Western Watersheds Project

CENTER for BIOLOGICAL DIVERSITY

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WILDERNESS WATCH

Keeping Wilderness Wild

Conservation CONGRESS

Jan Cutts August 5, 2019

District Ranger

Bridgeport Ranger District

HC 62 Box 1000

Bridgeport CA 93517

Via email comments-intermtn-humboldt-toiyabe-bridgeport@fs.fed.us and Via project website: https://cara.ecosystem-management.org/Public//CommentInput?Project=49993

RE: Bridgeport Southwest Rangeland Project

Dear District Ranger:

These second scoping comments are submitted on the Notice of Proposed Action (NOPA) on the Bridgeport Southwest Rangeland Project by Humboldt-Toiyabe National Forest (Forest) on behalf of Western Watersheds Project, the Center for Biological Diversity, Wilderness Watch, and Conservation Congress (collectively "conservation groups"). The conservation groups submitted extensive comments with attachments and references on June 4, 2018 in response to the initial scoping notice. Those comments, attachments and references are incorporated herein.

Western Watersheds Project (WWP) is a non-profit organization with more than 9,000 members and supporters. Our mission is to protect and restore western watersheds and wildlife through education, public policy initiatives and legal advocacy. Western Watersheds Project and its staff and members use and enjoy the public lands and their wildlife, cultural and natural resources for health, recreational, scientific, spiritual, educational, aesthetic, and other purposes.

The Center for Biological Diversity is a non-profit environmental organization dedicated to the protection of native species and their habitats through science, policy, and environmental law. The Center has over 1.6 million members and online activists with over 70,000 members throughout California and the western United States. The Center and its members have worked to ensure the conservation of the Sierra Nevada bighorn including by seeking protections for this endangered species from the risk of disease transmission from domestic sheep grazing in its habitat. The Center and its members have also worked to ensure protection for other listed, rare, and special status species in this area that may be adversely affected by the proposal to allow cattle grazing on these allotments including Bi-State sage grouse,[1] Sierra Nevada red fox, Yosemite toad, gray-headed pika (*Ochotona princeps schisticeps*), and rare plants.

Wilderness Watch is the leading national organization whose sole focus is the preservation and proper stewardship of lands and rivers included in the National Wilderness Preservation System (NWPS). The organization grew out of the concern that while much emphasis is being placed on adding new areas to these systems, the conditions of existing Wilderness and rivers are largely being ignored. We believe that the stewardship of these remarkable wild places must be assured through independent citizen oversight, education, and the continual monitoring of federal management activities. Wilderness Watch is committed to citizen oversight, public education and when necessary, legal and legislative action, to protect America's finest environmental legacy for present and future generations.

The Conservation Congress is a grassroots 501(c)3 nonprofit conservation organization incorporated in the state of California in 2004. We work to protect National Forest lands and native wildlife in northern California. The Conservation Congress is part of Voices for Public Lands (VPL), an informal coalition of public lands conservation groups united by a commitment to the values enumerated in VPL's Declaration of Principles for Public Lands. We believe these public lands that are owned by the American people and paid for with taxpayer dollars should have a strong public voice. Therefore, Conservation Congress especially provides a voice for the voiceless – the wildlife, trees, water and the interconnected ecosystems that cannot speak for themselves.

The conservation groups are concerned that the NOPA appears to show that the Forest Service may have pre-determined the outcome of this process before undertaking needed detailed environmental review regarding the potentially significant impacts of cattle grazing on these allotments. These high-elevation allotments are a hot spot of biodiversity providing habitat for many listed, rare and sensitive species (including, but not limited to, Sierra Nevada bighorn sheep, sage-grouse, Yosemite toad), that would be adversely impacted by cattle grazing on these lands. In addition, other resource conflicts include impacts to water quality, riparian areas, and recreation.

Because this area lies in the heart of the scenic Eastern Sierra region, close to Mono Lake and Yosemite National Park, and in a biodiversity hotspot, a full Environmental Impact Statement (EIS) needs to be prepared, as cattle grazing impacts to other resources would be significant. In addition, whether an EA or EIS is prepared, the Forest Service should consider at least one alternative that would administratively put these allotments in nonuse for the next 10-20 years to promote recovery of environmental resources, and an alternative that would include a plan amendment to close these allotments and not make them available for any livestock grazing to protect environmental resources.

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[1] The Bi-State sage grouse is currently proposed for listing and along with proposed critical habitat. https://www.federalregister.gov/documents/2019/04/12/2019-07252/endangered-and-threatened-wildlife-and-plants-threatened-status-for-the-bi-state-distinct-population

Desired Conditions

Based on forest plans as amended, the allotments in question are beginning to meet desired conditions because they have been rested since 2009, from all livestock grazing. Rangelands will be in satisfactory condition (Toiyabe National Forest 1986 Land and Resource Management Plan p., IV-4); riparian areas and meadows will be in late seral condition (2004 Sierra Nevada Forest Plan Amendment, p. 42); and rangelands in the project area that provide Bi-State greater sage-grouse habitat will meet the desired habitat conditions at the landscape scale (2016 Greater Sage-grouse Bi-State Distinct Population Segment Forest Plan Amendment, p. 37-38).

From our 2018 field visits to the allotments, we agree that most of the rangelands are in satisfactory condition, but this is precisely because they have been rested for from 4 to as long as 15 years. Grasses, forbs, meadows, riparian areas, and stream banks are recovering from all impacts of livestock grazing after permits were canceled. Riparian areas and meadows are improving from grazed conditions to late seral conditions. We would like to see these areas continue to improve to climax state or Potential Natural Community free of seral plant species, in order to provide better quality wildlife habitat. Introducing cattle grazing would bring back disturbances which could lower vegetation structure back to early seral stages.

Yosemite Toad and Sierra Nevada Yellow-legged Frog

As the attached maps show, Yosemite Toad and Sierra Nevada Yellow Legged Frog are present in this area with designated critical habitat adjoining or overlapping the allotments (See Map 1). As such, these species could be present on other portions of the allotment as well and full surveys at appropriate times of year should be conducted for the environmental review. In addition, because full fencing is not required in the proposal and cattle are proposed to be allowed to wander, it is highly unlikely that range riders will be able to contain cattle in these unfenced allotment boundaries completely. Therefore livestock could stray far and wide into critical habitat outside the boundaries, and further into Hoover Wilderness causing additional impacts to these resources. These issues must be fully addressed in the environmental review.

Proposed cattle grazing on the meadows, lake edges, and streams would have negative impacts from erosion of stream banks, trampling, lowering water quality, and removing vegetation that would impact amphibians. There are sensitive meadows along Tamarack Creek in Hoover Wilderness and Tamarack Lake, that would be within the new proposed allotment boundary, and we are concerned about cattle damage occurring there.

The Forest Service must undertake surveys for amphibians in this area, including the allotments and include that baseline data in an EIS.

The NOPA at 6 and 7 states: Riparian areas/Meadows will be grazed to early seral standards until monitoring indicates a different condition (30 percent or minimum six-inch stubble height). The riparian areas and meadows are currently at mid to late seral; stage, so grazing cattle at a rate that would take these habitats back to early seral state is a significant impact to Yosemite toads and mountain yellow-legged frogs. Early seral states indicate a percentage of bare ground, closely cropped vegetation, disturbance, and potential for erosion and spread of weeds. This is not acceptable.

Bi-State Sage-Grouse

The Forest lists a few standards and guidelines it proposes in the NOPA that reduce impacts of livestock water facilities, yet the Forest does not address the impacts to sage-grouse habitat of grazing meadows and riparian to early seral stages. This contradicts the Bi-State Sage-grouse plan amendment at 38, which states desired conditions for nesting habitat should allow for "[p]erennial grass height provides overhead and lateral concealment from predators," and "[g]rass forb heights provide lateral and overhead concealment."

