniae* negative on both PCR and serology. Our assessment is similar to the conclusions reached by Ramsey et al. (2017) that selective culling failed to contain or lessen pneumonia outbreaks in the East Fork Bitterroot or Bonner herds in western Montana (see also Edwards et al. 2010). Spatially-limited culling of animals during an outbreak in the Black Butte, Washington population in Hells Canyon was also unsuccessful at stopping disease spread (Cassirer et al 1996).

The lamb crop born in spring 2011 (13-15 months post culling) in the 2 sub-herds on the west side of the river had high survival, although *M. ovipneumoniae* was likely still present. East of the Yakima River, where no selective removals took place, lamb recruitment in subsequent years was variable. The disease, which was not detected east of the river during the winter 2009-2010 epizootic, caused less lamb mortality when it arrived in summer 2010. Since 2013, pneumonia has caused almost complete recruitment failure on each side of the river in at least 1 of the years monitored (Fig. 7).

Although all animals tested possessed the same strain of *M. ovipneumoniae* and all sub-groups have shown at least some evidence of poor lamb recruitment, sub-groups have displayed heterogeneity in the timing and severity of lamb recruitment failures (Fig. 7). This suggests variation in transmission to lambs, which could either reflect movement of animals among sub-herds, or temporal heterogeneity in immune response (Plowright et al. 2013). Co-infections with *Pasteurellaceae* (Table 2) or other aerobic or anaerobic bacteria could also play a role in disease severity. It would require additional concurrent sampling and monitoring to clarify

the reasons for the heterogeneity in long-term response we've noted among these sub-herds. The Yakima Canyon case-study is also noteworthy in exemplifying the difficulty biologists and veterinarians can face in detecting the presence of *M. ovipneumoniae* in adult animals and shows that serology is a more sensitive test than PCR for classifying the *M. ovipneumoniae* status of a population. In our 2013 sampling, we detected *M. ovipneumoniae* in nasal mucosae in none of 31 animals (and only confirmed the bacteria's presence in 1 of the 31 animals by testing for it in nasal washes from the 2 animals with levels considered indeterminate). In combination with our finding only 2 of 17 positive results during 2015 sampling, our best estimate is that prevalence of *M. ovipneumoniae* shedding in adults during the 2013-2015 period of poor lamb recruitment was 6%. At the same time, all 7 lambs collected lethally or found dead during May-September were *M. ovipneumoniae*-positive on PCR.

Asotin herd

As of 2016, it appears that the Asotin herd, although still small, has emerged from the disease event of early 2012, and is free of both *M. ovipneumoniae* and respiratory pneumonia (Table 4). Lamb recruitment 3 and 4 years post-outbreak has returned to pre-outbreak levels, and extensive capture and testing during 2012-2015 indicated very little if any *M. ovipneumoniae* circulating in the population. It is possible that our removal of a few positive animals, after the outbreak when prevalence of carriers was low, purged the population of carriers. The possibility that, with a small-sized herd, carriers died out without our intervention also cannot be ruled out. So far the population has experienced 4 consecutive healthy years after the outbreak. Since 1995, no other population in Hells Canyon has had more than 3 years of good lamb recruitment before recurrence of pneumonia (Manlove et al 2016). However, Asotin is part of a metapopulation of interconnected *M. ovipneumoniae* positive herds. A few more years of monitoring will be useful to assess longer term health status.

Tieton herd

Although it is impossible to know what would have occurred had we not euthanized the remaining Tieton herd individuals, available evidence suggests that neither pathogen nor disease had spread to the adjacent Cleman Mountain herd as of 2016 (Table 4). Based on proximity, and documentation of movement of sheep between the two herd ranges – as well as our experience in the Yakima River Canyon -- we believe that *M. ovipneumoniae* would likely have spread to the Cleman Herd and caused substantial mortality, had we not removed the remaining Tieton animals.

Our decision to remove the entire Tieton herd was not made lightly. Further (but not surprisingly), it was difficult to achieve in practice. It required 6 months and considerable effort to remove the remaining 57 sheep and much was learned. By mid-May when we were able to fully mobilize, the surviving individuals had begun retreating to rugged and forested terrain where finding and removing them was more difficult than the lower-elevation and more open habitats seemingly favored during winter.

One reason we contracted with USDA Wildlife Services to implement the removal was that most WDFW staff were equipped for humanely euthanizing animals at short distances. In contrast, Wildlife Services' use of a helicopter-based shot-gun marksman allowed relatively rapid disposition of animals even in the more remote areas. That said, even with this approach sheep became more difficult to remove with time. Wildlife Services helicopter-based shooting removed 24 animals during the first day but none thereafter, and all subsequent removals resulted from ground-based shooting due to a combination of poor weather and wary

behavior in response to helicopter flights. We speculate that bighorns in this herd were particularly sensitive to helicopters because some had been captured 3 of the previous 4 years by helicopter net gunning.

Following the departure of USDA Wildlife Services, we used standard trail cameras baited with salt to assess how many sheep were left in the area and location. We found that cameras helped us understand how many animals remained, but, because we were able to check them on foot only every 7-10 days, were of little help to shooters attempting to find and remove them. Cameras that send real-time images to computers or cell phone would have been useful.

We found the use of general hunters of limited utility in removing the last few remaining animals. Permitted tribal hunters realized quickly that remaining sheep were scarce, wary, and few, if any, were large rams. We believe this would have been the case with any non-selective process (i.e., had we permitted non-tribal hunters in addition). Instead, our success with non-WDFW staff in removing the few remaining animals depended on soliciting and selecting hunters with the specific motivation, equipment, and skills. The last ewe had become extremely wary of hunters and would not allow anyone within range of most rifles. We were fortunate in having an individual capable of consistent, lethal shots at >800 meters.

Management implications

Review of data collected during the outbreak and post-outbreak monitoring have provided some insights into how bacterial bronchopneumonia may be maintained and spread among bighorns during both invasion and persistent phases of the disease. Our data are far from capable of providing a thorough answer, but they can be used to support or explore various hypotheses. In both the Asotin and Yakima Canyon case studies, we documented widespread intra-herd transmission of *M. ovipneumoniae* during the acute invasion phase. Thus far, patterns observed in the persistence phase in both case studies are consistent with, although insufficient to confirm, the super-shedder hypothesis (Lloyd-Smith et al 2005, Fortin and Cassirer 2015). Data from Yakima Canyon suggest that infection status of individuals can change over time (several years). In both populations, we noted a return to healthy lamb recruitment coincident with documenting a low prevalence of *M. ovipneumoniae* carriers in the population. In the smaller Asotin herd it appears that the only carriers were removed and/or died or recovered naturally and the population is healthy. In the larger Yakima Canyon herd, which continued to experience poor (if heterogeneous) lamb recruitment due to pneumonia-induced mortality at least 6 years post-outbreak, carriers remain at low prevalence.

With the Yakima Canyon herd still large, we have time to attempt some experimentation, but the large size of the herd makes some potential options particularly difficult. Although removing any animal identified as a super-shedder would be possible, it would be prohibitively difficult to capture and test all of the estimated 173 ewes currently in the population (to say nothing of testing positive animals repeatedly). However, the sampling conducted during 2013 and 2015 suggests that the proportion carrying *M. ovipneumoniae* in their respiratory tracts, and presumably being responsible for disease persistence, is quite low. We do not know what percentage of 1 year-old animals are shedding or are in contact with lamb groups in Yakima Canyon (although see Manlove et al. 2017 for evidence that, in Hells Canyon, contact from infected yearlings rarely resulted in disease). To minimize the logistic challenges of large herd size, we could elect to perform experiments only on 1 or 2 sub-herds or on one side of the river. A complication is

that there may be sufficiently frequent movement among sub-herds that even should all the super-shedders within any focal sub-herd be identified and removed, the disease could be reintroduced from a separate, unstudied sub-herd. However, the spatial variability we observed in recruitment suggests that separation of sub-herds during lamb-rearing may restrict or delay pathogen transmission by chronic carriers.

As one of the focal herds in the inter-state Hells Canyon Initiative, research will continue on the Asotin herd, including continued health and demographic monitoring. Animals testing positive for *M. ovipneumoniae* will be removed (non-lethally if a captive facility can use them for research purposes). If no carriers are found and the lamb recruitment continues to show no evidence of pneumonia-related mortality, this will represent only the second population in Hells Canyon in over 3 decades that has cleared disease following a pneumonia outbreak (Coggins 1992, Cassirer et al, 2013, Manlove et al. 2016). Further testing of the super-shedder hypothesis is planned or ongoing in other populations and will help determine whether fadeout can be assisted by management actions or whether this was a natural stochastic purging of the pathogen.

That the Tieton herd exhibited rapid growth and attained a relatively high density (6.2 sheep per km^2) within a few years' time following reintroduction suggests that habitat conditions there were excellent. However, the continued presence of domestic sheep legally grazing on nearby federal allotments poses a risk of future pathogen transmission. In addition to the domestic sheep WDFW documented on core Tieton range just prior to the outbreak, the lessee lethally removed stray domestics in at least 2 other years. It is therefore prudent to await additional assurance that Tieton-herd bighorns would not come into contact with domestic sheep before conducting a reintroduction.

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The Montana-Wyoming Collaborative Bighorn Sheep Research Program

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ABSTRACT 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 the more successfully restored ungulate species. Two complementary long-term research programs have been initiated in Montana and Wyoming to help address the need for a better understanding of bighorn sheep ecology. These studies are designed on the premise that research insights that are broadly applicable for management and conservation are best obtained by addressing the same questions in multiple populations representing the range of variation realized by the species of interest. The studies were initiated in the Greater Yellowstone Ecosystem in 2009 and expanded to include bighorn populations throughout Montana in 2014. As of spring 2016, 17 bighorn herds were incorporated in the studies, which will continue until at least 2019. We selected herds to capture a wide range of variability in disease history, environmental settings, and herd attributes. We expected doing so would maximize variation in adult survival, recruitment, and population dynamics among herds. A multi-disciplinary team of agency biologists, academics, and graduate students are conducting the integrated studies that include investigations of health/physiology, spatial ecology, disease, genetics, and population dynamics (Fig. 1). A total of 476 animals (primarily ewes) have been captured via baited drop nets, ground-based chemical immobilization, and helicopter net gunning. Traditional physiological assessments including body weight, skeletal length, ultrasound rump fat measurements, body condition scores, and serum metabolite and hormone assays have been used to assess health, pregnancy, and body condition. We are also exploring the utility of nuclear magnetic resonance spectroscopy (NMR), an emerging technology in human medicine, in an attempt to develop a 'health panel' of metabolites and hormones that can provide a richer assessment of physiological status of ungulate populations. We have successfully developed NMR methods to identify and quantify a library of 53 biological molecules associated with a wide variety of physiological processes from <100 µl of serum. We are now evaluating associations between these data and reproduction, nutrition, body composition, and other physiological conditions. Genetic samples were also obtained from animals using FTA gene cards, whole blood, and/or tissue samples. High quality DNA is being

extracted from these samples, and is being used with the Ovine HD Single Nucleotide Polymorphism (SNP) array, with ~24,000 genetic markers informative for bighorn sheep, to address a variety of population- and individual-level genetic questions important for management. Preliminary findings suggest that levels of relatedness and inbreeding among herds are associated with management history. We are assessing pathogen communities hosted by each study population using Western Association of Fish and Wildlife Agencies (WAFWA) Wildlife Health Committee monitoring recommendations. In addition, we are collecting replicate swab samples from individual animals and using numerous diagnostic protocols to evaluate detection probabilities to help inform the development of more effective pathogen sampling protocols and interpretation of the resulting data. Telemetry is a tool that provides many ecological insights into spatial ecology, habitat selection, and demography and we have a goal of instrumenting a minimum of 20-30 adult ewes in each study population. We employ a dual collar strategy, instrumenting each animal with a GPS Store-on-board collar and a micro-VHF collar. The GPS collar collects fine spatial and temporal scale location data for ~2 years while the micro-VHF collar remains dormant. When the GPS collar falls off the animal at a preset time for collar recovery and data retrieval the micro-VHF begins to transmit, permitting an additional 4-5 years of monitoring for survival and coarse-scale movement data. As of spring 2016 we have instrumented 379 bighorn sheep and recovered the GPS data from approximately 190 of these animals. During the winter of 2016-17 we plan to capture and sample an additional 250 animals in Montana and Wyoming herds and instrument 160 of these animals with telemetry collars.

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KEYWORDS bighorn sheep, disease, genetics, health, Montana, *Ovis canadensis*, population dynamics, spatial ecology, Wyoming, Yellowstone National Park

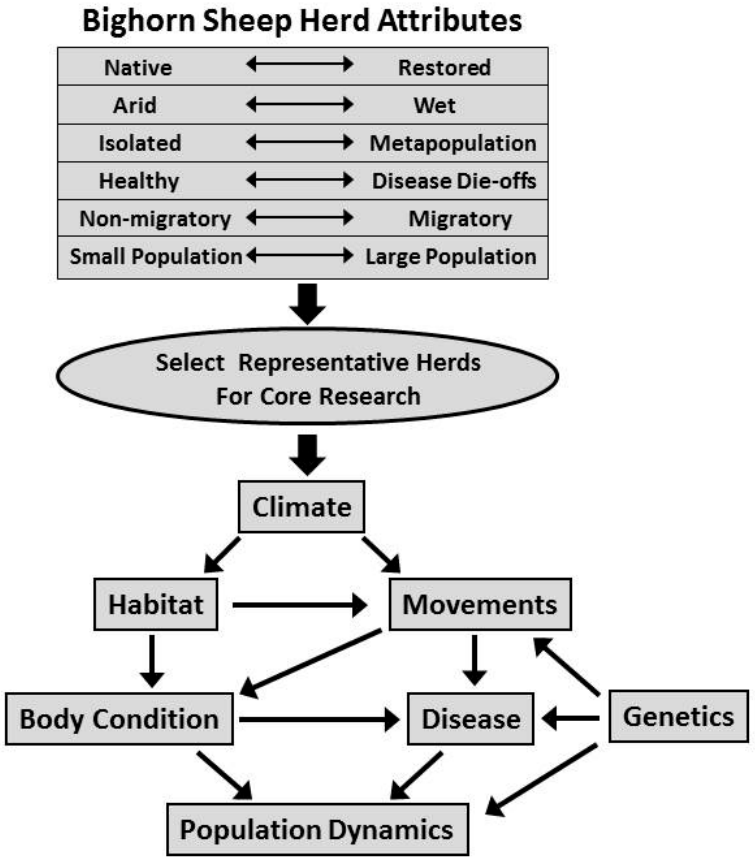

Figure 1. A conceptual diagram of the Montana-Wyoming bighorn sheep research program.

Evaluating Alternative Hypotheses to Explain Bighorn Sheep Respiratory Disease Events

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ABSTRACT Respiratory disease has been a major challenge for bighorn sheep conservation and is a dominant factor influencing management decisions of bighorn sheep. However, much about the disease process remains unknown. Decades of research have compiled considerable evidence that domestic sheep and goats transmit the disease to bighorn sheep. There is also strong evidence for several bacterial organisms commonly carried by domestic sheep and goats as causative agents for the disease. However, the bacterial agents linked to respiratory disease have been detected in several of the most robust bighorn sheep populations, such as the Missouri Breaks of Montana or the Greater Yellowstone Area of Wyoming. Further, the immediate cause of disease events often remains undetermined. We consider two general hypotheses to explain observed disease events in wildlife populations: 1) A disease event is caused by introduction of a novel pathogen from neighboring or sympatric host populations; or 2) A disease event is caused by certain conditions which trigger resident pathogens to increase in virulence or transmissibility. In the case of bighorn sheep respiratory disease, we consider resident pathogens to be pathogens that originated from domestic sheep or goats and likely caused a respiratory disease upon introduction to naive bighorn sheep populations, but continue to persist in a bighorn sheep population for an extended time period with minimal effects on the population. Although the extent to which these different hypotheses explain observed respiratory disease events in bighorn sheep is unknown, the appropriate management actions to address disease events caused by these processes are very different. Rigorous pathogen surveillance in populations with and without evidence of respiratory disease is necessary to evaluate the relative roles novel and resident pathogens play in causing respiratory disease. The pathogen community hosted by bighorn sheep populations prior to respiratory disease epizootics must be understood in order to conclude whether the epizootic was

caused by novel or resident pathogens. Limited evidence suggests that diagnostic protocols used to detect respiratory pathogens in bighorn sheep have low detection probability (i.e. sensitivity) and may falsely indicate some pathogens are not present in a sampled population. The detection probability of diagnostic protocols must be known in order to design rigorous respiratory pathogen surveillance programs. We collected multiple nasal swab and tonsil swab samples from 476 live bighorn sheep in Montana and Wyoming and employed several diagnostic protocols to detect *Mycoplasma ovipneumoniae* and relevant *Pasteurellaceae* organisms. This effort will estimate detection probabilities for diagnostic protocols commonly used by wildlife agencies to assess presence of respiratory pathogens in bighorn sheep, provide the ability to estimate confidence in negative test results at the population-level, and provide recommendations for rigorous pathogen surveillance programs. Initial findings confirm previous studies that suggested detection probability for *Pasteurellaceae* organisms is generally low (<40%), but varies among diagnostic protocols. In contrast, initial findings indicate that detection probability for *Mycoplasma ovipneumoniae* is generally high (>60%). The disparity in detection probability among the different pathogens may be explained by the availability of polymerase chain reaction (PCR) tests, which are not available through fee-for-service labs for *Pasteurellaceae* samples from bighorn sheep. These findings and concepts will be applied to a pathogen surveillance effort across Montana and Wyoming that aims to sample approximately 800 bighorn sheep from 17 populations (Figure 1).

