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

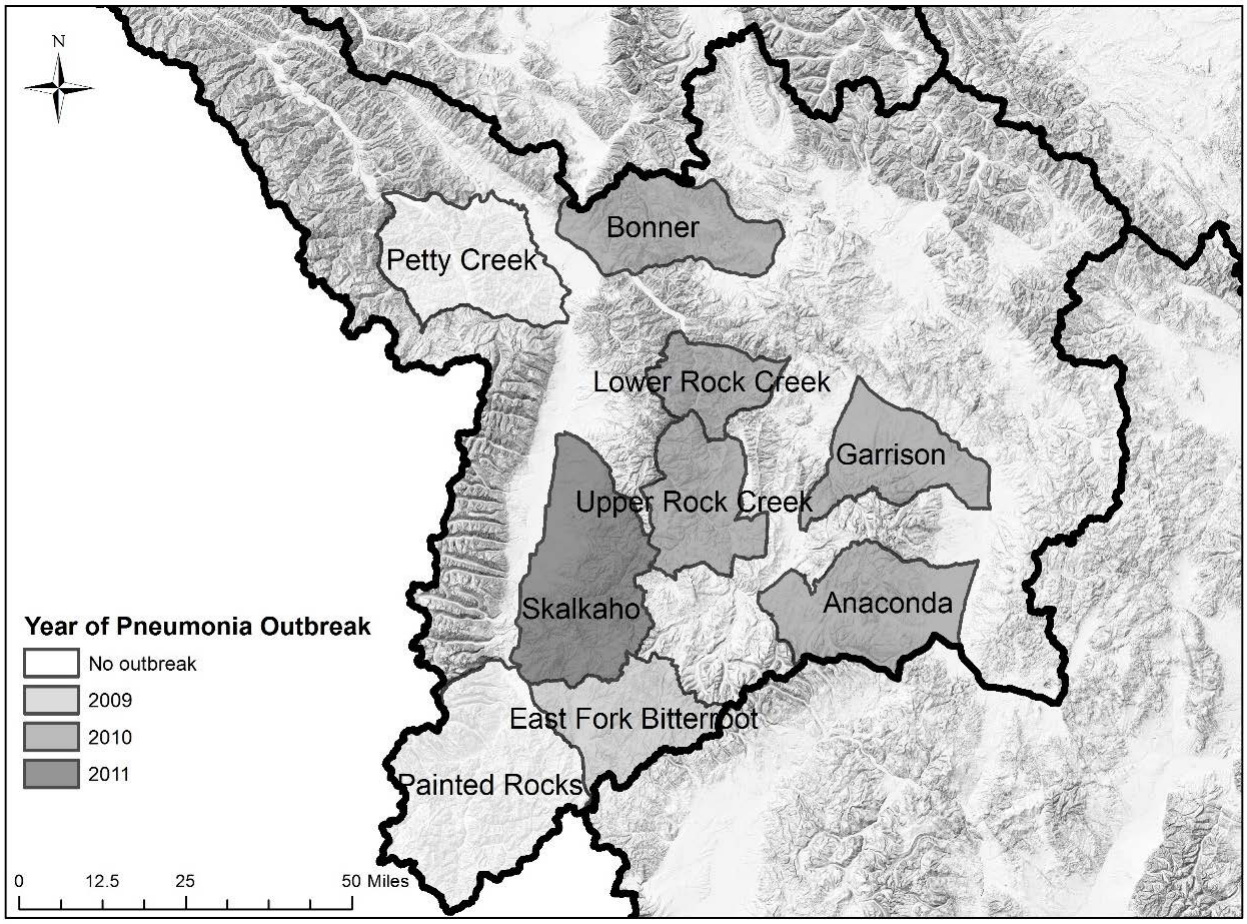

Figure 1. Map of bighorn sheep herds with pneumonia outbreaks during winter 2009-2011 and neighboring populations in Montana Fish, Wildlife and Parks, Administrative Region 2, western Montana.