Current sage-grouse habitat is improving and provides better cover of both perennial grasses and sagebrush, than adjacent grazed ranges.

Given the new information of declining populations,[2] and new status of the Bi-State Distinct Population Segment of sage-grouse recently coming under formal status review by US Fish and Wildlife Service for listing under the Endangered Species Act,[3] an EIS needs to be prepared, not an Environmental Assessment (EA).

Fencing standards and guidelines in sage-grouse habitat need to be better delineated. We found many old and downed barbed wire fences in the allotments, and the proposal to add new fences would add risk of collision mortality to sage-grouse.

Additional fences are proposed if needed (NOPA at 12), which would add impacts to bi-state sage-grouse. Fencing can be protective for some resources and also have significant impacts to wildlife by, for example, fragmenting habitat and by providing perching opportunities for predators. Existing fencing should be reduced or eliminated in Bi-State sage grouse habitat including occupied and recovery habitat and no new fencing should be allowed in these areas. Tagging barbed wire fences does not eliminate sage-grouse mortality—mortality is only reduced. Christiansen (2009) observes a 61% reduction in fence collisions with reflectors on fences, yet this still equates to mortality. Van Lanen et al. (2017) say: "Our results suggest that all three types of fence markers employed in our research were effective at reducing collision probabilities and confirmed our hypothesis, with stretches of marked fence having a 57% (27% - 87%) lower probability of containing ≥1 collision." But this means, broadly, that fence markers fail to prevent 43% of the collision mortalities from an unmarked fence. The best conservation action would be to remove fences where appropriate.

[2] Bi-State Sage-Grouse Local Area Working Group meeting, June 5, 2019, Walker CA.
[3] 84 FR 14909-14910

The NOPA did not address grass stubble height as it impacts sage-grouse. The height of grass cover in nesting and brood-rearing habitat is a key factor in determining the recruitment success of imperiled greater sage-grouse. Cattle graze down the native vegetation these rare birds use for food and for hiding their nests from predators. Ravens in particular are a problem predator for the Bi-State sage-grouse. In a study conducted in Montana and Wyoming (Doherty et al. 2014), populations showed significantly higher nest survival rates with higher average grass height. When grass heights averaged 7 inches, grouse nests in the Wyoming part of the study had a 75 percent chance of survival, while Montana nests had only a 47 percent survival rate when grass heights averaged 7 inches. The Montana nests had a 60 percent survival rate at 10.2 inches of grass cover, but didn't reach the 75 percent survival threshold until grass heights topped 15 inches.

This research is consistent with previous science that indicated that land managers should maintain at least 7 inches of grass height in sage grouse nesting and chick-rearing habitats in drier parts of the sage grouse range. California sage-grouse habitats would greatly benefit from similar stubble height standards, especially as ravens are a huge stressor on the Bi-State DPS, from such local factors as the un-covered Mono County trash dump in Long Valley, not far from the project site.

A 7-inch stubble height standard should be applied to both meadows and upland habitat.

Federally Endangered Sierra Nevada Bighorn Sheep

Because Critical Habitat overlaps the proposed allotments, and because cattle will effect bighorn by potential disease transfer and behavioral modification, impacts will be significant to this rare species. The NOPA did not acknowledge our concerns discussed in our prior scoping comment letter on this project. Therefore, the Forest must undertake a full Environmental Impact Statement and analyze these potential impacts.

Radio-collared bighorn sheep have been observed to wander from Lundy Canyon, and even on occasion into Bridgeport Valley.

West-wide, bighorn sheep populations have declined by more than 90% since the mid-nineteenth century, and bighorn sheep overall distribution has been reduced to less than 30% of the species' historic range.[4] The primary causes of historic bighorn sheep declines include livestock diseases, overhunting, and forage competition with livestock.[5]

Bighorn sheep remain at risk of disease from livestock pathogens throughout the West, with authorized grazing on public lands a limiting factor for many populations. Large areas of historic bighorn sheep habitat are unavailable for recolonization or artificial restocking due to the presence of livestock, including in California.

[4] U.S. Forest Service. (2009). Addition of Big Horn Sheep to the Forest Service Intermountain Region Sensitive Species List.
[5] Besser, T., Cassirer, F., Highland, M., Wolff, P., Justice-Allen, A., Mansfield, K., Davis, M., Foreyt, W. (2013). Bighorn sheep pneumonia: Sorting out the cause of a polymicrobial disease. Preventive Veterinary Medicine, 108, 85–93.

The Sierra Nevada subspecies of bighorn sheep was reduced to approximately 100 animals by the mid-1970s, and was added to the U.S. Fish and Wildlife Service Endangered Species list through an emergency declaration in 2000. Since this time, the population of Sierra Nevada bighorn sheep has grown to roughly 600 animals.

The allotments being analyzed for this project contain occupied Sierra Nevada bighorn sheep habitat. Cattle grazing has the potential to negatively impact bighorn populations: cattle are known to carry pathogens that can be transmitted to bighorn sheep, cattle may displace bighorn sheep from optimal habitats, reducing foraging efficiency, and cattle contribute to the spread of noxious weeds which outcompete native vegetation, degrade bighorn sheep habitat, and increase fire risk.

Cattle have been implicated in pneumonia-related die-offs of bighorn sheep, as well as in outbreaks of Bovine Viral Diarrhea and other diseases impacting wild sheep. Bovine respiratory syncytial virus (BRSV) and bovine parainfluenza virus 3 have been identified as co-agents in pneumonia outbreaks in bighorn sheep populations, affecting bighorn herds exposed to primary agents *Mycoplasma ovipneumoniae* and *Mannheimia haemolytica.*[6] [7] *Mannheimia haemolytica* originating in cattle is believed to have been a primary respiratory disease agent in at least one bighorn sheep pneumonia outbreak.[8] [9]

Sierra Nevada bighorn sheep are subject to management direction contained in FSM 2670. Therefore, the Forest Service must complete a Biological Evaluation to determine the likelihood of harm to bighorn sheep viability (FSM 2672.41*).* The Forest Service must "ensure that actions authorized, funded, or carried out by them are not likely to jeopardize the continued existence of any threatened or endangered species or result in the destruction or adverse modification of their critical habitats" (FSM 2670.11) and "[a]void **all** adverse impacts on threatened and endangered species and their habitats, except when it is possible to compensate adverse effects totally…" (FSM 2670.31) (Emphasis added).

Will proposed fences be wildlife-friendly to allow safe passage of bighorn sheep. Such fences require smooth-wire bottom strands and 18-inch lower wire height.

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[6] Dassanayakea, R., Shanthalingam, S., Herndon, C., Subramaniam, R. Paulraj K. Lawrence, Bavananthasivam, J., Cassirer, F., Haldorson, G., Foreyt, W., Rurangirwaa, F., Knowles, D., Besser, T., Srikumaran, S. (2010). Mycoplasma ovipneumoniae can predispose bighorn sheep to fatal Mannheimia haemolytica pneumonia. Veterinary Microbiology, 145, 354–359.
[7] Spraker, T., Collins, J., Adrian, W., Otterman, J. (1986). Isolation and serologic evidence of a Respiratory Syncytial Virus in bighorn sheep from Colorado. Journal of Wildlife Diseases, 22(3), 416-418
[8] Wolfe, L. Diamond, B., Spraker, T., Sirochman, M., Walsh, D., Machin, C., Bade, D., Miller, M. (2010). A bighorn sheep die-off in southern Colorado involving a Pasteurellaceae strain that may have originated from syntopic cattle. Journal of Wildlife Diseases, 46(4), 1262-8.
[9] NDOW. (2001). Bighorn Sheep Management Plan.