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KEY WORDS bighorn sheep, demography, die-offs, disease, health, Montana, *Ovis canadensis*, novel pathogen, pneumonia, recruitment, respiratory pathogens, Wyoming

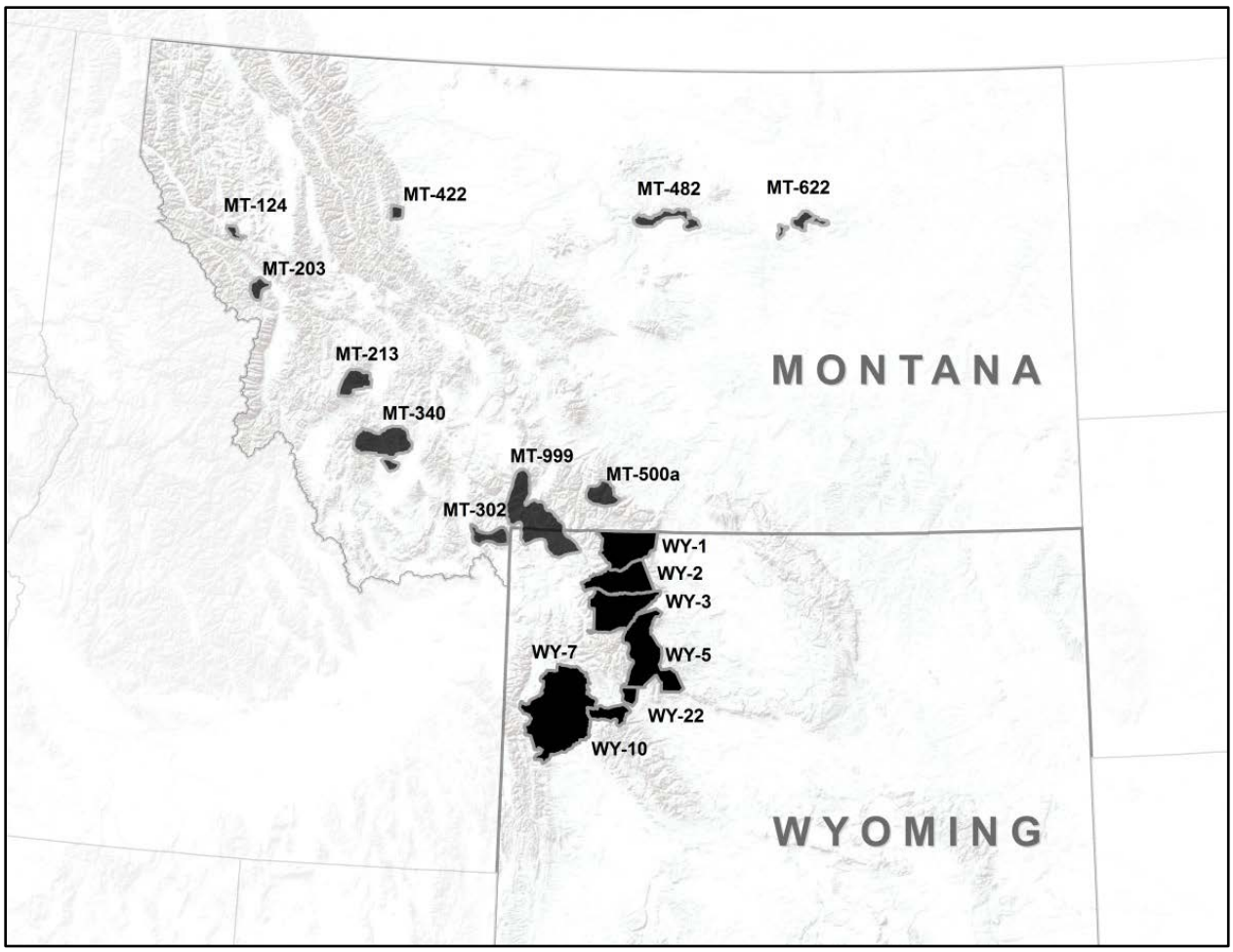

Figure 1. Bighorn sheep study populations in Montana and Wyoming to be collaboratively surveyed for respiratory pathogens. Labels correspond to the respective state's hunting districts.

Investigation of Pneumonia Mortalities in a *Mycoplasma*-positive Desert Bighorn Sheep Population and Detection of a Different Strain of *Mycoplasma ovipneumoniae*

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ABSTRACT Discovery of an emaciated and weak ewe on the banks of the Colorado River in northern Arizona was the first indication of a mortality event in the desert bighorn sheep population in the Black Mountains of Arizona. Herein we describe the documentation of an all age die-off due to *Mycoplasma ovipneumoniae* in a population of bighorns that was known to be positive for *M. ovipneumoniae*. We determined that the strain causing the mortality was different from the strain detected in previous surveillance. These results have implications for the management of bighorn sheep populations and disease monitoring.

In August 2015 an emaciated ewe was discovered in the Black Mountains, 5 miles south of Hoover Dam during a boat tour of the Colorado River by Arizona Game and Fish Department (AGFD, Figure. 1). Department personnel euthanized and necropsied the ewe in the field. Gross necropsy findings consisted of bilateral ventral consolidation of lung lobes, thickened bronchi containing white mucoid exudate, fibrinous adhesions between the lungs and the thoracic wall, and multiple white nodules within the lungs. Severe bacterial pneumonia with *M. ovipneumoniae* was diagnosed histologically, and supported by molecular testing of samples. The Black Mountain range is east of the Colorado River and extends from just north of Hoover Dam south to Interstate 40 (Figure 1). During population surveys in late September 2015, AGFD personnel observed 9 sheep carcasses and several sick sheep in the northern portion of the Black Mountains, approximately 7 miles east of the ewe's location. The bighorn sheep population in areas adjacent to the Colorado River was more than 75% below the 5-year aerial survey average.

The survey findings were strongly suggestive of a recent all age die-off, and the decision was made to conduct active disease surveillance in the affected management units (15CN and 15CS) west of US highway 93 and north of State Route 68. In November 2015 we captured and sampled 3 ewes from 3 different groups in the southern portion of the 15CS unit, approximately 30 miles south of the initial case's location. During the capture operations, many of the bighorns were exhibiting signs of respiratory infection, including the 3 sampled ewes. *M. ovipneumoniae* was detected by PCR from nasal swabs and *M. ovipneumoniae*-specific antibodies were detected with enzyme-linked immunosorbent assay (ELISA) in all 3 ewes. Two *Pasteurella* spp. were identified, one each in two of the ewes, and in one of these, the leukotoxin-A gene was detected.

Two weeks later, hunters reported coughing bighorns immediately south of State Route 68 in the adjacent game management unit, (approximately 58 miles of the location of the first ewe). As a result, we conducted additional surveillance in early December by selecting 3 ewes, one from each of 3 groups, for euthanasia. The ewes were in good body condition and did not display outward signs of disease. During necropsy, fresh and formalin fixed samples were collected from the cranial and caudal lung lobes bilaterally, as well as the right middle lung lobe. From the caudal lobes, we collected tissue near the main stem bronchus and the distal margin. The cranial and middle lung lobes of the ewes contained areas of consolidation, with thickened airways filled with mucopurulent exudate. No other abnormalities were detected on the necropsies. All 3 were diagnosed with acute bacterial pneumonia by histology. *M. ovipneumoniae*, *Bibersteinia trehalosi* (moderate to few), and *Fusobacterium necrophorum* (few to very many) were detected with PCR and microbial culture. Additionally, we collected swabs from 12 hunter-harvested bighorn rams from units 15C and 15D. *M. ovipneumoniae* was detected by PCR on 11 of the samples.

The Arizona Department of Game and Fish conducted disease surveillance on captured bighorns in these units during 2012 and in adjacent units annually from 2012 to 2014 during research and translocation projects. At that time, *M. ovipneumoniae* was detected by PCR conducted on nasal swabs in 5-10% of captured bighorns. Because we had detected *M. ovipneumoniae* in this population during these earlier surveillance events, we wanted to determine if the bighorns affected by pneumonia in 2015 were succumbing to the previously detected strain or a new strain, and if a new strain was detected, determine the source of the infection.

We performed strain-typing using partial sequences of four loci (16S, IGS, rpoB, and gyrB) from the *M. ovipneumoniae* in the affected bighorns ($n = 4$, Figure 2, Index case and Sampled ewes 1-3) in 2015 and from 3 bighorns from just east of Lake Mead (Figure 2, Grand Wash ewes 1-3) sampled as part of a population health assessment, and compared them to the sequences of *M. ovipneumoniae* detected in prior years and to organisms detected in bighorns captured south of the outbreak in November 2015. The strain identified in samples from 2012 to 2015 matched the strain identified in bighorn sheep sampled in the following southern Nevada mountain ranges in 2013, including the Spring Mountains, El Dorado Mountains, McCullough Range, and River Mountains (Figures 1 and 2). The *M. ovipneumoniae* detected in bighorns with pneumonia in 2015 differed from the previously identified strain and instead matched a strain initially detected in a mortality event at Old Dad Mountain (Providence Mountains, Figure 2, CA 2013 Mohave outbreak) in California in 2013. This strain was later detected during sampling efforts in response to detected disease events in the Spring Mountains (2013), River Mountains (2014), and Eldorado Mountains and McCullough Range (2015) of Nevada (Figure 2, NV2013-4 Mohave outbreak). Additionally, IGS sequences from the 3 euthanized ewes and 5 of 5 hunter-harvested rams matched the IGS sequence of the Mohave outbreak strain.

Prior to the detection of pneumonia in the ewe found on the Colorado River and the identification of mortalities during the subsequent fall surveys, the bighorn sheep population in the Black Mountains of Arizona was considered to be productive despite the presence of *M. ovipneumoniae*. The occurrence of respiratory disease in conjunction with the detection of a different strain of *M. ovipneumoniae* suggests that strains vary in pathogenicity and that exposure to one strain does not induce a broad immunity across strains. This is consistent with mycoplasma infections in domestic chickens and turkeys, as well as house finches (Kang et al. 2002, Hinz et al. 2003, Grodio et al. 2012), and has also been reported in bighorn sheep *M. ovipneumoniae* pneumonia (Cassirer et al. 2017).

The apparent movement of this strain of bacteria from Old Dad Peak to the Black Mountains as indicated by the sequential detections from California east to Nevada and Arizona suggests that the disease was likely introduced by the natural movement of bighorn sheep. Repeated introductions of the identical strain of mycoplasma into multiple populations of bighorns from multiple exposures to domestic sheep and goats are unlikely as mycoplasmas undergo frequent strain differentiation (Besser et al. 2012, Tulman et al. 2012, Spergser et al. 2013, Sulyok 2014). The events documented in this report provide additional clues to the epidemiology of bighorn sheep pneumonia and provide support for routine monitoring of bighorn sheep populations for the occurrence of pathogens with the inclusion of strain-typing and geospatial epidemiological modeling including population interaction parameters.

Biennial Symposium of the Northern Wild Sheep and Goat Council 20:68-72.

KEYWORDS Bighorn sheep pneumonia, *Fusobacterium necrophorum*, molecular epidemiology, *Mycoplasma ovipneumoniae*, strain-typing.

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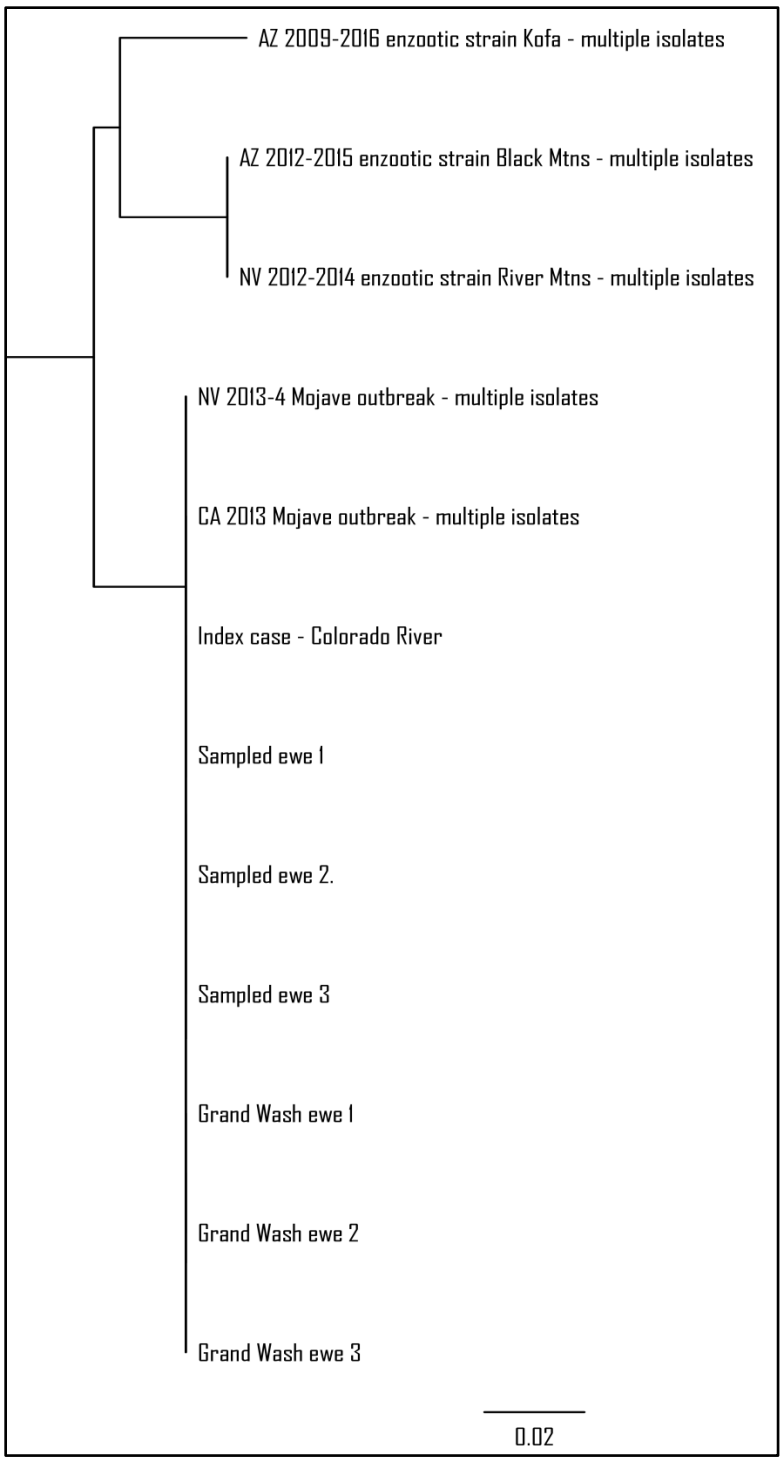

Figure 1. Location of disease event cases in the Black Mountains of Arizona and nearby mountain ranges in California and Nevada.