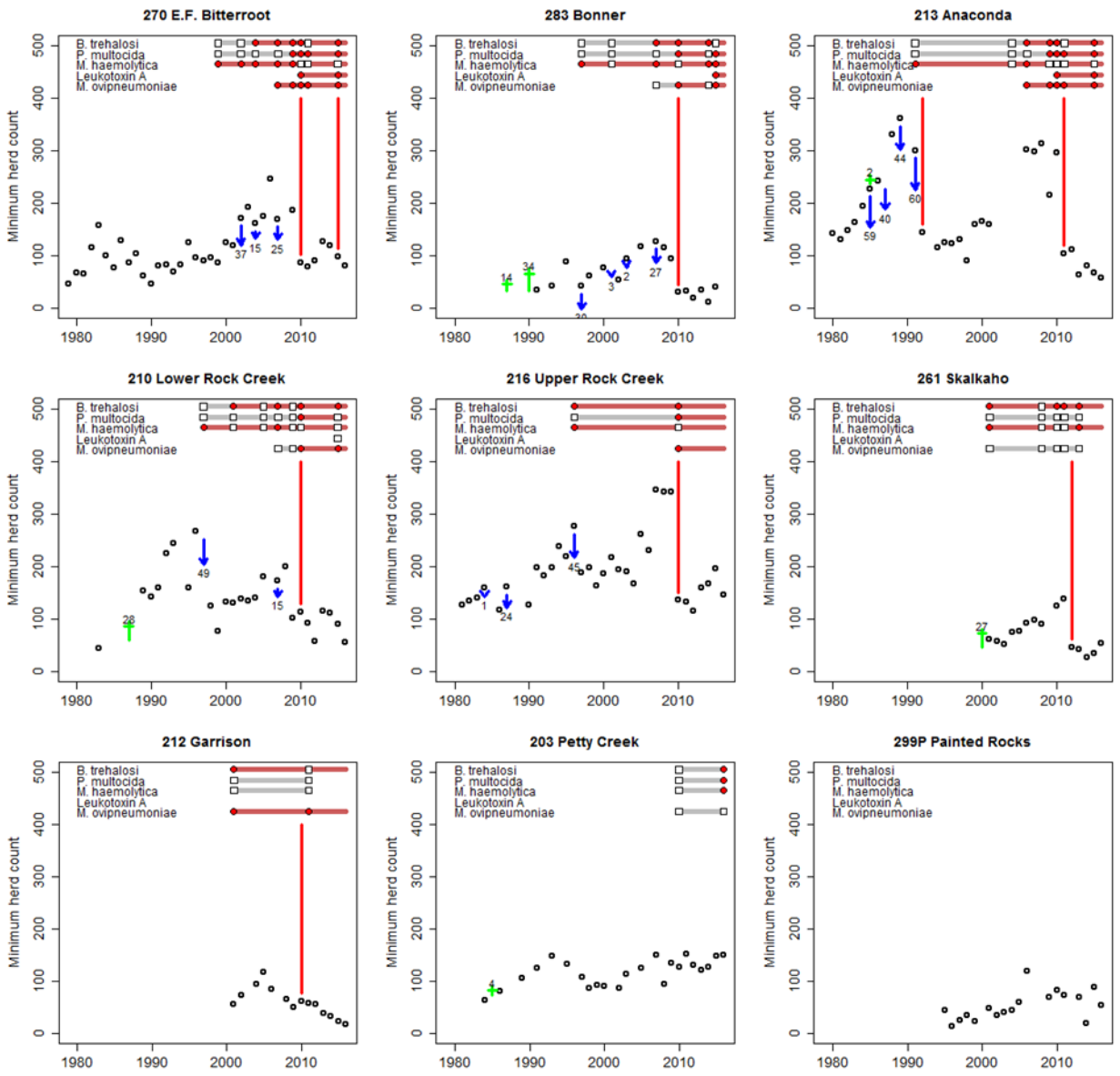

Figure 2. Plots of population size over time for 9 bighorn sheep herds within Montana Fish, Wildlife and Parks, Administrative Region 2, western Montana. Red vertical lines indicate the year of an observed all-age pneumonia die-off. Blue arrows indicate translocations out of the herd with the number 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.