The Forest Service must analyze and disclose the impacts of cattle grazing on the area's Sierra Nevada bighorn sheep population, including those from disease, displacement, and noxious weeds, and the Forest Service must take steps to preserve population viability and avoid any adverse impacts to the species or its habitat.

Rare Plants

Surveys for rare and sensitive plants should be undertaken during the appropriate seasons, and results and avoidance measures should be analyzed in an EIS. An EIS should also address best management practices to reduce and eliminate noxious weeds.

Aspen

We observed aspen communities along streambanks, in moist seeps in upland sites, and patches around snowdrifts. All aspen stands appeared to be in functioning condition, with abundant undergrowth. Cattle can heavily impact aspen stands by browsing on saplings, grazing on understory plants, breaking branches, disturbing and trampling the ground. The Forest should propose management guidelines to prevent cattle from impacting these recovering aspen stands, and halt any progression to at-risk status and loss of regeneration and diversity of age classes. Bare ground should not exceed 5%.

Riparian

Some riparian and aspen stands, as along Cameron Creek, may be functioning–at-risk due to indicators such as bare soil and presence of extensive stands of non-native bulbous bluegrass (*Poa bulbosa*). Most research indicates bulbous bluegrass requires disturbance to increase and can be an indicator of overgrazing by livestock; it is an indicator of deteriorating range conditions.[10]

Thus we have particular concerns with the Cameron Canyon Allotment. We recommend this allotment have a continued rest, as cattle grazing could push it to a non-functioning state, with a loss of resilience and possible resulting degraded state.

Meadows

The EIS must address how the Forest could prevent cattle from congregating on meadow communities, and cause bare ground, weed invasions, and over-utilization. We observed several meadow types around springs, seeps, and stream banks. Most appeared to be functioning or recovering but still at the threshold of moving back to functioning at risk. Small patches of cheatgrass have the potential to increase with cattle grazing. Undesirable species such as dandelion were abundant in some meadows, and bare ground still in evidence from past sheep grazing.

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[10] https://www.fs.fed.us/database/feis/plants/graminoid/poabul/all.html

The proposed Dunderberg Mine Allotment, in particular, has signs indicating function-at-risk, such as increased weedy forbs. This should be analyzed in an EIS.

Water Quality

Measures of water quality in all streams should be taken currently, and monitored in the future if cattle are permitted. These measures should include temperature, turbidity, nitrate, dissolved oxygen, and fecal coliform.

Cultural Resources

No mention is made of the high quality arborglyphs in aspen groves within the allotments, which should be protected from livestock. No proposal is given to fence these important areas off, or protect riparian areas and stream banks.

Hoover Wilderness and Inventoried Roadless Areas

Our first comments addressed these topics in some detail. Some of our suggestions dealing with ease of commenting were not incorporated in the NOPA. For example, the Hoover Wilderness boundary is not shown on Figure 3 in the NOPA. In fact, it is not shown on any map in the NOPA. Thus, it is difficult to assess where impacts could occur. The same is true for inventoried roadless areas, including those contiguous with the Hoover Wilderness.

While the NOPA states the EA will be looking at impacts to Wilderness and roadless areas, there is no recognition that grazing in Wilderness is a non-conforming use. In 1964, Congress passed the Wilderness Act "to secure for the American people of present and future generations the benefits of an enduring resource of wilderness."[11] The law provided statutory protections for wilderness areas and established the National Wilderness Preservation System. The Act, among other things, mandated that wilderness areas be administered in a manner that will leave them "unimpaired for future use and enjoyment as wilderness" and provide for "the protection of these areas" and "the preservation of their wilderness character."[12]

The Wilderness Act defines wilderness: "A wilderness, in contrast with those areas where man and his works dominate the landscape, is hereby recognized as an area where the earth and its community of life are *untrammeled by man*, where man himself is a visitor who does not remain."[13] Wilderness is "land retaining its *primeval character and influence*, without permanent improvements or human habitation, which is protected and managed so as to *preserve its natural conditions*...."[14] In addition, wilderness should be "*affected primarily by the forces of nature, with the imprint of man's work substantially*

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11 16 U.S.C. § 1131(a).
12 *Id.*
13 16 U.S.C. § 1131(c) (emphasis added).
14 *Id.* (emphasis added).

unnoticeable."[15]

The provision allowing grazing in the Wilderness Act is an exception to the general premise of the Act, which directs agencies to manage wilderness areas to preserve their wilderness character and natural conditions. The language concerning livestock grazing in wilderness is a mere forty words long: "Within wilderness areas in the national forests designated by this Act...the grazing of livestock, where established prior to September 3, 1964, shall be permitted to continue subject to such reasonable regulations as are deemed necessary by the Secretary of Agriculture." Thus, grazing which existed in wilderness areas when the Wilderness Act was enacted may continue. However, this proposal is not to continue the same "established" livestock grazing but to introduce a completely new type of livestock grazing by cattle in this high-elevation wilderness area that has only been previously grazed by sheep.

In other words, grazing is an exception to normal wilderness protections. It is a use that, by definition and practice, degrades Wilderness.

Further, the proposal is a grand experiment forced upon Wilderness and roadless areas:

> An initial calculation has been made to determine the occupancy rates, *but that rate and other aspects of the grazing management strategy may need to be adjusted as both the permittee and BRD learn the most effective and appropriate way to manage cattle grazing in this setting.* As a result, the proposed action would authorize flexible occupancy rates, season of use, and grazing management strategies as described below.

NOPA at 10, emphasis added. This is a tacit admission that the EA won't adequately analyze the impacts to Wilderness and roadless areas because those impacts are not known and, if the NOPA is to be believed, won't be known until years after the proposal has been approved. However, enough is known about cattle distribution, choice of areas, and utilization the make a much better analysis than the one that is projected.

Similarly, the idea that herding alone can distribute cattle has little basis in research. Few if any public land ranchers have the time or resources to have enough range riders to properly distribute cattle across the landscape to avoid problems. This could have a profound negative impact on Wilderness.

Sensitive meadows along Tamarack Creek would have cattle, as well as Tamarack Lake, would be within the new proposed allotment boundaries. Yet, as stated above, it is clear that the range riders will have a difficult time keeping cattle from wandering including further into the Hoover Wilderness. This should be analyzed in more detail in

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[15] *Id*. (emphasis added).

an EIS.

Recreation

The project area is very popular recreationally with visitors to the National Forest. The Virginia Lakes areas are a large draw, and the Trumball Campground is very well used. Dispersed camping along the Virginia Lakes Road is also a popular activity. We are concerned that without fences, and with few range riders, that cattle will wander into both the Trumball Campground and into dispersed campsites along routes in this area, and create negative visitor interactions with livestock.

In addition to the campgrounds, fishing opportunities, dispersed recreation, and wildflower viewing in the Virginia Creek and Dunderberg area, there is a resort near Little Virginia Lake. There are also a number of summer home cabins just below Big Virginia Lake. The cabin owners own their cabin but lease the land from the Forest Service. Leavitt Meadows Pack Station is located in the Virginia Creek drainage, as well as a number of private parcels, some with cabins and homes built on them. Water systems used by the campground, resort, pack station, and private parcels, and summer homes are precarious, and cattle grazing could well compromise water quality.

Robinson Creek area and campground is the most popular and visited recreation site in the entire Humboldt-Toiyabe National Forest. Cattle could impact this campground and the Twin Lakes area.