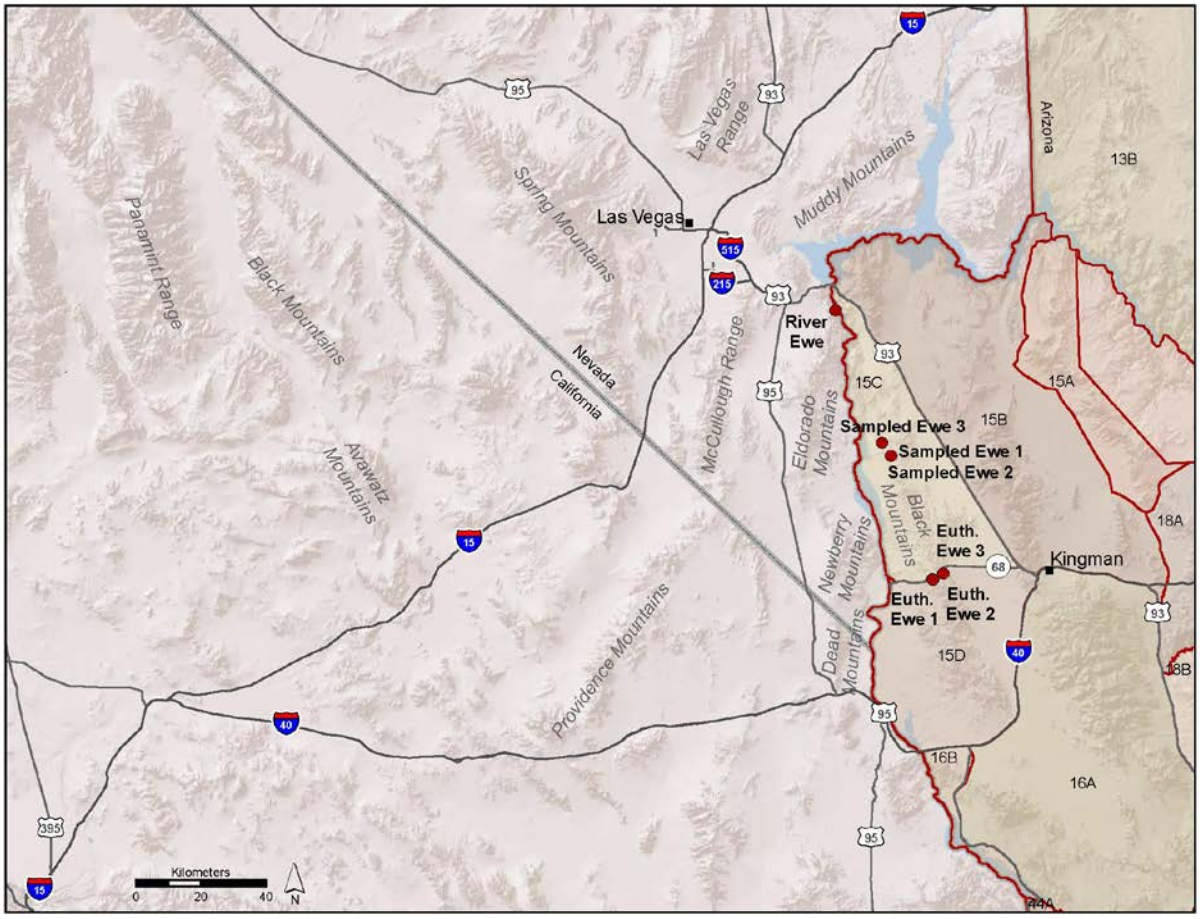

Figure 2. Phylogenetic tree of the *Mycoplasma ovipneumoniae* strains identified in southern California, Nevada, and Arizona prior to the all age die-off and from bighorns sampled during active disease surveillance after the die-off.

Understanding the Dynamics of *Mycoplasma ovipneumoniae* carriers in a Bighorn Sheep Population

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ABSTRACT We tested the hypothesis that pneumonia is maintained in bighorn sheep populations by asymptomatic carriers of the pathogen *Mycoplasma ovipneumoniae*. Our objectives were to (i) estimate the variation among individuals in the extent and duration of *M. ovipneumoniae* shedding, (ii) estimate the prevalence of chronic carriage, (iii) identify attributes that can be used to predict carriage, and (iv) determine whether a relationship exists between maternal chronic carriage and lamb survival. We collected upper respiratory samples and serum from over 80 individuals in the Lostine population in northeastern Oregon multiple times between 2009 and 2015. We report on patterns of *M. ovipneumoniae* shedding and exposure in adults, lambs, and yearlings relative to health, survival, and lamb recruitment.

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KEY WORDS carriers, disease shedding, *Mycoplasma ovipneumoniae*, Oregon

Disease introduction is associated with a phase transition in bighorn sheep demographics

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ABSTRACT Ecological theory suggests that pathogens are capable of regulating or limiting host population dynamics, and this relationship has been empirically established in several settings. However, although studies of juvenile disease were integral to the development of disease ecology, few studies show population limitation by a juvenile disease. Here, we studied twelve bighorn sheep (*Ovis canadensis*) populations and found a strong association between disease invasion and ensuing host population growth rates. While bighorn populations generally increased (λ =1.11) prior to disease introduction, most of these same populations experienced an abrupt change in trajectory at the time of disease invasion, usually followed by stagnant-to-declining growth rates (λ = 0.98) over the next twenty years. Disease-induced juvenile mortalities imposed strong constraints on population growth that were not observed prior to disease introduction, even as adult survival returned to pre-invasion levels. Simulations suggested that models with persistent juvenile disease qualitatively matched observed population trajectories, whereas models that only incorporated all-age disease events did not. We use these results to argue that pathogen persistence may pose a lasting, but under-recognized, threat to host populations, particularly in cases where clinical disease manifests primarily in juveniles.

Biennial Symposium of the Northern Wild Sheep and Goat Council 20:74.

KEY WORDS bighorn sheep, childhood disease, integrated population model, pathogen persistence, population projection matrix, vital rates, disease-induced mortality, wildlife disease, demographic trends

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Evidence for Strain-specific Immunity to Pneumonia in Bighorn Sheep

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ABSTRACT We used multi-locus sequence typing to document the introduction of a novel genotype (strain) of *Mycoplasma ovipneumoniae* into a free-ranging bighorn sheep population. Despite preexisting serologic antibodies and asymptomatic carriage of *M. ovipneumoniae* in this population, introduction of the new strain was accompanied by adult morbidity (100%) and pneumonia-induced mortality (33%) within the range observed in epizootics following exposure of naïve bighorn sheep. During the outbreak the new strain replaced the original strain in the population. To understand the broader context surrounding this strain introduction, we conducted a retrospective analysis of *M. ovipneumoniae* strains in 123 lung and upper respiratory tract samples from 14 interconnected populations within the region over nearly 2 decades. We identified 5 distinct genotypes of *M. ovipneumoniae* associated with all-age disease outbreaks between 1986 and 2014: a pattern consistent with spillover events from reservoir hosts. Some strains persisted and spread across populations, whereas others apparently faded out or were replaced. We use phylogenetic analysis to show that the strain associated with this outbreak was likely of domestic goat origin, whereas strains from other recent disease outbreaks in this metapopulation probably originated in domestic sheep. Lack of cross-strain immunity may account for a significant proportion of the disease outbreaks in bighorn sheep that continue to occur regularly despite over a century of exposure to pathogens carried by domestic sheep and goats. Enhanced efforts are indicated to avoid introducing new strains of *M. ovipneumoniae* into wild sheep populations, even if they have previously been exposed.

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KEYWORDS bighorn sheep, disease ecology, Hells Canyon, livestock-wildlife interface, molecular epidemiology, multi-locus sequence typing, *Mycoplasma ovipneumoniae*, *Ovis canadensis*

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Risk of Pathogen Spillover to Bighorn Sheep from Domestic Sheep and Goat Flocks on Private Land

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ABSTRACT Bighorn sheep (*Ovis canadensis*) across North America have suffered population losses due to polymicrobial pneumonia typically initiated by spillover events of bacteria from domestic sheep and goats. Because vaccination or treatment of individual animals remains an elusive goal and pneumonia often persists in bighorn herds for years or decades following infection, preventing contact between domestic and wild animals is widely accepted as the best prophylactic. For the past decade, most management efforts have focused on the risks associated with commercial sheep grazing on public lands; less attention has been paid to risks to bighorns from small flocks of domestic sheep and goats managed entirely on private land. We surveyed owners of 40 sheep or goat flocks located near bighorn sheep herds in central and southeastern Washington, USA during 2014 and 2015, to better understand their knowledge level, management practices, and willingness to reduce risks. Over one-third of sheep or goat owners had no knowledge of the potential for pathogen spillover to bighorns, but all were interested in reducing risk of interacting with bighorns, particularly by options that did not restrict their autonomy. We also sampled nasal mucosae of 137 animals in 24 flocks for presence of *Mycoplasma ovipneumoniae*, the bacterium most closely associated with bighorn pneumonia. *M. ovipneumoniae* was detected in 37.5% of sheep or goat flocks sampled, and animals had escaped their enclosures in 78% of these. Physical contact, and thus pathogen spillover from domestic sheep or goats living in small, private flocks in close proximity to bighorns is clearly a risk. We provide recommendations to agency staff on identifying, prioritizing and testing small herds, and then working with owners to reduce the risk of pathogen spillover.

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KEY WORDS bighorn sheep, disease, domestic goats, domestic sheep, landowner attitudes, *Mycoplasma ovipneumoniae*, *Ovis canadensis*, pneumonia.

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Mycoplasma ovipneumoniae Cross-strain Transmissions in Captive Bighorn Sheep

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ABSTRACT Bighorn sheep (*Ovis canadensis*) researchers and managers continually face dynamic challenges associated with population-limiting bronchopneumonia epizootics. Although the etiology of pneumonia is not completely understood, we consider *Mycoplasma ovipneumoniae* to be a primary pathogen responsible for bighorn sheep respiratory disease. Some individuals that have recovered from initial *M. ovipneumoniae* disease outbreaks become carriers of the *M. ovipneumoniae* strain type encountered, and these strains are usually unique in independent outbreaks. Our objectives are to present information resulting from accidental *M. ovipneumoniae* cross-strain transmission and explore factors that might influence the rate of individual bighorn sheep transitioning through the disease process. All experiments were conducted at the South Dakota State University Captive Wildlife Research Facility (SDSU CWRF). Free-ranging bighorn sheep used for this study were identified through collaborative efforts during the routine disease surveillance efforts of state wildlife managers and researchers, and selective removal of individuals from the wild to prevent further spillover infections to adjacent bighorn sheep populations (e.g., young rams that come into close proximity to domestic sheep flocks). We obtained adult bighorn sheep from source herds in Asotin, Washington (n = 9); Black Butte, Washington (n = 8); Lostine, Oregon, (n = 5); Sheep Mountain, Idaho (n = 2); Rapid Creek, South Dakota (n = 1); Badlands, South Dakota (n = 2); and Snowstorm Mountains, Nevada (n = 11). We assigned adults to pens based on source herds and sex, which were separated by ≥ 15m in an effort to prevent aerosol pathogen transmission across pens. We anesthetized and sampled all adults at 4-6 week intervals from January to March, and again from October to December or when no dependent lambs were present, annually during 2014–2015. We collected nasal and oropharyngeal swabs at each sampling event. We extracted DNA and used PCR to detect *M. ovipneumoniae* in nasal swabs or lung tissue. We used multi-locus sequence typing of the 16S-23S intergenic transcribed region (IGS) and 3 genes (16S, ryoB, and gyrB) to characterize strain types. We detected 4 strains of *M. ovipneumoniae* and refer to strain types by IGS length: 393 (Black Butte herd), 398 (Badlands and Rapid Creek herds), 400 (Snowstorm herd), and 404 (Asotin, Lostine, and Sheep Mountain herds). After cross-strain exposure, 84% (n = 32) of sheep actively shed 400 strain bacteria, which we infer contributed to high morbidity (100%; n = 38). We documented high adult pneumonia mortality (> 25%; n = 11) attributed to the 400 strain in upper respiratory tracts and in acute lung lesions at necropsy from July 2015 to February 2016. During this time, we documented a 4-fold increase in apparent *M. ovipneumoniae* prevalence (from 0.19 to 0.83), which we infer was the result of unintentional cross-strain transmissions that occurred in our study. We failed to detect more than 1 strain in any sample, and concluded that the 400 strain likely replaced all prior strains. We characterized the dynamics observed at SDSU CWRF using an epidemiological Susceptible-

Infected-Recovered (SIR) compartmental hazard-based model with spatial effects, initial strain type, and immune response as covariates influencing disease dynamics. We found support for models in which response of individual bighorn sheep to cross-strain transmission was a function of previous strain exposure. In general, bighorn sheep previously exposed to the 393 strain were less affected by cross-strain transmission in our study. In contrast, adults previously exposed to 398 and 404 strains were more susceptible to cross-strain infection and associated mortality. Our results underscore the importance of considering strain-specific *M. ovipneumoniae* exposure history when making bighorn sheep management decisions. Previously exposed bighorn sheep populations that experience spillover infections caused by a novel strain challenge may experience high adult morbidity and mortality similar to epidemics recorded in *M. ovipneumoniae* naïve populations upon first introduction of this pathogen. Spillover infections caused by the introduction of novel strains from adjacent free-ranging populations may impede bighorn sheep recovery in some areas.

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KEY WORDS cross-strain transmission, exposure history, *Mycoplasma ovipneumoniae*, strain

Disease Transmission between Sympatric Mountain Goats and Bighorn Sheep

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ABSTRACT In 2009-10, Rocky Mountain bighorn sheep (*Ovis c. canadensis*) herds in the adjacent East Humboldt Range (EHR) and Ruby Mountains (RM) in Elko County, NV suffered an all-age pneumonia die-off with an estimated loss of 90% in each herd. Sympatric mountain goats (*Oreamnos americanus*) also experienced pneumonia with an estimated 10-20% loss in both herds (see Wolff et al 2014, Anderson et al, 2017). *Mycoplasma ovipneumoniae* was confirmed as a contributing pathogen in both bighorn sheep and mountain goats, and the same strain was identified in both species and ranges. In 2013, after removing the remaining 15 bighorns from the EHR, 20 bighorn sheep from Alberta, Canada were translocated to this range to assess whether surviving mountain goats would pose a threat to naïve, sympatric bighorns. At the time of translocation (n = 20), and during subsequent sampling events in 2014 (n = 7) and 2015 (n = 13), all sheep sampled were negative for *M. ovipneumoniae* by both ELISA and reverse transcription polymerase chain reaction (RT-PCR) on nasal swabs. Concurrent sampling of the EHR mountain goats for *M. ovipneumoniae* by RT-PCR indicated a prevalence of 6% (n = 15) in 2013, 12% (n = 16) in 2014, and 18% (n = 11) in 2015. From 2010-15 winter aerial surveys, annual mountain goat kid ratios ranged from 0 to 17 per 100 adults ($\bar{x}$ = 7), with an estimated λ of 0.60 for the herd over the same time period. From 2013-15, we conducted summer ground observations on both species. In 2014 and 2015, we observed association of mountain goats with bighorns (as close as 2 meters apart). Between September and December 2015, clinical signs of respiratory disease were noted in the bighorns, and multiple mortalities observed. *M. ovipneumoniae* was identified in pneumonic lungs of bighorns, and 16S RNA IGS sequencing confirmed a match to the strain isolated from the mountain goats. These findings suggest that potential disease transmission between mountain goats and bighorn sheep should be considered where range overlap occurs.

Biennial Symposium of the Northern Wild Sheep and Goat Council 20:79.

KEYWORDS disease transmission, mountain goat, *Mycoplasma ovipneumoniae*, Nevada, *Oreamnos americanus*, *Ovis canadensis*

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Detection of M. ovipneumoniae in Pneumonic Mountain Goat Kids

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ABSTRACT Pneumonia epizootics resulting in all-age die-offs followed by sporadic reoccurring summer lamb mortalities due to polymicrobial pneumonia have been well documented in bighorn sheep (*Ovis canadensis*). Following a pneumonia epizootic in bighorn sheep and sympatric mountain goats (*Oreamnos americanus*) in Nevada, we documented respiratory disease in kids during late July and early August during aerial surveys in 2011-2016. We noted coughing, head shaking, neck extension, nasal discharge, incoordination, and lethargy among kids we observed. We diagnosed 7 of these mountain goat kids with gross and histologic lesions consistent with *Mycoplasma* sp. infection leading to polymicrobial bronchopneumonia. *M. ovipneumoniae* was detected in the lungs with real-time PCR from all 7 kids. This is the first diagnosis of bronchopneumonia due to *M. ovipneumoniae* in mountain goat kids. Because the overall pattern of disease appears similar to that seen with bighorn sheep, *M. ovipneumoniae* infection may affect kid recruitment in mountain goats.