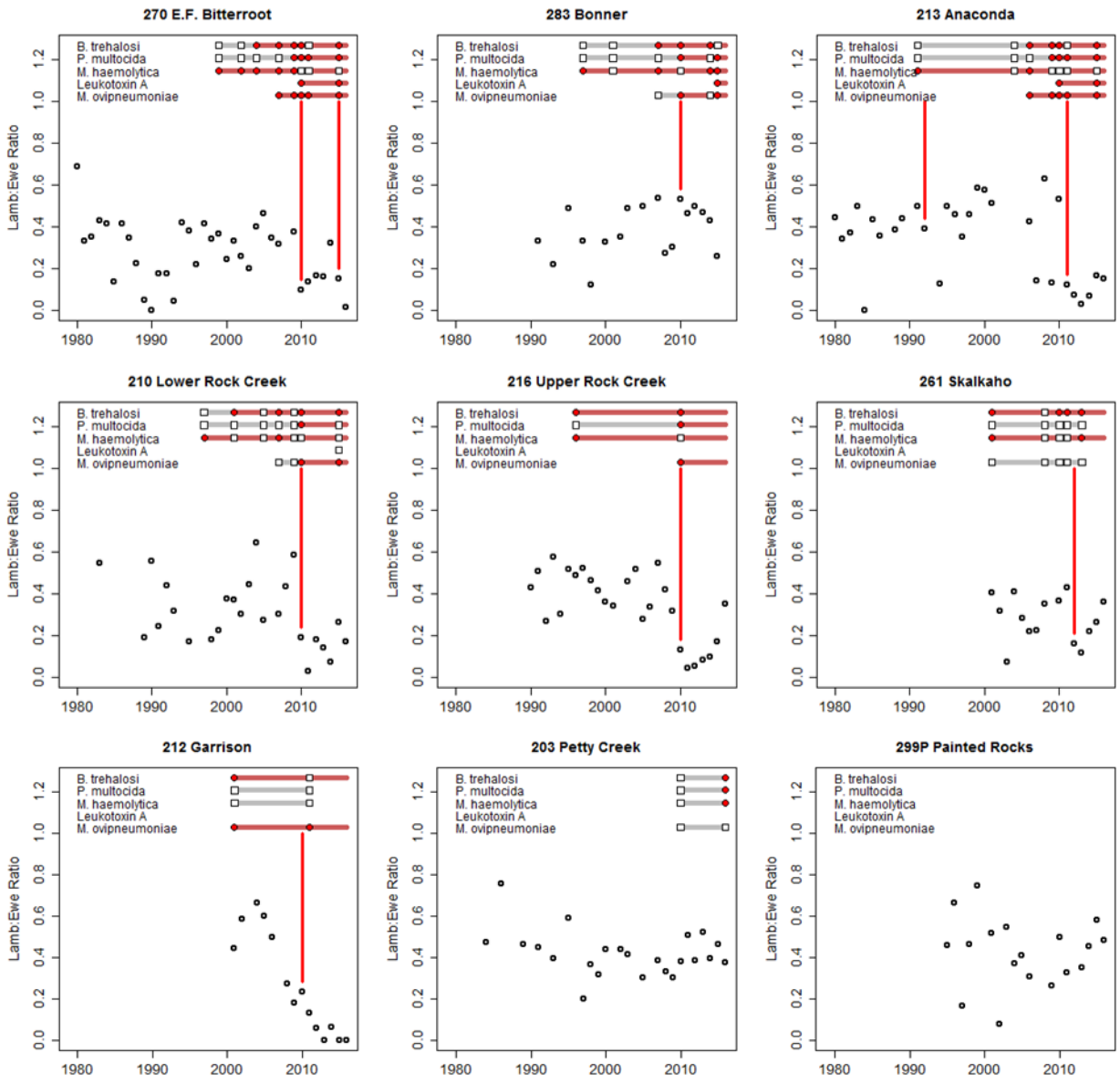

Figure 3. Plots of lambs per 100 ewes over time for 9 bighorn sheep herds within Montana Fish, Wildlife and Parks, Administrative Region 2, western Montana. Red vertical lines indicate the year of an observed all-age pneumonia die-off. Pathogen presence data is displayed at the top for *Bibersteinia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