Robinson Creek and the Twin Lakes part of the district are immediately downhill from the proposed grazing allotment. There are no fences or natural barriers which will prevent cattle from drifting into this important part of the economy for northern Mono County. There are numerous Forest Service campgrounds, which are busy and full for most of the summer. Fishing forms the foundation for recreation activities and all associated activities in this important area. In addition to the campgrounds, there are a number of resorts, restaurants, and boat rentals in the Twin Lakes area. There is a large concentration of summer homes and private cabins in the Twin Lakes area.

Water systems for homes, resorts, and campgrounds are a challenge to maintain. Trampling and cattle waste pose a significant threat to water quality throughout the proposed allotment, and adjacent areas.

None of the facilities scattered throughout the proposed allotment have fences or barriers to prevent cattle from wandering throughout the area. The idea of cattle using this area has never been proposed and they have never grazed this important recreation portion of the Bridgeport Ranger District.

In addition to the huge popularity of the Virginia Lakes and Twin Lakes areas, the Upper Summers Meadows Road area, including Cameron Canyon and Upper Summers Meadow, is a very popular recreation area itself. Dispersed camping, RV camping, fishing, arbor art visitations, four-wheel-drive activities on the designated roads, and fishing are all popular seasonal activities. The use is growing with the increasing popularity of Twin Lakes and the Virginia Lakes area. Recreational opportunities should be given high management priority given Californian's greater demand for front country activities.

The Environmental Impact Statement should analyze an alternative designation recognizing the importance and significance of all of the use, facilities, and the importance of the area to the local economy. A Special Recreation Management Area designation, which manages for no livestock grazing, should be considered administratively. Management prescriptions that allow for primitive and dispersed recreation, with protection of its natural resources, and that keep these allotments closed to sheep and cattle, would have much support from the public. This could help balance multiple uses on this area.

Capability and Suitability

Capability indicates acres capable of supporting cattle grazing based on forage production of at least 200 lbs. per acre, slopes less than 40%, and areas within 1 mile of perennial water. The Updated Capability Assessment[16] maps only 36% of the total allotment acreages as capable of supporting cattle. We observe that more acres are covered with unpalatable montane shrubland, and this also should be mapped in an EIS and excluded as capable. A reduced-acreage allotment alternative should thus be analyzed in an EIS. It is unclear why the Forest changed this capability assessment from 2014 or what basis was used. An EIS should disclose the methodology and data used to make this change.

An EIS is needed to fully address impacts of the proposed project

If a federal action "significantly affect[s] the quality of the human environment" the agency must prepare an EIS. 42 U.S.C. § 4332(c); *Anderson v. Evans*, 371 F.3d 475, 487 (9th Cir. 2004) (agency prepared an EA, court held an EIS was required). The CEQ regulations, 40 C.F.R. § 1508.27, define the term "significantly" for purposes of NEPA and provides that "significantly" "requires consideration of both context and intensity." *See also, Consol. Salmonid Cases*, 688 F. Supp. 2d 1013, 1019 (E.D. Cal. 2010). Many of the "significance" factors set forth in the NEPA Regulations are implicated by adding a new oil and gas well and pipeline in this area.

The context of the action includes "society as a whole (human, national), the affected region, the affected interests, and the locality." *Anderson v. Evans*, 371 F.3d 475,

[16] Updated Capability Assessment for the Bridgeport Southwest Rangeland Project, 5/7/2019, accessed at https://www.fs.usda.gov/project/?project=49993.

487 (9th Cir. 2004); 40 C.F.R. § 1508.27(a). The Forest Service must address the context of the proposed action in the environmental review. As explained above, the context shows that the proposed action will have a significant effect as it is could undermine conservation of listed, rare and imperiled species on public lands that provide a rare biodiversity hot spot and could significantly impair world-class recreation opportunities in the high Sierra Nevada.

The consideration of "intensity" required by section 1508.27 refers to the severity of impact. 40 C.F.R. § 1508.27(b); *Anderson v. Evans*, 371 F.3d at 487. In order for an EIS to be required, the public "need not demonstrate that significant effects will occur. A showing that there are 'substantial questions whether a project may have a significant effect' on the environment is sufficient." *Anderson v. Evans*, 371 F.3d at 488; *Consol. Salmonid Cases,* 688 F. Supp. 2d at 1033.

An EIS is required here under several of the intensity factors because, as detailed above, cattle grazing could have adverse consequences to listed, rare, and special status species, water resources, riparian areas, recreation, and other resources. Each of the following intensity factors may be triggered here. 40 C.F.R. § 1508.27(b)(3) (unique characteristics of the area including proximity to historic or cultural resources or ecologically critical areas); § 1508.27(b)(4) (degree to which effects are likely to be highly controversial); § 1508.27(b)(5) (degree to which effects are highly uncertain or involve unique or unknown risks); § 1508.27(b)(6) (degree to which the action may establish a precedent for future actions with significant effects by allowing a major change in the type of grazing and impacts within wilderness); § 1508.27(b)(7) (whether the action is related to other actions with individually insignificant but cumulatively significant impacts); § 1508.27(b)(8) (may adversely affect significant scientific resources); § 1508.27(b)(9) (adverse effects on endangered species or their critical habitat); § 1508.27(b)(10) (threatens violation of the ESA and the Wilderness Act). These and other impacts must be fully evaluated in an EIS.

For example, the potential for disease transmission to bighorn sheep from cattle is known as are the potentially devastating impacts of such disease transmission, this is a unique risk that must be adequately addressed in an EIS before any decision can be made. 40 C.F.R. § 1508.27(b)(5) (degree to which effects are highly uncertain or involve unique or unknown risks). In addition, there is a substantial dispute about the importance and effect of opening these lands to cattle grazing and the proposal is significant under this intensity factor as well. 40 C.F.R. § 1508.27(b)(4) (effects controversy).

The cumulative significance factor is also triggered here: "Whether the action is related to other actions with individually insignificant but cumulatively significant impacts. Significance exists if it is reasonable to anticipate a cumulatively significant impact on the environment. Significance cannot be avoided by terming an action temporary or by breaking it down into small component parts." 40 C.F.R. § 1508.27(b)(7). The NEPA decision-making process is designed to address significant effects that could otherwise be masked by "the tyranny of small decisions." *See Kern v. Bureau of Land Mgmt*, 284 F.3d 1062, 1078 (9th Cir. 2002); *Pac. Coast Fed'n of Fishermen's Ass'ns v. Nat'l Marine Fisheries Serv.*, 265 F.3d 1028 (9th Cir. 2001).

The information available to date alone shows that the proposed action would impair habitat, water quality, and other values that must be addressed in a through environmental review; therefore, BLM should prepare an EIS. Many of the "significance factors," both of context and intensity, are triggered by the proposal including the potential effects on listed species and critical habitats, impacts to other species and habitats, impacts to water resources and riparian areas, the controversy regarding cattle grazing in this area, and impacts to recreation and visual resources. Therefore, an EIS is required.

Proposed Action

Neither Multiple Use Sustained Yield Act (MUSYA) nor the existing Forest Plans require that livestock grazing be the primary use of these lands or be authorized at all where it conflicts with other uses. Given the presence of listed and special status species may be impacted the Forest Service must consider prioritizing those uses over cattle grazing and closing the allotments for 10-20 years to support species and water resource recovery. The NOPA mentions the operative forest plans including the Sierra Nevada Forest Plan Amendment (SNFPA) but appears to only note the sections on livestock grazing. These plans also have other forest wide and resource specific requirements (standards and guidelines) for conserving and protecting water quality, riparian conservation areas, buffer areas, and aquatic/riparian resources including wet meadows that must be considered. In addition, the forest plans provide standards and guidelines for protecting and maintaining special status species habitats as a priority that the HTNF must take into account in the environmental review.