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KEYWORDS mountain goat, *Mycoplasma ovipneumoniae*, Nevada, *Oreamnos americanus*

Bighorn Sheep Sinus Tumors, an Update

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ABSTRACT In 2009, bighorn sheep sinus tumors were discovered within a herd of seven Rocky Mountain bighorn ewes in Colorado, USA that were culled due to a history of at least 10 years of failed lamb recruitment. Since discovery, at least 38 cases of sinus tumors have been identified in at least 10 free ranging bighorn herds in Colorado. Additional cases have been identified in Rocky Mountain bighorns from Wyoming, Nevada, and Nebraska, as well as one herd of desert bighorn sheep in California, and one herd of California bighorn sheep in Nevada. The disease has been shown to be infectious experimentally and likely has moved across the landscape through natural and artificial movements of bighorn sheep. Although sinus tumors alone do not appear to affect adult survival or lamb recruitment, sinus tumors in combination with other typical respiratory pathogens have been consistently identified in Colorado bighorn herds that are struggling with dismal lamb recruitment. Theoretically, sinus tumors may affect the susceptibility of adult bighorns to pneumonia through interference with normal clearance mechanisms of the upper respiratory tract. We provide the illustration below to alert biologists, managers, or others who may have access to bighorn sheep skulls or horn, to the possible presence of skull and sinus tumors.

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KEYWORDS co-infections, infectious, solid mass, tumor, upper respiratory sinuses

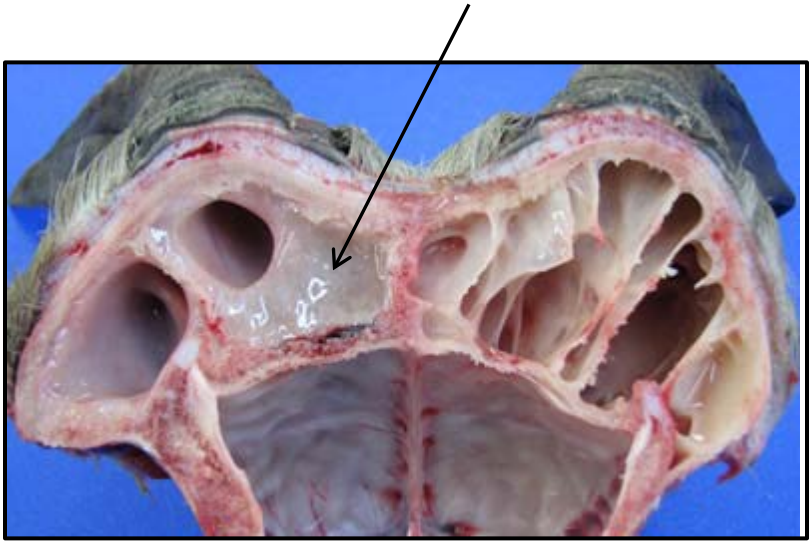

Figure 1. Tumor formed in the right-side of the forehead (contrast the sinus occluded by the tumor, left side arrow, with sinus free of tumor, right side).

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An Improved Method for Culturing *Mycoplasma ovipneumoniae* from Field Samples

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ABSTRACT To better understand the distribution, occurrence, and role of *Mycoplasma ovipneumoniae* in the epidemiology of respiratory disease in bighorn sheep, techniques that accurately and consistently detect this organism in wild sheep populations are needed. We reviewed published techniques and compared commercially available growth media to optimize the growth of *M. ovipneumoniae* in our laboratory. Penicillin, amphotericin B, thalium acetate, and phenol red were added to tryptone soya broth. Incubating field samples at 37 C in 10 % CO_2, for 48 hr, followed by direct plating onto solid media, improved detection, culture success, and overall agreement between culture and detection by polymerase chain reaction.

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KEY WORDS bighorn sheep, culture, modified TSB-1, mountain goat, *Mycoplasma ovipneumoniae*, *Ovis canadensis*, *Oreamnos americanus*, polymerase chain reaction (PCR) assay, pneumonia

Mycoplasma ovipneumoniae causes persistent infections that are difficult to detect, diagnose, or cure in susceptible hosts (Razin 1996). Some investigators consider *M. ovipneumoniae* to be a primary cause of bronchopneumonia in North American bighorn sheep (*Ovis canadensis*; Besser et al. 2008, 2012, 2013), whereas others consider this agent a contributing pathogen that, when present can predispose to or exacerbate respiratory disease caused by pathogenic *Pasteurellaceae* (Dassanayake et al. 2010; Wolfe et al. 2010). Recent efforts to better understand the occurrence, distribution, role, and perhaps control of *M. ovipneumoniae* in bighorn respiratory disease have been hampered by inconsistency in detection methods and between investigating laboratories. It follows that such efforts would collectively benefit from more reliable approaches for detecting this agent in field samples. Here we describe work focused on optimizing media, techniques and environmental conditions for growing and detecting *M. ovipneumoniae* in diagnostic

samples collected from bighorn sheep under field conditions.

Sample area

We collected samples from seven free-ranging bighorn sheep and three free-ranging mountain goat herds from Wyoming, and fourteen free-ranging bighorn sheep herds from Colorado. Captive bighorn sheep from both the Wyoming Game & Fish Department and the Colorado Parks & Wildlife research facilities; three mule deer, two domestic sheep, and one domestic goat from Wyoming were also sampled.

Methods

In total, we analyzed 1,186 samples in this study, including 1,005 nasal swabs and 181 various tissues and swabs. Samples were collected from October of 2010 through July of 2015 and included animals of both sexes and all age classes. Samples were received and processed within 48 hr of collection.

SP4-G

Using a general *Mycoplasma* spp. culture protocol provided by Dr. Tom Besser (Washington State University, personal communication) and procedures outlined by Nicholas et al. (2008), we used nasal swabs (sterile polyester tipped applicators; Puritan#25-806 1PD, Guilford, Maine, USA) and "SP4 with glucose" broth (SP4-G; Hardy Diagnostics #R86, Santa Maria, California, USA) for *M. ovipneumoniae* detection. Samples were transported on cold packs, in Port-A-Cul™ tubes (modified Carey-Blair; Becton Dickinson #221606, Franklin Lakes, New Jersey, USA; n = 703), or early during the study, in 3ml Amies media without charcoal in a 15 x 103mm Triforest culture tube (Triforest Enterprises, Irvine, California, USA; n = 153). Swabs were removed from the transport media and placed into individual tubes of SP4-G. Tubes were incubated with caps loosened at 37 C with 10% CO_2 for 4 days. After incubation, one plate of Columbia Blood Agar (CBA) with 5% sheep blood (Hardy Diagnostics #A16, Santa Maria, California, USA) was inoculated with 100 µl of broth. Inoculum was spread across half of the plate with a dry sterile polyester swab, and then streaked for isolation across the remaining half of plate. We incubated plates at 37 C with 10 % CO_2 for 7 days, and checked for growth daily. A 250 µl aliquot of broth was removed for DNA extraction on day four. DNA was extracted according to extraction kit instructions (E.Z.N.A. Tissue DNA Kit, Omega Bio-Tek, Inc, Norcross, Georgia, USA). DNA was analyzed using primers and PCR protocol published by McAuliffe et al. (2003), and optimized in our lab using the following protocol: initial denaturation for five minutes at 94 C, 32 denaturation cycles for 30 sec each at 94 C, annealing at 57.5 C for 30 sec, and extension at 72 C for 30 sec. The final extension was 72 C for 5 min. Samples were kept at 4 C until analyzed by agar gel electrophoresis.

TSB-1

As an alternative, we experimented with switching from SP4-G to tryptone soya broth (TSB-1; Patel et al. 2008), and incorporated amphotericin B, penicillin, and thallium acetate (Razin 1996) to inhibit contamination. Samples were cultured in 2 ml of this modified TSB-1 (mod TSB-1) in 5 ml round-bottom tubes (BD Falcon 352054, Franklin Lakes, New Jersey USA). Culture and PCR protocol remained the same.

During this study, we made minor changes to improve the culture protocol. Spreading inoculum on the CBA plate was facilitated by a polyester swab soaked in corresponding broth sample instead of a dry polyester swab. Culture plates were read for 5 days, instead of 7 days. Also, 1 ml (i.e., 1,000 µl) of broth was aliquoted for PCR instead of 250 µl, because this matched the protocol used by the Washington Disease Diagnostic Laboratory (WADDL) in Pullman, WA, USA. A negative control of modified TSB-1 was

also used to ensure no contamination of the stock modified TSB-1. This control consisted of 1 ml of modified TSB-1 that was incubated and analyzed via PCR under the same conditions and protocols as samples.

Results

SP4-G

Several initial *Mycoplasma* culture isolates were identified by gross characteristics (i.e., small, round, center-less areas of hemolysis) and confirmed as *M. ovipneumoniae* via sequencing of the polymerase chain reaction (PCR) product and comparison with published *M. ovipneumoniae* sequences (National Center for Biotechnology Information [NCBI] Basic Local Alignment Search Tool [BLAST]; Wyoming State Public Health Laboratory, Cheyenne, Wyoming). We identified all subsequent culture isolates based on gross characteristics only. Using the initial *M. ovipneumoniae* enrichment protocol, with SP4-G (Table 2) as the standard culture broth and a 4- day incubation period, 33% (42/129) of nasal swabs were positive for *M. ovipneumoniae* by PCR. This culture method provided 26% (11/42) culture success (recovery rate) when compared to PCR results. In addition to a low recovery rate, this method was also associated with substantial bacterial contamination with 42% (53/125) of CBA plates examined exhibiting gross evidence of contamination.

Modified TSB-1

Switching from SP4-G to tryptone soya broth (TSB-1; Patel et al. 2008), and incorporating amphotericin B, penicillin, and thallium acetate (Razin 1996) nominally increased culture success with a 2-day incubation period (Fisher's exact P=0.582; Table 1); there was a significant difference in amount of contamination (Fisher's exact P=0.0108; Table 1). A final formulation for modified TSB-1 is found in Table 3. To assess our optimized modified TSB-1 media and incubation time, a total of 856 nasal swabs, collected from routine surveillance of free-ranging and captive animals, were enriched with modified TSB-1 and incubated for 48 hr. Of these, *M. ovipneumoniae* was detected by PCR in 32.6% (279/856) samples. In addition, 65.6% (183/279) of the PCR-positive samples also yielded observable *M. ovipneumoniae* via CBA culture. Various tissues and swabs obtained from necropsy were also enriched in modified TSB-1 for 48 hr, cultured and analyzed by PCR. Culture success rates are summarized in Table 4.

Discussion

We modified established media and protocols in order to optimize growth of *M. ovipneumoniae* in our laboratory. Samples incubated for 48 hr in our modified TSB-1 at 37 C and 10 % CO_2 appeared to optimize growth and detection of *M. ovipneumoniae*. We note, however, that we were unable to identify the individual components of the different protocols that may have contributed to the improvement we observed. Because information from the field was often limiting, we were also unable to characterize our samples by the level (if any) of physical manifestation of disease. We compared 1,037 samples over five years using this improved protocol and culture broth to enhance the culture and PCR detection of *M. ovipneumoniae* to better understand its prevalence and distribution in bighorn sheep and mountain goat herds.

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Table 1. Culture recovery and contamination in duplicate swabs enriched in SP4-G or modified TSB-1 (bighorn sheep from Wyoming and Colorado). Culture recovery rate is the number of culture positive divided by the number PCR positive. Days incubated is designated by 'd'.

Incubation Time and Enrichment broth	Total Samples	PCR Positive	Culture Positive	Culture Recovery Rate	Contamination Positive	Percent Contamination
2 d – SP4-G	13	9	7	77.8%	5	38.5%
2 d - mod TSB-1	13	10	9	90.0%	0	0%
4 d – SP4-G	23	13	2	15.4%	12	52.2%
4 d - mod TSB-1	23	13	5	38.5%	2	8.7%

Table 2. SP4 with glucose broth (SP4-G; Hardy Diagnostics, Santa Maria, California, USA).

Amount	Component
10g	Pancreatic Digest of Casein
5.0g	Pancreatic Digest of Gelatin
3.5g	PPLO Broth without CV
50mg 5mg	Polymixin B Amphotericin B
170ml	Fetal Bovine Serum
1,000,000units	Penicillin
50mL	CMRL 1066 Medium (10X)
35.0ml20ml 5g/L	Yeast Extract Yeastolate 10% Glucose
690mL	Deionized H_20

Table 3. Modified TSB-1. Shelf life is 3 months at 4°C.

Amount	Component
30g	Tryptone Soya Broth (Oxoid Ltd, Basingstoke, Hampshire, England) (pancreatic digest of casein (17.0g/L), enzymatic digest of soya bean (3.0g/L), sodium chloride(5.0g/L), di-potassium hydrogen phosphate(2.5g/L), glucose(2.5g/L))
10g	D-Lactose Monohydrate (Sigma, St. Louis, Missouri, USA)
200mL	Porcine Serum – heat inactivated (Rocky Mountain Biologicals, Missoula, Montana, USA)
7.25mg	Amphotericin B (Sigma, St. Louis, Missouri, USA)
1,323,661units	Penicillin G Potassium Salt (Sigma, St. Louis, Missouri, USA)
23.27mL	Thallium Acetate solution (10mg/mL(de-ionized H_20) (Sigma, St. Louis, Missouri, USA)
18mg	Phenol Red (Sigma, St. Louis, Missouri, USA)
1,000mL	Deionized H_20

Table 4. Overall culture recovery in samples enriched in modified TSB-1with thalium acetate for 48 h (Wyoming: bighorn sheep (721), mountain goats (40), mule deer (3), domestic sheep (1) and domestic goat (3); Colorado: bighorn sheep (267), domestic sheep (2)).

Sample Type	Culture Positive	PCR Positive	Culture Recovery Rate	Total Samples Tested
Nasal Swab	183	279	65.6%	856
Lung	32	78	41.0%	123
Bulla	5	19	26.3%	33
Sinus	1	3	33.3%	11
Liver	0	1	0%	12
Sinus Tumor	0	1	0%	1
Pericardium	0	1	0%	1

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Mycoplasma ovipneumoniae Strains Associated with Pneumonia Outbreaks in North American Bighorn Sheep

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ABSTRACT Epizootic all-age pneumonia outbreaks (and subsequent recurrent lamb pneumonia outbreaks) significantly limit many North American bighorn sheep (*Ovis canadensis*) populations but vary widely in severity and duration. We conducted a pilot study to determine whether *Mycoplasma ovipneumoniae* genetic strain types were associated with outbreak severity, using 1) *M. ovipneumoniae p113,* the gene encoding a putative *M. ovipneumoniae* adhesin, and 2) multi-locus sequence typing (MLST) based on partial DNA sequences of four loci (the small ribosomal subunit, the 16S-23S intergenic spacer, and *rpoB* and *gyrB* housekeeping genes). For both of these comparisons, we examined whether clusters of genetically similar *M. ovipneumoniae* strains shared similar outbreak severity, using data from the Wild Sheep Working Group of the Western Association of Fish and Wildlife. The *p113* locus data and the MLST data each produced several well-supported clusters, but these clusters were not detectably associated with outbreak severity. Our study may have been limited by several factors, including detection bias towards severe outbreaks, insufficient sequence data, and perhaps the impact of selection pressure on *p113,* which is a cell surface protein. Future research may provide better assessment of the role of *M. ovipneumoniae* genetic strain types in contributing to outbreak severity.

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KEYWORDS bighorn sheep, epizootic pneumonia, *Mycoplasma ovipneumoniae, Ovis canadensis,* phylogenetics, virulence, wildlife disease

When developing solutions for complex and persistent wildlife disease, it is important to understand the factors that influence the pathology, transmission dynamics, and severity of the disease. Since the introduction of pneumonia causing pathogens to bighorn sheep in North America in the mid-nineteenth century, bighorn sheep *(Ovis canadensis*) have been limited by all-age epizootic pneumonia and annual recurrent lamb pneumonia events that combine to create significant population limiting effects (Cassirer et al. 2013, Manlove et al. 2016). Our understanding of bighorn sheep pneumonia is still developing and there are many aspects of the disease that require investigation, including the observed variation in disease severity and persistence. Here we report the results of a pilot study designed to analyze potential relationships between the genetics of *Mycoplasma ovipneumoniae* (*M. ovipneumoniae*) and disease severity (Besser et al. 2013).