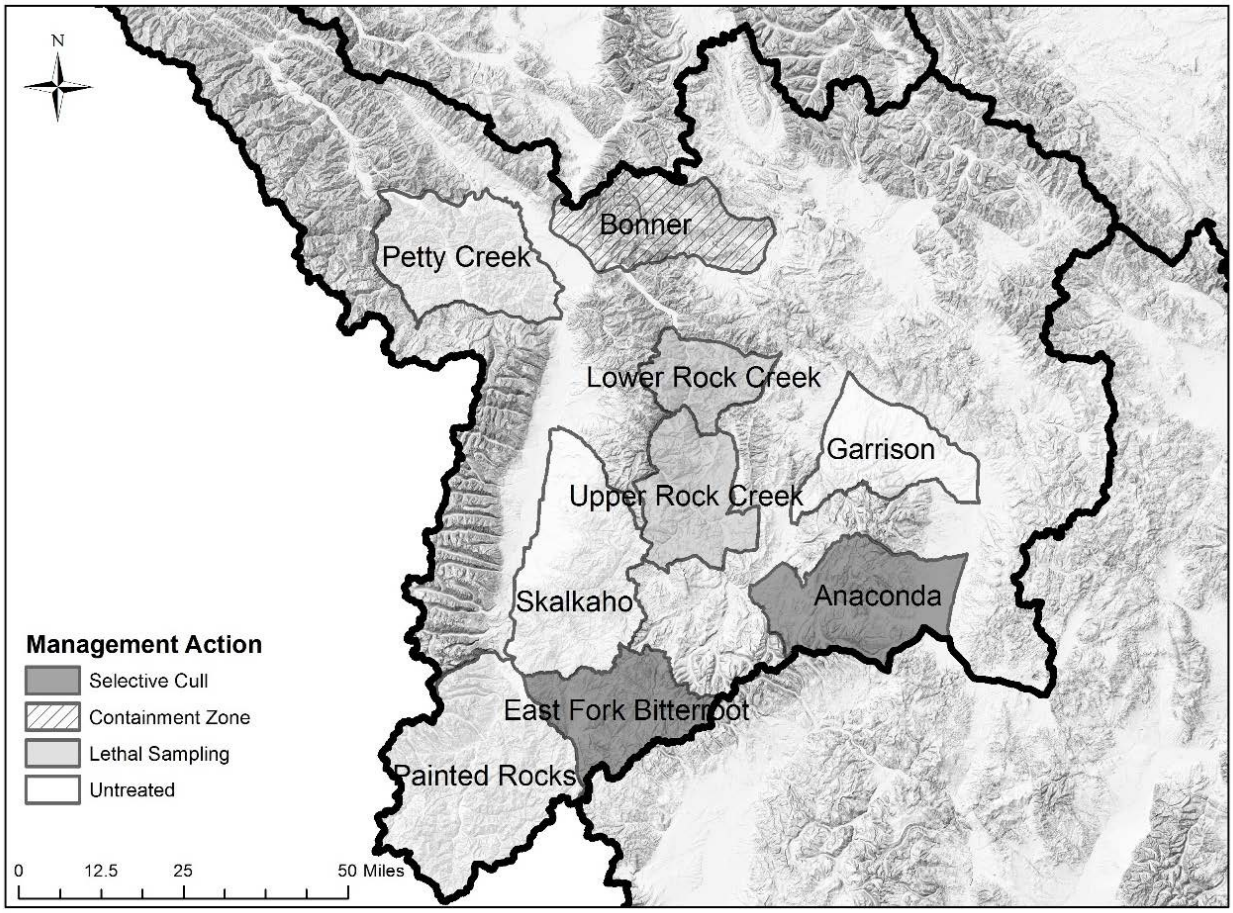

Figure 4. Map of bighorn sheep herds and the corresponding management action applied during the 2009-2010 pneumonia outbreak in Montana Fish, Wildlife and Parks, Administrative Region 2, western Montana. A selective culling effort was initiated in Anaconda, but was abandoned because the pneumonia outbreak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