Even looking solely at multiple uses under MUSYA, recreation and natural resource protection must be given at least equal weight to livestock grazing and impacts cannot be ignored—decreasing the number of AUMs from the proposed amount and reducing the seasons or years grazed must also be considered. Resting the allotments for an additional 5 years should also be considered along with other alternatives that would keep the allotment closed for 10 or 20 years to protect other resources and allow recovery of native species.

Allotments are proposed to be divided up into pastures and grazed in a deferred or rest rotation system. Instead of new fences, range riders would keep cattle in these pastures. An EIS should be prepared since the presence of range riders could have impacts on bighorn sheep use of habitat and water sources, and impacts on sensitive wildlife periods.

The Forest proposes flexible occupancy rates, season of use, and grazing management strategies for cattle, *after* the public review process and Record of Decision. This is an unacceptable as so many sensitive and listed species are present here—adjusting cattle grazing parameters at a later time could greatly impact federally threatened and endangered species, and all flexible management proposals need to be analyzed during an EIS review in front of the public. The Forest should not defer these important management decisions to a later date.

Draft versions of any new proposed Allotment Management Plans should be included in the EIS.

Proper use criteria should be defined more specifically for vegetation types and habitats. For example, maximum allowable utilization percentages should be defined for aspen stands, wet meadow, dry-to-moist meadow, willow riparian, upland sagebrush-perennial grass communities, and higher-elevation *Ceanothus*-bitterbrush-sagebrush-conifer communities. Streambank alteration should take into account downstream impacts to Lahontan cutthroat trout populations.

Sage-grouse lekking, nesting, and brood-rearing should be a priority that is analyzed for season of use and start dates for grazing on the allotments.

Trailing is mentioned in the NOPA at 11. Impacts of trailing cattle through already-grazed pastures should be analyzed on vegetation cover, height, riparian vegetation, stream banks, sage-grouse habitat, bighorn sheep, Yosemite toads, and other species of concern.

Soil protection, weed control, and vegetation rehabilitation best management practices (BMPs) should be detailed in an EIS.

Three existing but poorly functioning water developments would be reconstructed to provide reliable stock watering points (NOPA at 12). The Forest should analyze more fully the impacts of new water sources in sage-grouse and Sierra Nevada bighorn sheep habitat in an EIS.

Discussions with Forest staff indicate that only one range rider is proposed to keep cattle within unfenced allotment boundaries. This is not adequate to keep cattle from wandering further into designated Wilderness, or into adjacent critical habitat units and those potential impacts must be fully addressed in the environmental review. Property owners in the community of Twin Lakes have made their concerns known about cattle wandering into the community as well.

Additional Issues

The NOPA at 13 says there is a potential for damage in areas of concentrated livestock use (e.g., around water developments, trailing routes, and along fences). The significant impacts of this issue should be fully analyzed in an EIS.

The NOPA at 14 says there is potential for cattle to concentrate around wetlands and in riparian areas despite restrictions on such use, and significantly impact several listed and rare species. The significant impacts of this issue should be fully analyzed in an EIS.

The pika should be considered as a species of concern for this project.

The NOPA at 16 says there is potential for impacts on wilderness character due to human activity and livestock use. The significant impacts of this issue should be fully analyzed in an EIS.

Impacts of new cattle grazing in this scenic region to recreation, wilderness, and Inventoried Roadless Areas should also be analyzed in an EIS. The impacts of cattle grazing to recreation will be significant, with dung, flies, and potential for negative user interface with unfenced cattle. The Virginia lakes area and Twin lakes areas have become increasingly popular with hikers, campers, and other visitors to the National Forest, wilderness, and adjacent parks.

An EIS should also analyze a reduced allotment size alternative that completely avoids critical habitat units and provides a buffer to such units.

Conclusion

Because of the great number of valuable resources, rare and listed species, potential significant cattle impacts to Sierra Nevada bighorn sheep, sage-grouse, Yosemite toads, and other species, plus the high recreational values of the area, we request that a full Environmental Impact Statement be analyzed for these allotments. . Because of the very high recreational value of the area, and the hot spot of rare and sensitive species, we ask that these allotments be canceled due to significant resource conflicts.

We appreciate the opportunity to assist the Humboldt-Toiyabe National Forest by providing additional scoping comments for the Bridgeport Southwest Rangeland Project environmental review. Please keep each of our groups informed of all further substantive stages in this and related NEPA processes and documents sent to the contacts below. us

Yours sincerely,

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Attachments:

Map 1: Allotment boundaries, proposed boundaries, "capability suitability" areas from NOPA, and designated critical habitats

Map 2: Allotment boundaries, proposed boundaries, "capability suitability" areas from NOPA, with threatened species sightings within 2 kim

Map 3: Allotment boundaries, proposed boundaries, "capability suitability" areas from NOPA, with CNDDB observations within 2 km

References (attached):

Besser, T., Cassirer, F., Highland, M., Wolff, P., Justice-Allen, A., Mansfield, K., Davis, M., Foreyt, W. 2013. Bighorn sheep pneumonia: Sorting out the cause of a polymicrobial disease. Preventive Veterinary Medicine, 108, 85–93.