In the all-age epizootic form of

bighorn sheep pneumonia, there is considerable variation in outbreak mortality (Cassirer et al. 2013). Because there is typically only a single *M. ovipneumoniae* genetic strain associated with each outbreak, we hypothesized that variation in outbreak severity may be attributable, in part, to differences in strain-specific genetic repertoires that are potentially associated with virulence (Besser et al. 2012). To evaluate strain-specific *M. ovipneumoniae* genetics, we utilized two approaches. First, we analyzed the partial sequence of *p113,* which encodes the protein P113, a putative *M. ovipneumoniae* adhesin thought to be involved in binding to host respiratory epithelial cilia. The sequenced region included bases 375-659 of the 3,240 bp gene, predicted to encode amino acids 92-186 of the extracellular domain (NCBI 2015, Yang et al. 2014). Second, we analyzed the overall. *ovipneumoniae* phylogeny as assessed by multi-locus sequence typing (MLST) developed for a separate study (Cassirer et al. 2017). The MLST included sequence data from four loci: the small ribosomal subunit (343 bp), the 16S-23S intergenic region (390– 420 bp), *rpoB* (562 bp), and *gyrB* (400 bp). In both of these approaches, we looked for association between *M. ovipneumoniae* genetic strain clusters and the reported severity of the associated outbreaks from which they were detected.

METHODS

Study areas

We included bighorn pneumonia outbreaks where both mortality estimates and information on *M. ovipneumoniae* DNA were available (n = 40; Fig. 1). Geographically these outbreaks ranged from North Dakota to southern Nevada, and from Washington to Nebraska. These outbreaks spanned a 29-year period from 1985 to 2014. Climate, topography, disturbance regimes, vegetation character, and other ecological factors vary widely amongst all populations (Fig. 1).

Experimental design

Epizootic all-age die offs commonly characterize the first observations of disease after initial introduction of an *M. ovipneumoniae* strain to a bighorn population (Plowright et al. 2013). Typically there is only one *M. ovipneumoniae* strain per epizootic, so we used outbreak data compiled by the Western Association of Fish and Wildlife Agencies' Wild Sheep Working Group to identify outbreaks from which banked *M. ovipneumoniae* DNA extracts were available (Besser et al. 2012). For each *M. ovipneumoniae* strain, we identified the year of the outbreak, the population involved and its location, and the estimate of percent mortality associated with the outbreak (Table 1).

Laboratory technique

Gene amplification: We amplified a 285 bp fragment of *p113* from each *M. ovipneumoniae* strain using the PCR technique reported by Yang et al. (2014). For each strain, the reaction mixture included 2.0 µL of genomic DNA, 12.5 µL Qiagen master mix, 1.0 µL each of *p113* forward and reverse primers (10 µM each), and 8.5 µL deionized water. Thermocycling conditions included initial denaturation (15 min at 95 C), 45 cycles of denaturation (30 sec, 95 C), annealing (30 sec, 56 C), and extension (30 sec, 72 C), and a final extension (7 min, 72 C). The positive control was *M. ovipneumoniae* strain 'Crony' and the negative control was a DNA extract of deionized water.

Gel electrophoresis: We separated *p113* PCR product amplicons by gel electrophoresis to confirm the appropriate size. Gels (80 mL TAE, 1 g. agarose, 4.0 µL EtBr) were electrophoresed (60 min, 100 v, 261 mAmps) with the 15 wells including size ladders (product, source) in lanes 1 and 15, and samples (5.0 µL) of positive controls (lane 13), negative controls (lane 14), and test samples (lanes 2-12). Following electrophoresis, gels were imaged (Alpha

Imager HP system, ProteinSimple, San Jose, CA) to confirm the presence of a DNA product equivalent in size to the positive control in each test sample lane.

Preparation of amplicon for sequencing: We treated *p113* PCR products (20.0 μL) with 0.4 μL ExoI (20 U/μL), 1.6 μL FastAP (1U/μL), 0.4 μL 10x FastAP RB buffer, and 1.6 μL deionized water, and incubated them for 20 min at 37 C, followed by 15 min at 80 C). Treated products (20.0 μL) were submitted for sequencing to Eurofins Genomics Company, Louisville, KY.

Sequence analysis: We used Sequencher 5.1 software (Gene Codes Corporation, Ann Arbor, MI) to assemble forward and reverse DNA sequence reads, and to trim the assembled sequences. Sequencing chromatograms were manually examined and edited to remove unsupported insertions or deletions, and to correct ambiguous base calls due to artifacts.

Sequences were aligned in Clustal Omega (Clustal Omega 1.2.1, www.ebi.ac.uk/Tools/msa/clustalo/, accessed 1 November 2015).

Phylogenetic analysis

Phylogeny construction: We formatted aligned *p113* and MLST sequences to NEXUS file format and used these for analyses in Bayesian phylogenetic analytical software (MrBayes http://mrbayes.sourceforge.net/, accessed 4 March 2016). The number of generations in the analyses was 6 million for *p113* and 8 million for MLST, and burn-in was 25% of generations. The evolutionary model is a general time reversible model with a proportion of invariable sites and a gamma shaped distribution of substitution rates across sites. We used Dirichlet (non-informative) priors. Convergence was assessed by standard deviation of split frequencies (*p113*: 0.005; MLST: 0.008). For cluster identification, clusters were selected by clade probability>90%, lumped to ensure at least 3 strains per clade. Where multiple well-supported nodes existed within a clade, we attempted to maximize the number of well-supported clusters each containing at least 3 strains. The topologies of the phylogenetic trees for *p113* and MLST were compared to subjectively identify concordant clusters.

Statistical analysis: We conducted a simple analysis of variance of estimated outbreak percent mortality by cluster to detect possible association between outbreak mortality rates and genetic clusters detected within either *p113* or MLST sequence phylogenies (RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/). Our null hypothesis predicted that average mortality estimates would not vary significantly among genetic clusters, and our research hypothesis predicted that average mortality estimates would vary significantly among genetic clusters. We used the median outbreak severity (estimated 45% mortality) to define the cut-point between severe and moderate/mild outbreaks.

Selection analysis

To evaluate differences in selection pressure on *p113* relative to two *M. ovipneumoniae* housekeeping genes, *rpoB* and *gyrB*, we determined the average dN/dS ratios for each set of sequences. The dN/dS ratio is the ratio of non-synonymous mutations (mutations that change the amino acid sequence) to synonymous mutations (mutations that do not change the amino acid sequence), a measure that is possible because of the degeneracy in the genetic code: multiple codon sequences may encode a single amino acid residue. dN/dS greater than 1.0 denotes positive selection (when non-synonymous changes are favored), equal to 1.0 denotes neutral selection or drift, and less than 1.0 denotes constraining selection against altered amino acid sequence (Graur and Li 2000).

Table 1. Population outbreak strain samples and their corresponding year of outbreak, state in which outbreak was first reported, population, estimated outbreak percent mortality, and membership in *M. ovipneumoniae p113* and/or *M. ovipneumoniae* MLST phylogenetic clusters.

Year	State	Population	Population Abbreviation	Outbreak Mortality (%)	*p113* Cluster	MLST Cluster
1985	NV	Desert Sheep Range	DSR	75	3	1
1991	MT	Anaconda	AN	75	2	4
1994	MT	Tendoys	TE	87		2
1995	OR	Wenaha	WE	54		1
1995	WA	Black Butte404**	BB404	55		
1997	MT	Taylor Hilgard***	TH	75		
1997	ND	Ice Box Canyon	IBC	87		2
2000	ID/OR	Sheep Mtn***	SM	51		
2003	SD	Custer State Park	CU	75*		3
2004	NE	Fort Robinson	FR	54		1
2006	WA	Black Butte415***	BB415	35		
2007	CO	Fossil Ridge	FO	74	1	3
2008	MT	Elkhorns	EL	83	1	3
2009	CA	White Mountains	WH	Mild	3	3
2009	MT	Bonner	BO	71*		4
2009	MT	East Fork Bitterroot***	EFB	52*		
2009	MT	Lower Rock Creek	LRC	54	2	4
2009	WA	Yakima Canyon	YC	34*		2
2010	MT	Sun River389	SR389	34		4
2010	MT	Sun River398	SR398	34	3	3
2010	NE	Barrel Butte	BA	17	3	3
2010	SD	Spring Creek	SC	20*	3	3
2010	UT/NV	Pilot Leppy405	PL405	44		1
2010	UT/NV	Pilot Leppy398	PL398	44	3	3
2011	NV	Pancakes	PA	14	2	4
2011	NV	Snowstorms	SN	50		4
2012	NV	Grant Range	GR	45	2	3
2012	NV	McCullough	MC	27	3	1
2012	NV	Slate Mountain	SL	28		3
2012	WA	Asotin Creek	AS	36		1
2013	MT	Cinnabar	CI	21	1	3
2013	MT	Gardiner	GA	21	1	3
2013	MT	Mt. Everts	ME	21		3
2013	NV	Spring Range401	SP401	19		4
2013	NV	Spring Range406	SP406	19	3	
2013	WA	Tieton	TI	Severe		4
2014	ND	Sheep Creek	SC	30		3
2014	NE	Sowbelly	SO	40	3	3
2014	NV	Santa Rosa	SA	40		3
2014	WA	Black Butte393	BB393***	33		

Notes to Table 1: Strain clusters identified in both the *M. ovipneumoniae p113* and *M. ovipneumoniae* MLST phylogenies are denoted numerically (1, 2, 3, 4) and strain membership in a certain phylogenetic cluster is listed in columns 6 and 7; *culled (percent mortality value may have been higher or lower if symptomatic individuals had not been culled); **outbreak occurred at Sheep Mountain ID/OR; ***strain did not resolve into any phylogenetic cluster identified in our analysis.

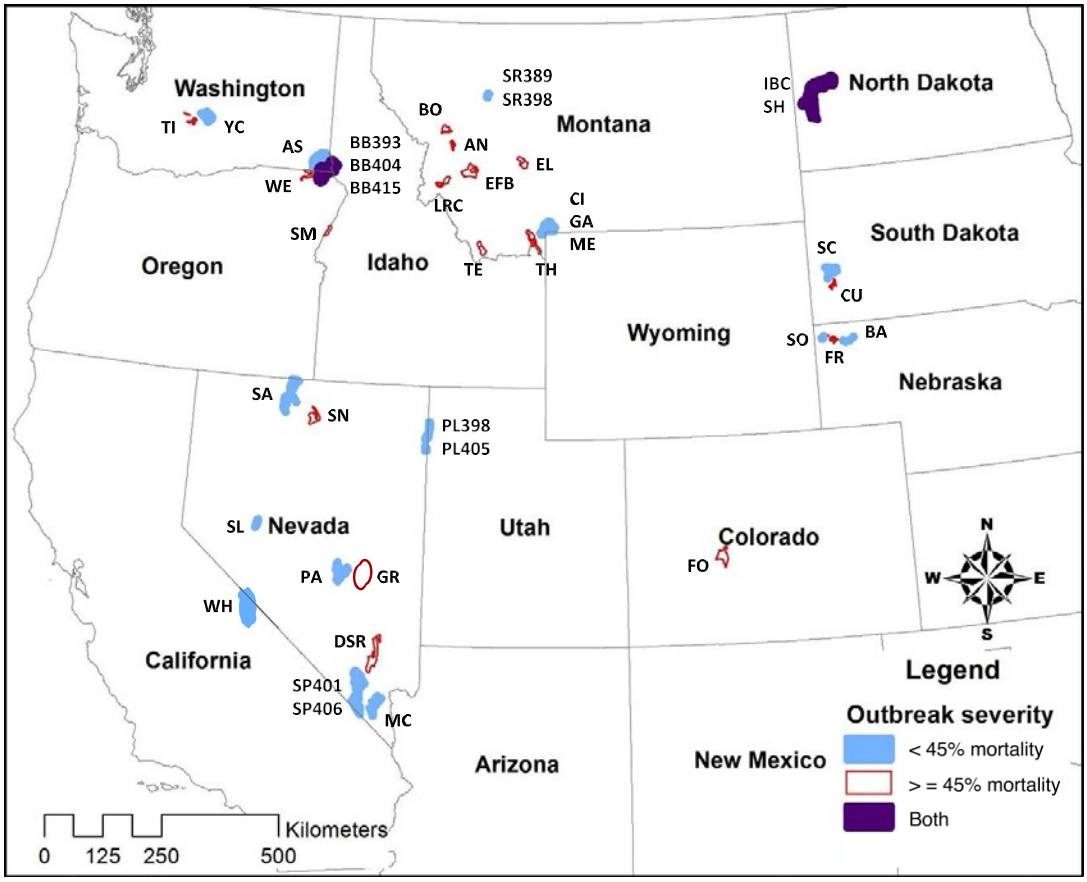

Figure 1. Geographic distribution of the 40 bighorn sheep populations in North America in which epizootic pneumonia outbreaks occurred with severity documented by the Western Association of Fish and Wildlife Agencies (WAFWA) Wild Sheep Working Group (WSWG), and for which *M. ovipneumoniae* DNA extracts were available. See Table 1 for population identification codes.

RESULTS

We produced 285 bp DNA fragment sequences of *M. ovipneumoniae p113* for each population, and they were highly polymorphic. The *M. ovipneumoniae p113* phylogeny produced only a few well-supported clades, and the mean mortality estimates in each cluster were not significantly different [F = 0.628; df = 2,14; P = 0.548] (Fig. 2). *M. ovipneumoniae* MLST sequences produced slightly more well-supported clades; however, these were also not significantly associated with outbreak severity [F = 1.54; df = 3,27; P = 0.228] (Fig. 3).

Our dN/dS analysis indicates that the three *M. ovipneumoniae* genes we investigated (*p113, rpoB,* and *gyrB)* are under strong constraining selection, but that the two housekeeping genes *rpoB* and *gyrB* exhibit stronger constraining selection than *p113* (Table 2). Of the three genes, *p113* exhibits the highest frequency of positive selection occurring, with 2.43% of sites under strong positive selection (ω (+) = 1.54). For *rpoB,* 0.89% of sites were under positive selection (ω (+) = 2.04) and for *gyrB,* 0.91% of sites were under positive selection (ω (+) = 2.15).

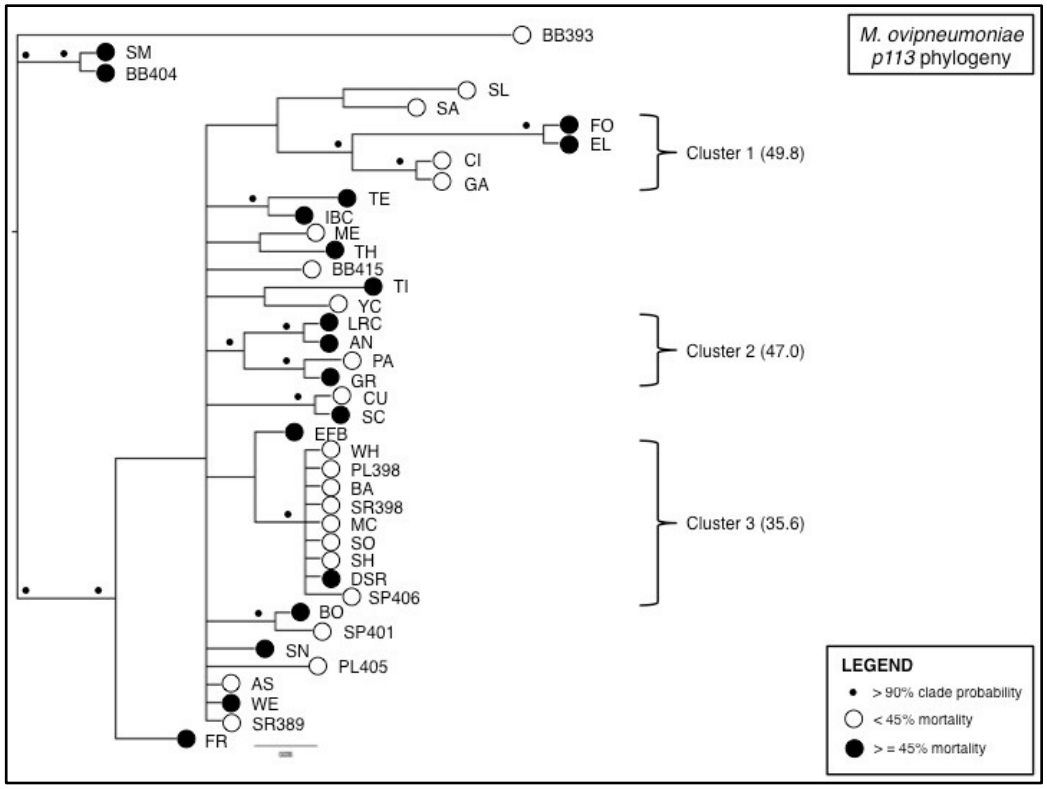

Figure 2. Bayesian *M. ovipneumoniae p113* phylogeny with strain clusters 1-3 identified and supported by >90% clade probability values. Average estimated mortalities for each cluster are listed parenthetically after the cluster number. See Table 1 for population identification codes.

Table 2. Summary data for selection at three *M. ovipneumoniae* loci (*p113,* and housekeeping genes *rpoB* and *gyrB*) using Bayesian analysis to determine dN/dS ratios.

	p113		rpoB		gyrB	
Parameter	**Mean**	**Variance**	**Mean**	**Variance**	**Mean**	**Variance**
p113						
ω (-)[1]	0.0441	0.000242	0.00221	0.00000248	0.0131	0.0000303
ω (N)	1.00	0.00	1.00	0.00	1.00	0.00
ω (+)	1.54	0.445	2.04	1.21	2.16	0.879
π (-)[2]	0.929	0.000893	0.962	0.000184	0.970	0.000372
π (N)	0.0470	0.000756	0.0290	0.000167	0.0211	0.000363
π (+)	0.0243	0.0004.81	0.00893	0.0000877	0.00912	0.0000942

[1] ω denotes the dN/dS ratios for negatively (-), neutral (N), and positively (+) selected sites; [2]π denotes the frequencies of the site categories (negatively (-), neutral (N), and positively (+) selected sites).

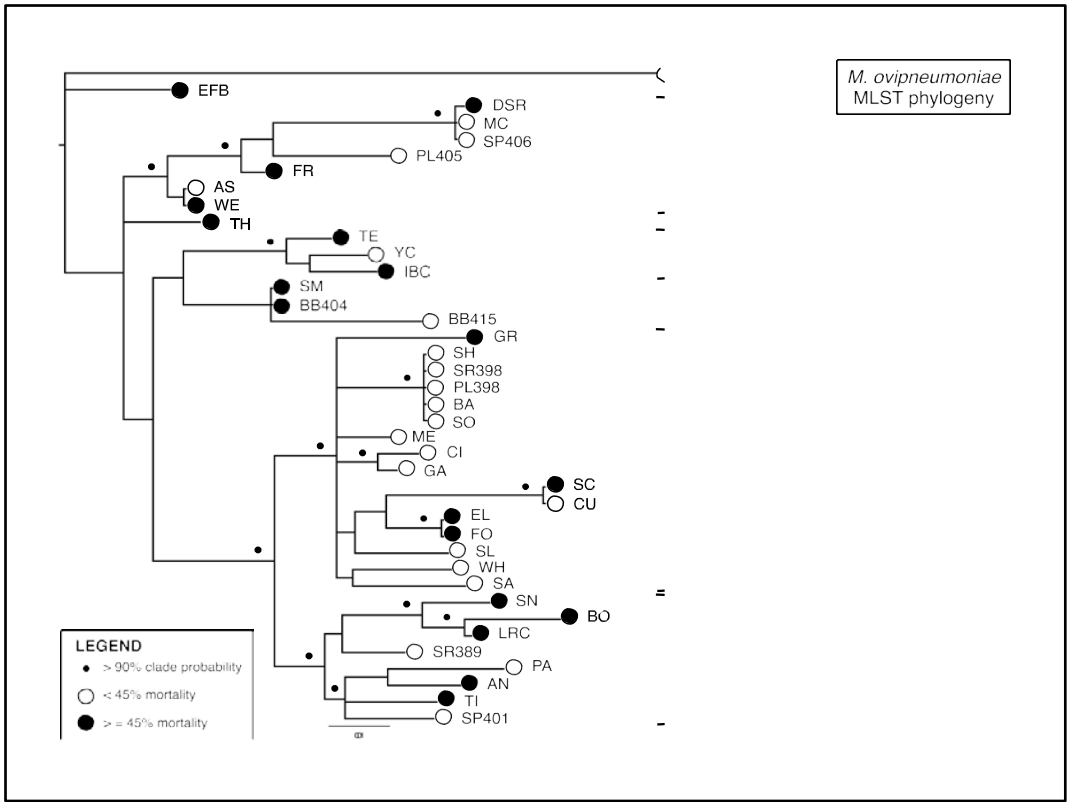

Figure 3. Bayesian *M. ovipneumoniae* MLST phylogeny with strain clusters 1-4 identified and supported by > 90% clade probability values. Average estimated mortalities for each cluster are listed parenthetically after the cluster number. See Table 1 for population identification codes.

DISCUSSION

We did not detect association of *M. ovipneumoniae* genetic clusters with outbreak severity, as least as we were able to characterize severity. We detected fewer well-supported clades based on *p113* sequences than MLST sequences. Neither *p113* nor MLST clusters were significantly associated with outbreak severity.

This was a pilot study, and as such, included several significant limitations that could be addressed by future, more extensive investigations; these include, a possible bias towards high severity in the outbreaks selected for inclusion in the study (under-representing *M. ovipneumoniae* genetic clusters that may be associated with less severe disease), the inclusion of insufficient *p113* sequence to detect genetic clustering, and the possibility that *p113*, if actually a cell surface gene associated with virulence, may undergo selection for immune escape that may obscure phylogenetic association with virulence. An important limitation of this study is likely bias towards severe outbreaks. Outbreak detection in the field and accurate disease-induced mortality estimates depend on the level of disease surveillance and sample recovery. It is likely that there was variation in the intensity of monitoring at the population and individual levels and that mild disease outbreaks would not have been recognized for inclusion in the WSWG data.

If P113 is a virulence determinant due to its hypothesized role in interaction with respiratory epithelial cilia receptors, association of its amino acid sequence with virulence may well be limited to the receptor-ligand region. Given that the ligand region is not currently identified, it is very possible that it is encoded outside the 285 bp regions we analyzed, since the complete *p113* gene sequence is 3,240 bp (Yang et al. 2014). Finally, if antibody responses to P113 reduce its ability to mediate interaction with cilia, one would expect strong genetic selection on *M. ovipneumoniae* to avoid these immune responses that could confound any association between *p113* phylogeny and virulence.

There are also other factors that affect outbreak severity that may blur an underlying association of genetic clusters with virulence. These include host genetics and nutrition, host social contact networks, and climatic conditions as confounding factors that, along with pathogen virulence, may contribute to outbreak percent mortality estimates (Hudson et al. 2002, Manlove et al. 2014, Plowright et al. 2013, Tompkins et al. 2011).

Progress in determining whether genetic variability of *M. ovipneumoniae* is associated with the severity of bighorn sheep pneumonia all-age outbreaks will require continued efforts to identify specific bighorn sheep populations in which the introduction of *M. ovipneumoniae* is associated with low all-age mortality. More complete sequencing of *p113*, other virulence-associated genes, or generation of whole genome sequences will provide a stronger basis for these comparisons. In addition to their potential to improve our understanding of the factors that contribute to the variation in severity of pneumonia outbreaks in bighorn sheep, such studies will also offer the potential to improve outbreak response, and may contribute to development of candidate *M. ovipneumoniae* vaccines (Hudson et al. 2002, Sells et al. 2015, Tompkins et al. 2011, Weiser et al. 2012, Zeigler et al. 2014).

ACKNOWLEDGEMENTS

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Mycoplasma ovipneumoniae originating from Domestic Goats Triggers Mild Bronchopneumonia in Experimentally Exposed Naïve Bighorn Sheep (and Domestic Goats)

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ABSTRACT We conducted a series of experimental exposure studies to investigate the virulence of *Mycoplasma ovipneumoniae* carried by domestic goats (*Capra aegagrus hirca*) for naïve bighorn sheep (*Ovis canadensis*). Bighorn sheep (n = 6) from an *M. ovipneumoniae*-free population were transported to Washington State University and divided into two groups of 3 animals held in pens >1 km apart. Domestic goats were purchased from local private operations. Experiment 1: Following the addition of 3 naturally *M. ovipneumoniae*-colonized domestic goats to one of the pens, the 3 comingled bighorn sheep developed symptoms of chronic respiratory disease and, at necropsy 100 days later, all exhibited focal bronchopneumonia. During this period, the isolated, non-comingled (control) bighorn sheep in the second pen remained healthy. Experiment 2: Three *M. ovipneumoniae*-free domestic goats were comingled with the 3 remaining bighorn sheep and observed for a 100-day period. There was no evidence of respiratory disease in either bighorn sheep or goats during Experiment 2. Experiment 3: Goat-origin *M. ovipneumoniae* was introduced into the same commingled domestic goats and bighorn sheep that had been used for Experiment 2. During the following 100 days, all 3 domestic goats and all 3 bighorn sheep in Experiment 3 developed signs of chronic respiratory disease, and at necropsy all of the animals exhibited focal bronchopneumonia. See Figure 1 for a schematic summary of these three experiments. The results indicate that the goat-origin *M. ovipneumoniae* strains used in these experiments were capable of causing respiratory disease symptoms and pneumonia lesions in susceptible bighorn sheep and domestic goats. *Pasteurellaceae* bacteria encoding *lktA*, the gene encoding the leukotoxin virulence factor, were detected in all animal groups both prior to and after each experiment, and so were not clearly involved with the observed respiratory disease. The disease observed in Experiments 1 and 3 was notably milder than that reported in previous experiments conducted with domestic sheep-origin strains of *M. ovipneumoniae*. Nasal discharge from infected bighorn sheep, although consistently observed, was relatively scant and non-purulent compared to previous experimental infections of bighorn sheep with domestic sheep strains of *M. ovipneumoniae.* Coughing was also consistently observed among bighorn sheep in Experiment 1 and among both bighorn sheep and domestic goats in Experiment 3, but the frequency, severity, and duration of coughing episodes was markedly reduced compared to previous experiments with sheep strains. Finally, no respiratory disease deaths occurred during these studies, whereas previous comingling studies of bighorn sheep with domestic sheep have been associated with nearly 100% bighorn sheep mortality.

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KEYWORDS bighorn sheep, comingling experiments, domestic goats, enzootic pneumonia, *Mycoplasma ovipneumoniae*

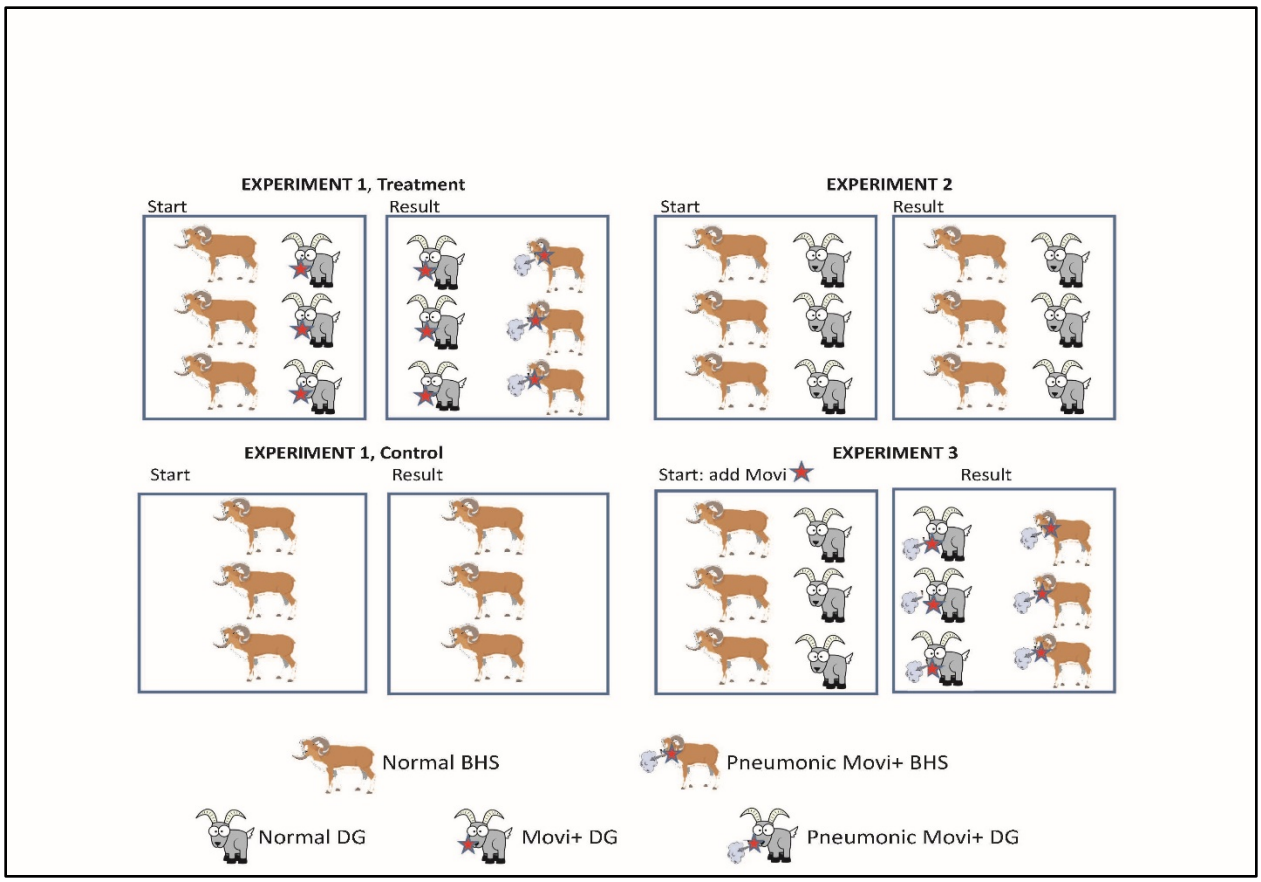

Figure 1. Schematic diagram of experiments described herein.