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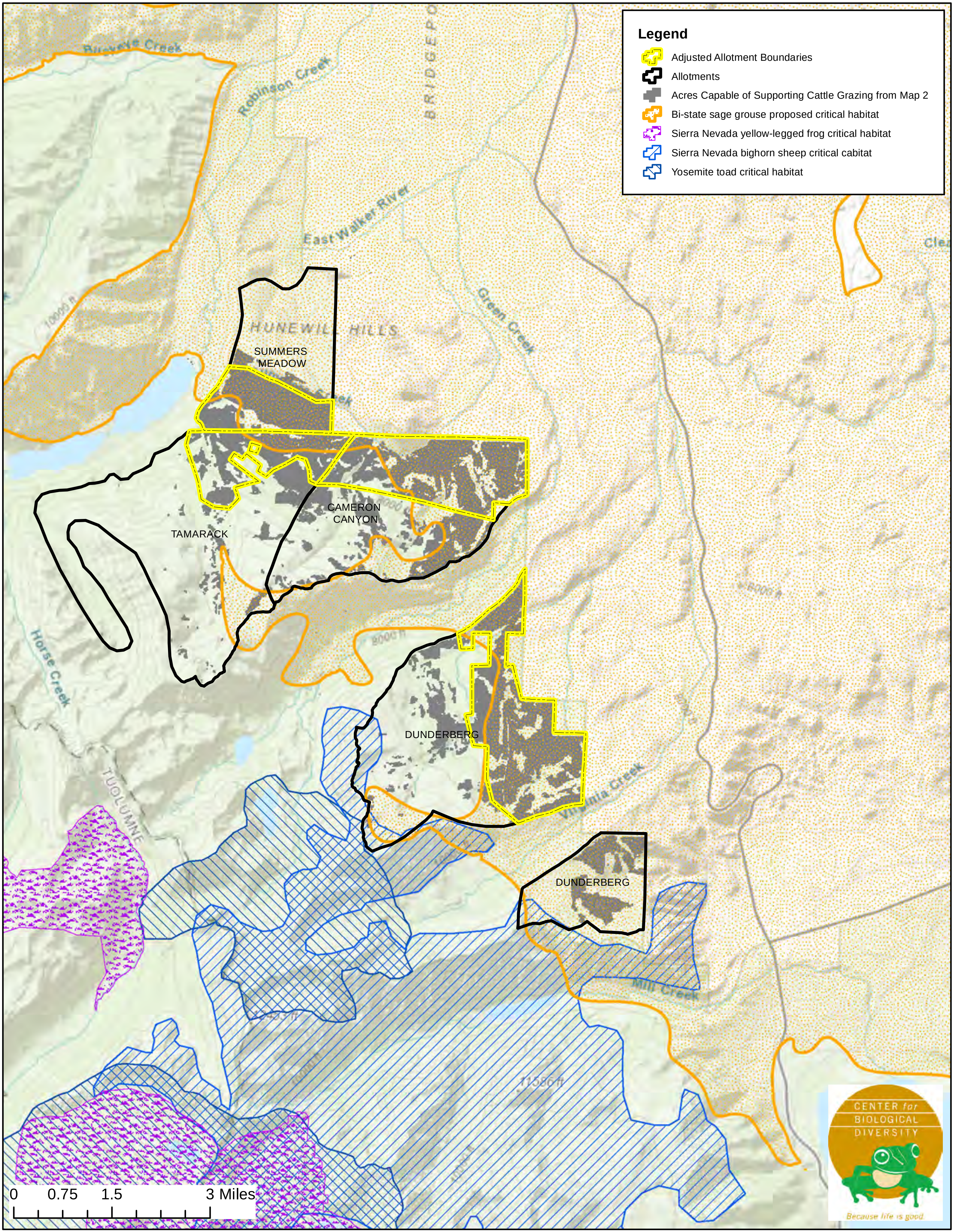
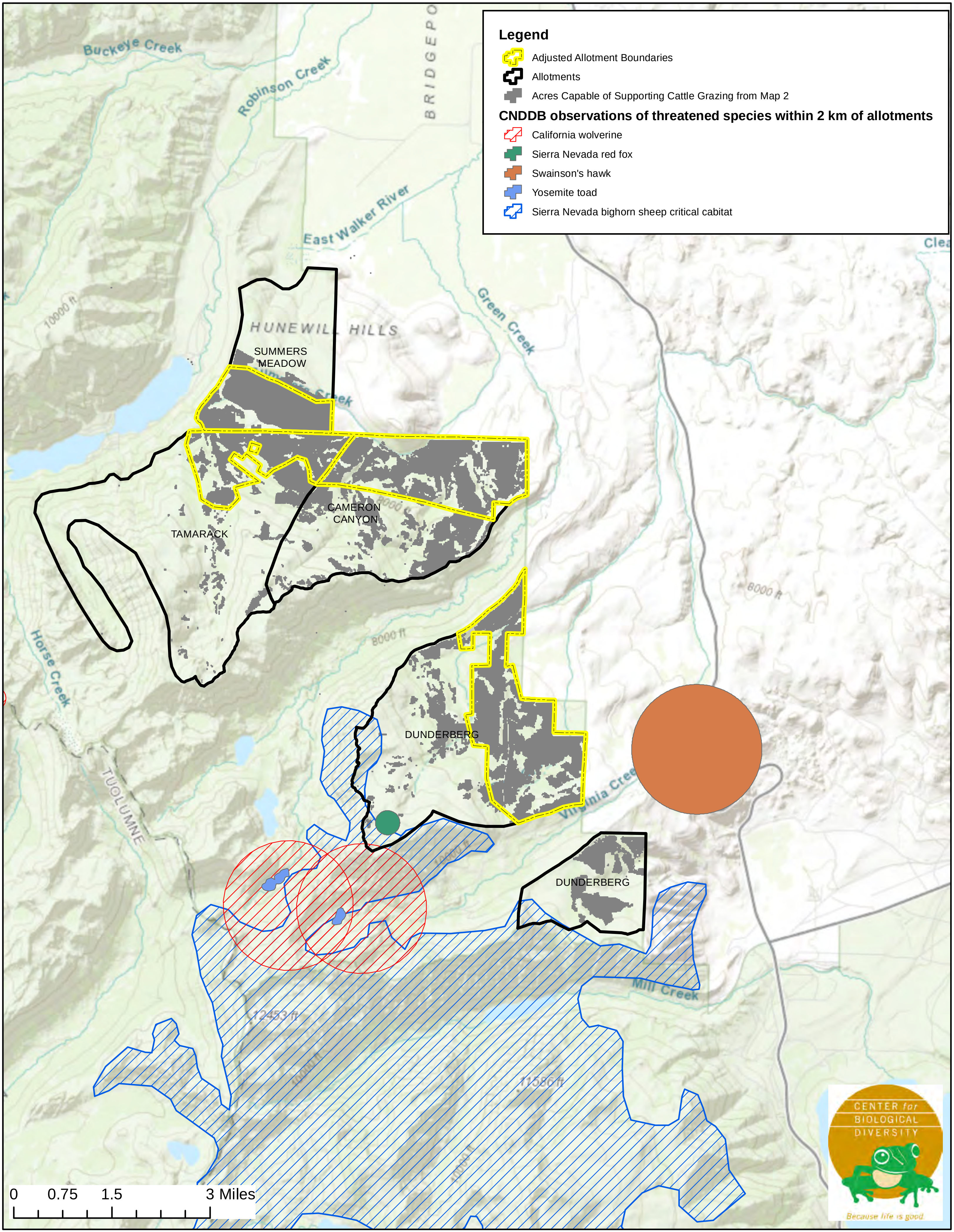
Attachments