Potential Disease Agents in Domestic Goats in Idaho and Oregon and their Relevance to Bighorn Sheep Management

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ABSTRACT Domestic goats are raised for meat, milk, and hair production, and are also used as herd goats for rangeland weed control and as pack animals. There is some controversy about the relative risk of disease transmission between domestic goats and free-ranging bighorn sheep (Foreyt 1994a, 2009; Miler et al. 2008). Domestic sheep, domestic goats, and wild bighorn sheep are all susceptible to a pneumonia that is multifactorial with variable consequences depending on the specific pathogen and the host species. We report here on a survey with the objectives of evaluating the health status and disease exposure of pack and herd domestic goats, and using this information to develop risk management criteria for situations in which domestic goats may come into contact with bighorn sheep. We defined herd goats as animals that were assembled and used for various purposes, including pets, companions, raised for 4H, or rangeland weed control, and defined pack goats as animals that were assembled and used for packing. We sampled 43 herd goats from 7 herds, and 48 pack goats from 11 groups. Blood was collected by jugular venipuncture and serum harvested after centrifugation. Serum was tested for antibodies to Anaplasmosis, Bluetongue (BT), Bovine Respiratory Syncytial Virus (BRSV), Bovine Viral Diarrhea (BVD), *Brucella ovis*, Caprine Arthritis and Encephalitis (CAE), Epizootic Hemorrhagic Disease (EHD), Infectious Bovine Rhinotracheitis (IBR), Leptospirosis, and Parainfluenza 3 (PI3). Feces were collected by digital removal from the rectum and tested for the presence of gastrointestinal parasites by flotation in saturated sugar solutions (Foreyt 1994b). Nasal and oropharyngeal swabs were collected and submitted for Pasteurellaceae culture. Goats in this study were in generally good health, although most goats did harbor various pathogens and parasites. The pack goats were exclusively male (4 intact and 44 wethers). Herd goats consisted of 30 females, 12 wethers, and 1 intact male. The average age of pack goats was 7.4 years and of herd goats was 3.1 years. The majority of animals and herds had low to no titers to most pathogens assessed. No goats had antibodies against Anaplasmosis, IBR, or Leptospirosis. One herd goat was seropositive for *Brucella ovis* and three herd goats were seropositive to BVD and PI3. Antibodies to BT and EHD were prevalent in pack goats (25 of 48, 52.1%, seropositive to BT and 26 of 48, 54.2%, seropositive to EHD) but not in herd goats (2 of 41, 4.8% seropositive to both viruses; χ^2=23.2, df =1, P = 0.001). Animals with positive titers came from 9 of 11 (82%) pack goat herds and 1 of 7 (14%) herd goat herds. Antibodies to CAE were found in 7 pack goats belonging to 5 herds and in 7 herd goats belonging to 3 herds, with the majority of seropositive animals from 2 pack goat herds and 1 herd goat herd. Only herd goats had individual animals with high or moderate levels of coccidia oocysts. Two pack goats and 7 herd goats had *Nematodirus* spp. ova. Individuals in 6 goat herds (3 pack and 3 herd) had greater than 30 eggs/g for *Strongylus* spp. Eggs of *Trichuris* spp. were found in 4 pack goats and 5 herd goats with only 1 pack goat having a high egg count. Oropharyngeal swabs yielded isolates of one or more Pasteurellaceae species from 43 of 48 (89.5%) pack goats and from 41 of 43 (95.3%) herd goats. The frequency of Pasteurellaceae isolation was similar between herd and pack goats (χ^2 = 2.5, df = 1, P = 0.41). Isolates of *Bibersteinia trehalosi* were found in 33 of 48 (68.8%) pack goats and 34 of 43 (79.1%)

herd goats, with no significant differences between herd types (χ^2 = 1.2, df = 1, P = 0.26). The majority of *B. trehalosi* isolates were biogroup 2 (21 of 33 (64%) for pack goats and 27 of 34, (79%) for herd goats), and very few were hemolytic (1 of 33, 3.0%) and 7 of 34 (20.5 percent, isolates from pack and herd goats, respectively). *Mannheimia haemolytica* was not found in any of 48 pack goats, but was isolated from 14 of 43 herd goats (33 percent) (χ^2 = 9.8, df = 1, P = 0.002). Isolates from both groups of goats were predominantly the unnamed *Mannheimia* species. Low numbers of *M. glucosida, M. ruminalis,* and *M. varigena* were found in both groups of goats. Most isolates of *Mannheimia* spp. from pack and herd goats were hemolytic (22 of 26, 84.6%) and 36 of 54, 66.7%), respectively. Based on biogrouping, most isolates from pack goats (20 of 26 isolates (77%) and herd goats (40 of 54 isolates, 74%) were of high to moderate disease potential for bighorn sheep (Jaworski et al. 1998). Goats in this study were exposed to, or had evidence of the presence of several pathogens that have been reported from bighorn sheep. Both pack and herd goats in this study were found to have respiratory bacteria that have been associated with pneumonia in bighorn sheep (Ward et al. 2002, Jaworski et al. 1998). The prevalence of Pasteurellaceae isolated from domestic goats in this study is comparable to others (Ward et al. 2002) and these results can be used to define the typical oropharyngeal flora of domestic goats. Differences between pack and herd goats in this study are likely due to differences in age structure, herd size, and the extent of interaction between goats from different sources. Contact between bighorn sheep and domestic goats under field conditions has been documented (Rudolph et al. 2003, 2007; Jansen et al. 2006). Infectious keratoconjuntivitis (pink eye) has been transmitted from domestic goats to bighorn sheep under range conditions (Janson et al. 2006) and *Mannheimia* spp. have been shown to be shared between bighorn sheep and feral goats, although the sharing was limited to interaction among 3 animals and did not appear to be involved in the large bighorn sheep die-off occurring in the area (Ward et al. 1997, Jansen et al. 2006, Rudolph et al. 2007). However, due to the possibility of transmission, management of domestic goats in areas in or near bighorn sheep habitat should be conducted to minimize the risk of the spread of disease agents. Recommendations for management of pack goats should include avoiding direct contact between goats and bighorn sheep, using a tether or lead rope at all times when in the presence of free-ranging bighorn sheep, and keeping goats under close control when in areas in which bighorn sheep could be present. Parasite control is highly recommended as a best management practice and should be required prior to use of goats in bighorn sheep habitat to minimize the risk of parasite transmission to bighorn sheep.

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KEYWORDS bighorn sheep, disease risk, domestic goats, microbiology, *Ovis canadensis*, Pasteurellaceae

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Maternal Investment Partitioning of Bighorn Sheep: Do Chronic Shedders Exhibit an Inferiority Complex?

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ABSTRACT Life history theory predicts how natural selection should shape the way iteroparous individuals such as bighorn sheep (*Ovis canadensis*) should partition resources to optimize their survival and reproductive success. Reproduction is energetically costly due to maternal care, provisioning milk intake, and vigilance. Maternal care results in a fitness deficit for the dam causing a maternal investment trade-off between current reproduction and the long-term survival of the dam. In species in which male reproductive success exceeds that of females, extra parental investment would favor sons over daughters. The Trivers-Willard hypothesis predicts that dams in superior health will invest more heavily in the progeny sex with greater expected lifetime reproductive success. Adult ewes housed at the South Dakota State University Captive Wildlife Research Unit have known *Mycoplasma ovipneumoniae* shedding histories; thus, animals used in this study vary relative to their health status. Our research objective was to compare offspring investment and progeny sex ratios of individual bighorn sheep females as a function of the overall time in our study in which they actively shed *M. ovipneumoniae*. We predicted that bighorn sheep dams will differentially partition parental investment based on the female's current pathogen shedding status, with shedding-negative ewes investing greater resources in offspring than would shedding-positive ewes (that latter strategy would conserve resources for subsequent reproduction). We also predicted that shedding-negative females would differentially partition greater resources to male than to female lambs in order to increase reproductive payoff, whereas shedding-positive ewes would produce more female than male lambs (females being less costly to raise than males).

To categorize each ewe by shedding status, we used individual serial samples collected from 2014–2015 to estimate overall *M. ovipneumoniae* apparent prevalence, using 1 of 3 classes: Negative (≤ 25%), Intermittent (26-74%), or Chronic (≥75%). From May–September 2015, we recorded the duration and frequency of suckles allowed by ewes categorized as shedding-negative (n = 8), shedding-intermittent (n = 6), and shedding-chronic (n = 4) ewes. We indexed daily maternal investment by estimating the amount of milk allocated to lambs. We did this by multiplying the mean suckling duration by the mean "ewe rate" (acts-per-active-hour). We used analysis of covariance (ANCOVA) to investigate the effect of pathogen shedding status on the weekly means of rate and duration of suckles. These means were weighted by number of successful suckles to account for *M. ovipneumoniae*-associated lamb mortality. We recorded lamb

sex and used a Pearson's χ^2 test to determine if *M. ovipneumoniae* shedding-negative ewes produced a greater number of male progeny than shedding-chronic ewes.

Our preliminary findings suggest that maternal investment was similar among the 3 shedding groups, i.e., that *M. ovipneumoniae* pneumonic infected ewes exhibited more fitness plasticity than previously supported in the literature. Infected ewes invested as much in their offspring as did uninfected ewes. Our preliminary results indicated no difference in progeny sex born to ewes of varying health status, suggesting that shedding-positive ewes may favor current reproduction over long-term survival. Identifying life history trade-offs between *M. ovipneumoniae* shedding dams and investment in their offspring provides information critical to management of declining bighorn sheep populations.

Biennial Symposium of the Northern Wild Sheep and Goat Council 20: 103-104.

KEY WORDS disease, maternal investment, *Mycoplasma ovipneumoniae*, pathogen, progeny

Seasonal Resource Selection by Introduced Mountain Goats in the Southwest Greater Yellowstone Area

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ABSTRACT Mountain goats (*Oreamnos americanus*) are among the least studied North American ungulates. Aided by successful translocations from the early to mid-1900's, introduced populations have greatly expanded within non-native ranges, yet there remains a paucity of empirical studies concerning their habitat requirements and potential distributions. The lack of studies presents a formidable challenge to managers tasked with monitoring mountain goat expansion and mitigating for any potential negative impacts posed to native species and communities. We constructed summer and winter resource selection models using GPS data collected during 2011–2014 from 18 (14 female and four male) mountain goats in the Snake River Range of the southwest Greater Yellowstone Area. We used a generalized linear mixed-model approach and evaluated landscape and environmental covariates at multiple spatial grains within four related suites. The multi-grain resource selection function greatly improved model fit, indicating that mountain goat resource selection was grain dependent in both seasons. In summer, mountain goats largely selected rugged and steep areas at high elevations and avoided high solar radiation, canopy cover, and time-integrated NDVI. In winter, mountain goats selected lower elevations characterized by steep and rugged slopes on warm aspects and avoided areas with high canopy cover, NDVI amplitude, and snow water equivalent. Slope was the dominant predictor of habitat use in both seasons, although mountain goats selected for steeper slopes in winter than in summer. Regional extrapolations depicted suitable mountain goat habitat in the Snake River, Teton, Gros Ventre, Wyoming and Salt Ranges centered around steep and rugged areas. Winter range was generally characterized by the steepest slopes within a more broadly distributed and generally less steep summer range. Further research should examine the spatial and temporal overlap with native populations to further our understanding of resource selection dynamics and the potential for introduced mountain goats to alter intraguild behavioral processes of sympatric species, namely the Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*).

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KEYWORDS mountain goats, multi-grain analysis, *Oreamnos americanus*, resource selection function (RSF); Yellowstone

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Bighorn Sheep Movements and Mineral Lick Use in Waterton-Glacier International Peace Park

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ABSTRACT We used bighorn sheep telemetry data collected in Glacier National Park, Waterton Lakes National Park, and the Blackfeet Reservation in northwestern Montana to examine bighorn sheep movements and use of known mineral licks. Over 168,400 GPS locations were collected between 2002 and 2011 on 94 bighorn sheep individuals from 17 different social groups. We examined the proximity of bighorn sheep telemetry locations to 13 known mineral licks to describe timing and frequency of mineral lick use. After estimating bighorn sheep kernel home ranges, we evaluated how movements toward the lick, timing, and frequency of use varied depending on location of the mineral lick relative to bighorn sheep home ranges. Of the 86 adult sheep with sufficient data to detect mineral lick visits, 76 individuals had GPS locations near known mineral licks, primarily between May and August. We found that social groups consistently used the same mineral lick and visitation rates were generally influenced by distance between the mineral lick and home range, with the highest rates when the mineral lick was within the home range. Duration of mineral lick visits was variable, but ewes generally visited mineral licks more frequently and for a longer duration than rams. Given that nearly all animals used mineral licks, population estimates, or at least minimum population size, may be obtainable from sampling at them.

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KEYWORDS abundance, mineral lick, movements, salt lick, seasonal resources

Spatial Responses of Bighorn Sheep to Forest Canopy in North-central Washington

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ABSTRACT Fire suppression has allowed conifers to encroach into historically open grasslands and shrublands across western North America. Woody encroachment may reduce habitat quantity and quality for bighorn sheep (*Ovis canadensis*), which rely on open escape terrain. We examined the influence of conifer canopy cover, along with topography and forage resources, on habitat selection by bighorns in north-central Washington. Our study took place where thinning and prescribed fire treatments have been applied to encroaching forest to restore historic landscape conditions within and adjacent to existing bighorn habitat. To model habitat selection of bighorns using Resource Selection Functions, we estimated Utilization Distributions (UDs) from Global Positioning System (GPS) locations of 21 radio-collared bighorns (14 females and 7 males) using the Brownian bridge movement model. We defined seasons as lambing (1 May to 15 June), summer (16 June to 15 September), and winter (1 December to 29 February), and created 99% home ranges from UDs for each individual bighorn sheep for each season (as well as an annual UD for each animal). We generated random points within each 99% home range to represent available habitat. We used logistic regression to compare bighorn GPS locations (used) to random points (available) after assigning them to habitat variables that we created in a geographic information system. As we predicted, bighorn sheep selected areas with lower tree canopy cover, even when controlling for topography and potential foraging habitat. Bighorns also selected for steeper slopes; however, distance to escape terrain, aspect, ruggedness, slope X ruggedness, distance to forage, and distance to escape terrain X distance to forage, as well as categories of Tasseled Cap greenness, varied in their ability to predict habitat selection by bighorn sheep. Bighorn sheep in our study area selected habitats with lower canopy cover than generally available. Restoring or maintaining open habitat in areas with woody encroachment may influence movements and increase the value of habitat for bighorn sheep.

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KEYWORDS bighorn sheep, forest encroachment, habitat, *Ovis canadensis*, resource selection function, habitat, Washington

Pattern of Herbivory, Nitrogen Content, and Biomass of Bluebunch Wheatgrass on a Mountain Sheep Habitat in Central Idaho

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ABSTRACT Bluebunch wheatgrass (*Pseudoregneria spicata* [Pursh] A. Love) is a major forage species for mountain sheep (*Ovis canadensis*) in central Idaho. Observed condition of this forage species is high, prompting an investigation of herbivory levels and subsequently nutrient content and biomass of this species. Mean amounts of tissue removed from wheatgrass plants on a slope frequently used by mountain sheep ranged from 5.3% to 26.8% from 1992-1996. Nitrogen levels ranged from 0.7-1.4% from 1998-2007 in plants collected in late June after seed-set. Higher levels of N occurred in growth following wildfire burns. Above-ground growth of bluebunch wheatgrass ranged from 11.3 to 102.1 gm/m2 and was highly correlated with spring precipitation. While herbivory on this major forage species was low to moderate, nitrogen levels may vary enough to affect mountain sheep population trends without appreciably affecting productivity of their major forage species.

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Annual Changes in Bluebunch Wheatgrass Biomass and Nutrients Related to Climate and Wildfire

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ABSTRACT Current year's growth (biomass) and nutrient levels of bluebunch wheatgrass (*Pseudoroegneria spicata*), a highly palatable bunchgrass in western North America, were evaluated over 20-year and 10-year periods, respectively. Three study sites representing a range of variation in conditions were located on south-facing slopes. Annual biomass ranged from 5.6 to 109.0 gm m-2 on individual sites with means for all sites of 42.7 gm m-2 (range 17.5–73.3 gm m-2), with April and May precipitation best predicting the variation. Variation was highest on the site lowest in elevation and highest in biomass. A fire in August 2000 that burned all study sites suppressed biomass for the following two years, aided by lower than average precipitation. The highest elevation site had higher mean values of Cu, Mg, N, K, P, S, and Zn than the two lower sites, but the greatest range of values occurred on one of the two lower sites for Ca, Fe, K, Mg, N, P, and S. Combinations of temperature and precipitation predicted Ca, K, N, P, and Zn values, while Cu and Fe were predicted with total monthly precipitation, and Mg and S were predicted with mean monthly temperature. Values of Cu, Fe, K, N, P, S, and Zn were higher than expected for one to two years following the 2000 fire, while Ca and Mg did not show any responses to the fire. Predictions for biomass and nutrient content apply to the range of conditions, temperatures and precipitation observed over the study period. The predictions may be useful in assessing responses to changes in climate, and are helpful in explaining variation in herbivore populations relative to changes in forage quality and quantity.

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KEY WORDS biomass, climate, fire, nutrients, *Pseudoroegneria spicata*

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Production and Nutrient Content of Two Shrub Species Related to Fire in Central Idaho

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ABSTRACT Nutrient content and weight of current year's growth of *Cercocarpus ledifolius* Nuttall and *Physocarpus malvaceus* (Greene) Kuntze in central Idaho were obtained during early July in the years 1987–2007. The purpose of this work was to determine whether there was significant variation between years and whether mean monthly temperatures and total monthly precipitation could predict the variation. A wildfire in August 2000 caused *P. malvaceus* to vigorously resprout. Significant differences between years occurred for all nutrients for both species. October temperatures best predicted weight of current year's growth in *C. ledifolius*, whereas prediction equations for nutrients involved spring temperatures and precipitation, primarily for June. January mean temperature and December precipitation best predicted weight of current year's growth, and spring mean monthly temperatures best predicted nutrient levels in *P. malvaceus.* Future changes in production and nutrient content of these species that are not predicted may be related to climate change.

Biennial Symposium of the Northern Wild Sheep and Goat Council 20:110.

Western North American Naturalist 75(4):446-45 (2015)
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Population Genetics of the World's Thinhorn Sheep (*Ovis dalli)*

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ABSTRACT Thinhorn sheep (*Ovis dalli*) populations throughout northwestern North America are managed to maximize hunting, viewing, and sustenance opportunities. Central to effective conservation management is the identification of biologically relevant groupings, which can inform game management units. The identification of genetically discrete population units is essential for the delineation of true population boundaries, which can then form the basis for monitoring and the development of specific conservation action. We investigated the worldwide population genetics of thinhorn sheep by profiling ~2000 harvested thinhorn rams from across the species' range using 153 single-nucleotide polymorphism (SNP) markers. We used the genetic profiles to characterize the distribution of genetic variation and identify population boundaries across the entire geographic range of thinhorn sheep. We were also able to re-examine the current subspecies (Dall's and Stone's sheep) boundaries for thinhorn sheep and found that current boundaries likely do not reflect the evolutionary history of the species. Results from this study will be used to update the thinhorn management and harvest policies in British Columbia and Yukon, Canada.

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KEYWORDS Dall's sheep, *Ovis dalli*, phylogeography, population structure, Stone's sheep, thinhorn sheep

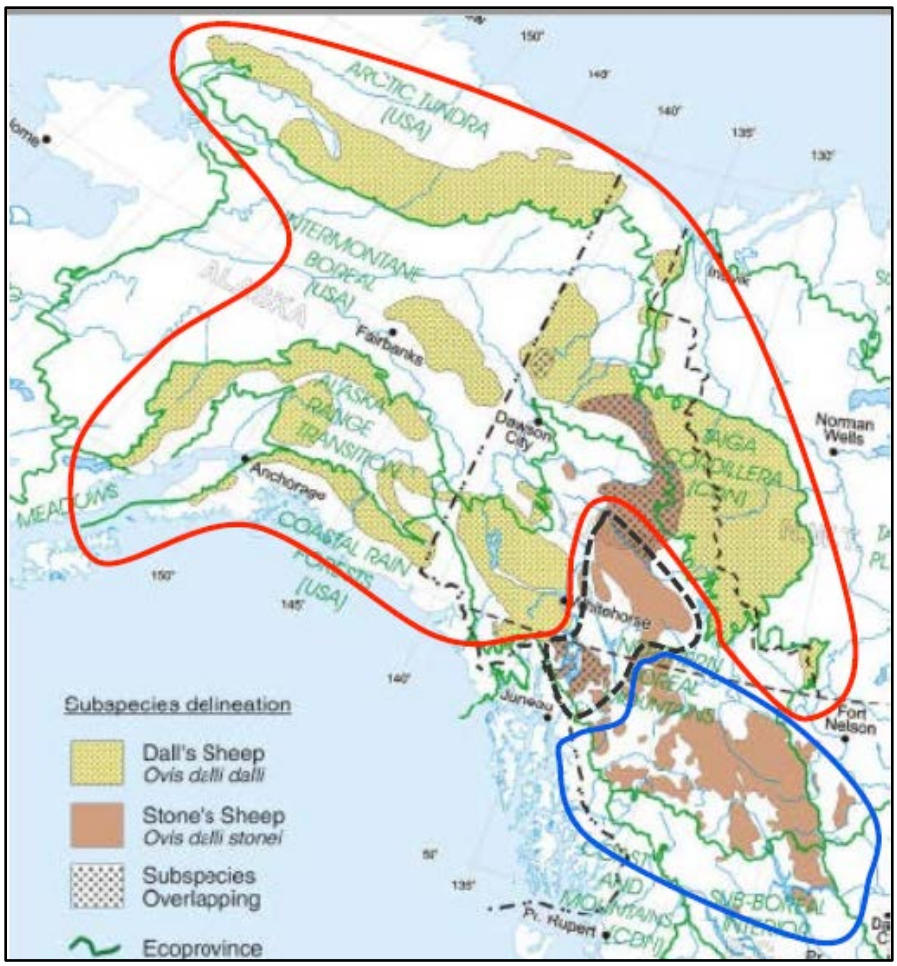

Figure 1: Map comparing subspecies genetic and current subspecies boundaries for thinhorn sheep. Red lines represent the approximate genetic boundaries for Dall's sheep (*Ovis dalli dalli*), blue for Stone's sheep (*O. d. stonei*). Dotted line represents admixed sheep.

Genetic Linkages Among *Mycoplasma ovipneumoniae* Strains in Wild and Domestic Sheep and Goats

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ABSTRACT Epizootic pneumonia has contributed greatly to historical declines and extirpations of bighorn sheep populations, and is now hampering their re-establishment. We examined the genetic diversity and phylogeographic structure of the bacterium *Mycoplasma ovipneumoniae* in reservoir (domestic sheep and goat) and spillover (bighorn sheep and mountain goat) hosts affected by bronchopneumonia across the western United States. We obtained *M. ovipneumoniae* isolates (n = 343) from diverse geographical and host sources and used a multi-locus sequence-based genetic typing (MLST) approach that targeted four polymorphic *M. ovipneumoniae* loci: the 16S-23S intergenic spacer (IGS), the small ribosomal subunit (16S), *gyrB*, and *rpoB*. We integrated pathogen sequence data with host species, location, and sampling year in population genetic and phylogeographic analyses to examine *M. ovipneumoniae* genetic diversity and relatedness among hosts and locations, and to evaluate patterns of pathogen spillover and persistence in bighorn sheep populations. Our preliminary results indicated that genetic diversity of *M. ovipneumoniae* strains was higher in domestic sheep than strains found in bighorn sheep. There was no evidence for geographic clustering of *M. ovipneumoniae* strains from domestic sheep. Instead, these strains were found distributed among strains derived from wildlife throughout the primary *M. ovipneumoniae* phylogenetic clade. Taken together, the genetic diversity and the relationships among strains suggest that there have been repeated *M. ovipneumoniae* spillover events from domestic to bighorn sheep. We also identified genetic linkages within and between neighboring bighorn sheep outbreaks, but because we have limited sampling from domestic sheep (relative to the *M. ovipneumoniae* strain diversity observed in this group), these outbreaks may alternatively be linked through unsampled domestic sheep sources. Domestic goats formed a genetically distinct clade, and there were only three observed instances of *M. ovipneumoniae* spillover from domestic goats into bighorn sheep. These data will enable a broad-scale molecular epidemiologic investigation of *M. ovipneumoniae* transmission dynamics and inform the development of effective management strategies for both controlling the disease and promoting the re-establishment of bighorn sheep.

Biennial Symposium of the Northern Wild Sheep and Goat Council 20:113.

KEYWORDS bighorn sheep, mountain goat, *Mycoplasma ovipneumoniae*, phylogenetics, pneumonia, spillover, transmission

An Initial Assessment of the Potential of Genomic Analysis to Help Inform Bighorn Sheep Management

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ABSTRACT Genetic research may be a useful approach for understanding factors that could impact productivity and restoration of bighorn sheep herds. For example, genetic consequences of inbreeding in small populations can impact recruitment, and local adaptations can influence translocation success. This modest pilot study quantified genetic attributes of bighorn sheep populations with a range of different herd histories in Montana and Wyoming to investigate genetic similarity and differences, genetic heterogeneity, and genetic distance. We used an Ovine array containing about 700,000 single nucleotide polymorphisms (SNPs) with approximately 24,000 markers that are informative for Rocky Mountain bighorn sheep. This technique represented a significant advancement in genetic analysis of bighorn sheep, because most previous studies have used microsatellites and less than 200 genetic markers. The Ovine array provided the capability to conduct whole genome genotyping of bighorn sheep and can increase understanding of population genetics. In addition, the Ovine array provides the potential to map informative SNPs to genomic areas of known function. We analyzed 11 to 16 individuals from each of 4 different populations that we predicted would differ in genetic characteristics due to population dissimilarities that potentially impacted their genetics. We examined differences in whether herds were native or reintroduced, population size, history of genetic bottlenecks, degree of connectivity, and augmentation history. The 4 populations we selected (Tendoys, Stillwater, and Glacier National Park (GNP) in Montana and the northeastern Greater Yellowstone Area (GYA) in Wyoming) provided a spectrum of herd attributes (Figure 1). The Tendoys herd was a small, introduced population on historical bighorn sheep range, which was started by two transplants in the 1980s. Additional transplants occurred in 1997 and 2002, following die-offs that occurred in 1993 and 1999. The Tendoys herd had strong potential for past bottlenecks to impact herd genetics and was also likely isolated from other bighorn sheep herds. In addition, we opportunistically included 2 samples from bighorn sheep that had been translocated from Wild Horse Island to the Tendoys. The Wild Horse Island herd was started by the transplant of 9 bighorn sheep in the 1930s-1940s and grew in number over time to a medium size, remaining isolated from other populations. Thus, there was a strong potential for past bottlenecks to impact genetics of this herd due to the small number of founding animals. Wild Horse Island bighorn sheep frequently serve as a source population for translocations, and examining this population could be informative for herds with initial reintroductions or augmentations from Wild Horse Island. The Stillwater population is a small, native herd with a moderate possibility for potential bottlenecks, and it may have some connectivity with the East GYA metapopulation. During the mid-1970s and early 1980s, the Stillwater population size ranged from 50 to 60, and the herd size decreased to below 40 in the mid-1980s and less than 30 sheep on average from 1989-1999. In recent years, there was

an increase in the population to a medium size. The GNP population represented a large, native herd that had high connectivity and mild potential for past bottlenecks. In addition to the Montana herds, we analyzed bighorn sheep across the east GYA in Wyoming (Wyoming hunt units 1-2). The Beartooth-Absaroka metapopulation also served as a baseline comparison of a large, native herd with high anticipated connectivity and genetic diversity. We are seeking to address important questions about the potential impacts of past management histories of individual bighorn sheep herds on their genetic attributes. We present the results and plan future research to evaluate the potential for links between genetics and herd demography. Genetic analyses may serve to improve knowledge of bighorn sheep populations and have potential implications for bighorn sheep management.

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KEYWORDS bighorn sheep, genetic bottleneck, genomics, *Ovis canadensis*, population management

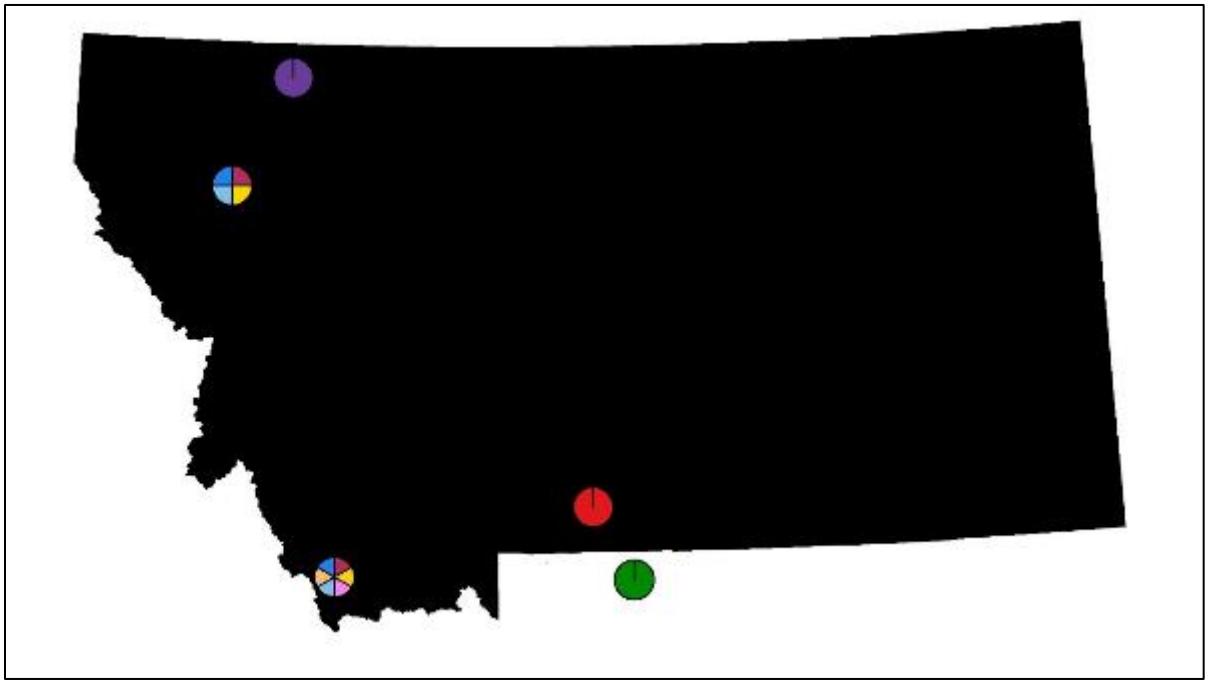

Figure 1. Hypothesized genetic composition of bighorn sheep herds in Montana and the east Greater Yellowstone Area (GYA) that were selected for this pilot study. Circles with one color symbolize native and large herds, as these herds have only one potential genetic source. Circles with multiple colors symbolize reintroduced and augmented herds, as these have multiple potential genetic sources caused by reintroduction and/or previous augmentations from multiple source populations.

Associations Between Allelic Diversity and *Mycoplasma ovipneumoniae* Shedding History in Bighorn Sheep

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ABSTRACT Epizootic bronchopneumonia is a respiratory disease of high morbidity and mortality and is considered one of the most significant limiting factors of bighorn sheep (*Ovis canadensis*) populations. Furthermore, decreasing genetic diversity within bighorn sheep populations also is a concern. Understanding the complex dynamics between disease and genetic diversity in bighorn sheep populations is of growing importance. One primary pathogen under investigation is *Mycoplasma ovipneumoniae*. *M. ovipneumoniae* is a bacterium associated with respiratory disease in sheep and goats worldwide. Polymorphic Major Histocompatibility Complex (MHC) allelic variants have been associated with susceptibility and resistance to infectious diseases in domestic and bighorn sheep (Larruskain et al. 2010, O'Brien and Evermann 1988). Recent studies have found associations between internal parasite loads and genetic diversity (Luikart et al. 2008). However, the association between allelic variation and *M. ovipneumoniae* shedding patterns in bighorn sheep has not been addressed. Our objective was to compare allelic variation from 5 *M. ovipneumoniae*-exposed populations and associate the genetic diversity of individual bighorn sheep within pathogen shedding groups. Shedding status was categorized by repeated monitoring of *M. ovipneumoniae* presence/absence through collection of nasal and oropharyngeal swabs at ≤ 6 week intervals for 8 consecutive months. Individuals testing negative for *M. ovipneumoniae* on PCR in ≥ 75%, 74-26%, and ≤ 25% of repeated test samples were categorized as negative, intermittent, and chronic shedder, respectively. We compared loci to determine if heterogeneity was differentially correlated with *M. ovipneumoniae* shedding pattern dynamics. We predicted that individuals with higher heterozygosity and/or unique polymorphisms in MHC alleles would be more resistant to *M. ovipneumoniae* pathogens. We sampled 27 bighorn sheep, and amplified these samples at 3 microsatellite loci in candidate genes (ADCYAP1, TCRG4 and MMP9). We tested associations between microsatellite genotypes and shedding phenotypes using Pearson's χ^2. We used Kruskal-Wallis one-way analysis of variance to test for significant differences between the 3 shedding statuses and the associated allelic

diversities within those shedding statuses. Preliminary results indicate that allelic diversity among negative ($\bar{x}$ = 1.00) and intermittent ($\bar{x}$ = 1.27) shedders were similar (average $P = 0.61$); whereas, chronic shedders ($\bar{x}$ = 2.50) had higher allelic diversity and differed from negative ($P < 0.01$) and intermittent ($P < 0.01$) shedding genotypes. In future analyses, we will include 15 microsatellites (8 neutral loci, 3 loci in genes and 4 loci in candidate genes) and individual bighorns from 4 additional *M. ovipneumoniae*-exposed populations. By identifying the effect of genetic diversity and specific loci on disease resistance, we hope to provide managers with scientific evidence to sustain successful long-term bighorn sheep translocations and augmentations while decreasing livestock producer-wildlife conflicts.

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KEYWORDS genetic diversity, microsatellites, *Mycoplasma ovipneumoniae*