Map 1

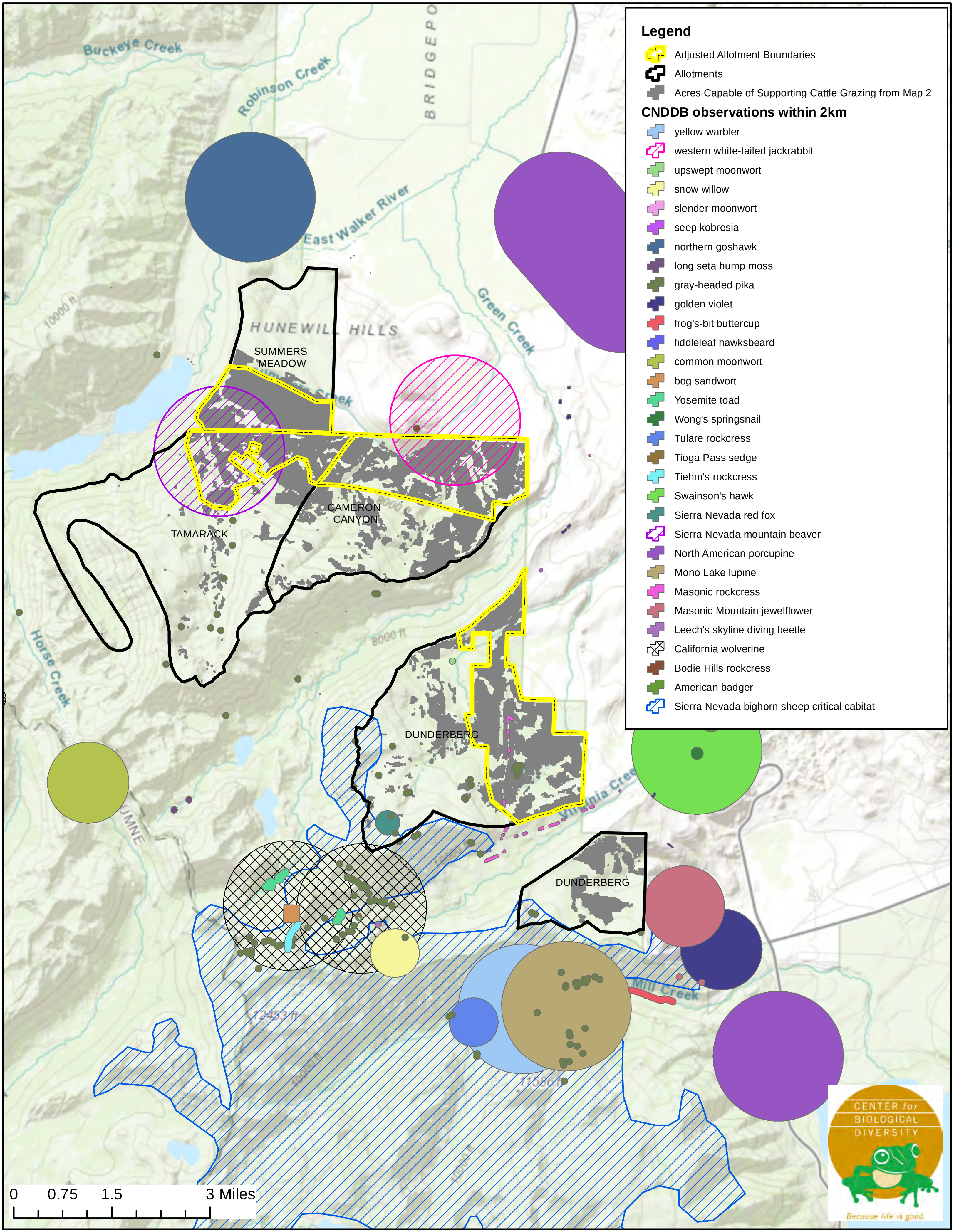


Legend
Adjusted Allotment Boundaries
Allotments
Acres Capable of Supporting Cattle Grazing from Map 2
Bi-state sage grouse proposed critical habitat
Sierra Nevada yellow-legged frog critical habitat
Sierra Nevada bighorn sheep critical cabitat
Yosemite toad critical habitat
Buckeye Creek
Robinson Creek
BRIDGEPO
East Walker River
Green Creek
HUNEWILL HILLS
SUMMERS MEADOW
CAMERON CANYON
TAMARACK
Horse Creek
DUNDERBERG
DUNDERBERG
Virginia Creek
Mill Creek
TUOLUMNE
0
0.75
1.5
3 Miles
CENTER for BIOLOGICAL DIVERSITY
Because life is good.

Map 2


Legend
Adjusted Allotment Boundaries
Allotments
Acres Capable of Supporting Cattle Grazing from Map 2
CNDDB observations of threatened species within 2 km of allotments
California wolverine
Sierra Nevada red fox
Swainson's hawk
Yosemite toad
Sierra Nevada bighorn sheep critical cabitat
SUMMERS MEADOW
TAMARACK
CAMERON CANYON
DUNDERBERG
DUNDERBERG
HUNEWILL HILLS
Buckeye Creek
Robinson Creek
East Walker River
Green Creek
Horse Creek
Mill Creek
TUOLUMNE
0 0.75 1.5 3 Miles
CENTER for BIOLOGICAL DIVERSITY
Because life is good.

Map 3


Legend
Adjusted Allotment Boundaries
Allotments
Acres Capable of Supporting Cattle Grazing from Map 2
CNDDB observations within 2km
yellow warbler
western white-tailed jackrabbit
upswept moonwort
snow willow
slender moonwort
seep kobresia
northern goshawk
long seta hump moss
gray-headed pika
golden violet
frog's-bit buttercup
fiddleleaf hawksbeard
common moonwort
bog sandwort
Yosemite toad
Wong's springsnail
Tulare rockcress
Tioga Pass sedge
Tiehm's rockcress
Swainson's hawk
Sierra Nevada red fox
Sierra Nevada mountain beaver
North American porcupine
Mono Lake lupine
Masonic rockcress
Masonic Mountain jewelflower
Leech's skyline diving beetle
California wolverine
Bodie Hills rockcress
American badger
Sierra Nevada bighorn sheep critical cabitat
SUMMERS MEADOW
TAMARACK
CAMERON CANYON
DUNDERBERG
DUNDERBERG
HUNEWILL HILLS
Buckeye Creek
Robinson Creek
East Walker River
Green Creek
Horse Creek
Mill Creek
BRIDGEPO
0 0.75 1.5 3 Miles
CENTER for BIOLOGICAL DIVERSITY
Because life is good.

References

Epizootic Pneumonia of Bighorn Sheep following Experimental Exposure to *Mycoplasma ovipneumoniae*

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Abstract

Background: Bronchopneumonia is a population limiting disease of bighorn sheep (*Ovis canadensis*). The cause of this disease has been a subject of debate. Leukotoxin expressing *Mannheimia haemolytica* and *Bibersteinia trehalosi* produce acute pneumonia after experimental challenge but are infrequently isolated from animals in natural outbreaks. *Mycoplasma ovipneumoniae*, epidemiologically implicated in naturally occurring outbreaks, has received little experimental evaluation as a primary agent of bighorn sheep pneumonia.

Methodology/Principal Findings: In two experiments, bighorn sheep housed in multiple pens 7.6 to 12 m apart were exposed to *M. ovipneumoniae* by introduction of a single infected or challenged animal to a single pen. Respiratory disease was monitored by observation of clinical signs and confirmed by necropsy. Bacterial involvement in the pneumonic lungs was evaluated by conventional aerobic bacteriology and by culture-independent methods. In both experiments the challenge strain of *M. ovipneumoniae* was transmitted to all animals both within and between pens and all infected bighorn sheep developed bronchopneumonia. In six bighorn sheep in which the disease was allowed to run its course, three died with bronchopneumonia 34, 65, and 109 days after *M. ovipneumoniae* introduction. Diverse bacterial populations, predominantly including multiple obligate anaerobic species, were present in pneumonic lung tissues at necropsy.

Conclusions/Significance: Exposure to a single *M. ovipneumoniae* infected animal resulted in transmission of infection to all bighorn sheep both within the pen and in adjacent pens, and all infected sheep developed bronchopneumonia. The epidemiologic, pathologic and microbiologic findings in these experimental animals resembled those seen in naturally occurring pneumonia outbreaks in free ranging bighorn sheep.

Citation: Besser TE, Cassirer EF, Potter KA, Lahmers K, Oaks JL, et al. (2014) Epizootic Pneumonia of Bighorn Sheep following Experimental Exposure to *Mycoplasma ovipneumoniae*. PLoS ONE 9(10): e110039. doi:10.1371/journal.pone.0110039

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper.

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Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Bighorn sheep are a North American species that has failed to recover from steep declines at the turn of the 20th century despite strict protections and intensive management, and two populations (Sierra Nevada and Peninsular) are currently classified as endangered [1]. Epizootic pneumonia is limiting bighorn sheep population restoration and as such, the etiology is of considerable interest. The first appearance of the disease in a population is typically in the form of epizootics that affect animals of all ages and is sometimes accompanied by high (>50%) mortality rates. Subsequently, epizootics affecting primarily lambs may occur for decades [2]. Various causes have been proposed for this disease, including lungworms (*Protostrongylus* sp.) [3–6], Pasteurellaceae, especially *Mannheimia (Pasteurella) haemolytica,* [7–12] and more recently, *Mycoplasma ovipneumoniae* [13–16]. In a recent comparative review of the evidence supporting each of these possible etiologies we concluded that *M. ovipneumoniae* was most strongly supported as the primary epizootic agent of bighorn sheep pneumonia [14]. However, the only two previous experimental challenge studies with *M. ovipneumoniae* either did not reproduce disease [13] or were confounded by challenges with other agents [16]. The objective of this study was to improve upon previous investigations to better assess the outcome of experimental introduction of *M. ovipneumoniae* to naïve bighorn sheep.

Methods

Ethics statement

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and in conformance with

United States Department of Agriculture animal research guidelines, under protocols #03854 and #04482 approved by the Washington State University (WSU) Institutional Animal Care and Use Committee. As described in those protocols, euthanasia was performed by intravenous injection of sodium pentobarbital for animals observed to be in severe distress associated with pneumonia during the study and prior to necropsy examination for surviving animals at the end of each experiment.

Experimental aims

Experiment 1 was conducted to investigate the transmission of *M. ovipneumoniae* to bighorn sheep and their subsequent development of disease, using an infected domestic sheep source. Experiment 2 was conducted to investigate experimental direct *M. ovipneumoniae* infection of a single bighorn sheep and the subsequent transmission of this agent to conspecifics. Both experiments were conducted in multiple pens separated by short distances, which allowed investigation of transmission to both commingled and non-commingled animals.

Experimental animals

All experimental animals originated from herds and flocks unexposed to *M. ovipneumoniae* as determined by repeated testing with both serology on blood serum and PCR on enriched nasal swab cultures (using the methods described later in the 'Microbiological testing' section). In Experiment 1, three hand-reared bighorn sheep (yearling rams BHS #82 and #89 and yearling ewe BHS #07) that originated from a captive flock at WSU and three purchased domestic sheep (adult ewes DS #00 and #01 and yearling ewe DS #LA) were co-housed in three 46 m^2 pens, with one domestic and one bighorn sheep per pen. Pens were separated by 7.6–12 m. Experiment 1 animals had all been commingled in a single pen for 104 days immediately prior to the beginning of this experiment, as previously described [15]. One of the four bighorn sheep used in that prior study had died of *M. haemolytica* pneumonia, while the other three, which had demonstrated no signs of respiratory disease in that study, were used in experiment 1. In Experiment 2, wild bighorn sheep captured from the Asotin Creek population in Hells Canyon were housed in two 700 m^2 pens, 7.6 m apart, with three animals per pen (Pen #1: adult ewe BHS #40, yearling ewe BHS #38, and yearling ram BHS #39; Pen #2: adult ewes BHS #41 and #42 and adult ram BHS #C). The study pens had either never previously housed domestic or bighorn sheep (pen 1 in experiment 1; both pens in experiment 2) or had been rested for greater than one year since their previous occupancy by any *M. ovipneumoniae* infected sheep (pens 2 and 3 in experiment 1) prior to these experiments.

Experimental design

Experiment 1. A domestic ewe (DS #00) was placed in isolation and experimentally infected with *M. ovipneumoniae*. The inoculum consisted of ceftiofur-treated (100 ug/ml, 2 hrs, 37°C; Pfizer, Florham Park, NJ) nasal wash fluids from a domestic sheep naturally colonized with *M. ovipneumoniae* [16]. Following ceftiofur treatment, no aerobic bacterial growth was observed from the nasal wash fluids cultured under conditions expected to permit growth of *M. haemolytica, B. trehalosi,* or *P. multocida* (Columbia blood agar with 5% sheep blood, 35°C, overnight, 5% CO_2). DS #00 was then challenged with the treated nasal wash fluid by infusion of 15 ml in each nares, 10 ml orally and 5 ml into each conjunctival sac. Subsequent nasal swab samples obtained on days 1, 2, 4 and 7 post-challenge were all PCR positive for *M. ovipneumoniae* using the method described later in the 'Microbiological testing' section confirming that the experimental infection had been successful. On post challenge day 7, DS #00 was introduced into pen #1 with BHS #82. Following commingling, DS #00 and BHS #82 were restrained for collection of nasal swab samples on days 1, 2, 4, 7, 14, 21, 28, and subsequently at 30 day intervals until the experiment was terminated. Rectal temperatures were recorded from both sheep approximately twice each week. Sheep in pens #2 (BHS #89 and DS #01) and #3 (BHS #07 and DS #LA) were restrained for rectal temperature determination and collection of nasal swabs for microbiology at approximately monthly intervals. All pens were observed daily for clinical signs of respiratory disease. The experiment was conducted October 2009–January 2010.

Experiment 2. BHS #39 was inoculated with *M. ovipneumoniae* just prior to its release into pen #1 with non-inoculated BHS #38 and #40. Non-inoculated BHS #C, #41, and #42 were housed in pen #2 on the same day. The inoculum for BHS #39 was prepared as described for that used in experiment 1 but originated from a different domestic sheep source. In lieu of computation of colony forming units, which is not possible for *M. ovipneumoniae* due to inconsistent growth on plated media, viable *M. ovipneumoniae* counts in the inoculum were determined using most probable number (MPN) using a custom 3×4 format: Triplicate enrichment broth tubes were inoculated at each of four decimal dilutions (10^{-2}–10^{-5}) of the treated nasal wash fluid [17], incubated (72 hrs, 35C) then PCR was used to detect growth of viable *M. ovipneumoniae*. The treated fluid was determined to contain 930 MPN/ml (95% confidence interval, 230 to 3800 MPN). Two of the bighorn sheep (BHS #38 and #39) in pen 1 were recaptured by drive net on day 21 of the experiment for nasal swab sampling to detect *M. ovipneumoniae* infection; otherwise, no live animal sampling was conducted in experiment #2 to reduce the risk of traumatic injury of the wild bighorn sheep involved. The experiment was conducted December 2011–June 2012.

Biosecurity. In both experiments, routine biosecurity measures included: 1) the pens containing the single *M. ovipneumoniae*-challenged animals (exposed pens) were located downwind of the prevailing wind direction from the pens containing no experimentally *M. ovipneumoniae* exposed animals (clean pens), 2) order of entry rules were established so that on any single day exposed pens were routinely entered by animal care staff for feeding and cleaning only after all work in clean pens had been completed, and 3) personal protective equipment (coveralls and boots) used in exposed pens were either not reused, or were sanitized prior to use in clean pens.

Clinical scores. Clinical score data were determined using the following cumulative point system: observed anorexia (1), nasal discharge (1), cough (2), dyspnea (1), head shaking (1), ear paresis (1) and weakness/incoordination (1).

Microbiological testing. Routine diagnostic testing performed by the Washington Animal Diagnostic Laboratory (fully accredited by the American Association of Veterinary Laboratory Diagnosticians) included detection of *M. ovipneumoniae*-specific and small ruminant lentivirus-specific antibodies in serum samples using competitive enzyme-linked immunosorbent assays (cELISA) [14,18,19], detection of *M. ovipneumoniae* colonization by broth enrichment of nasal swabs followed by *M. ovipneumoniae*-specific PCR testing of the broths [20,21], detection of Pasteurellaceae in pharyngeal swab samples by aerobic bacteriologic cultures, and detection of exposure to parainfluenza-3, border disease, and respiratory syncytial viruses by virus neutralization antibody assays applied to serum samples.

PCR tests specific for detection of *M. haemolytica*, *B. trehalosi*, and *P. multocida*, and *lktA* (the gene encoding the principal virulence factor of *M. haemolytica* and *B. trehalosi*) were applied to DNA extracted from pneumonic lung tissues using previously described primers (Table 1) and methods with minor modifications. All reactions were conducted individually in 20 µL volumes containing 80–300 ng of template DNA. For *M. haemolytica*, *B. trehalosi*, *lktA* and *P. multocida*, reactions contained 0.5 units of HotStar Taq DNA polymerase (Qiagen), 2 µL 10x PCR buffer (Qiagen), 4 µL Q-solution (Qiagen), 40 µM of each dNTP (Invitrogen). The *M. ovipneumoniae* reaction used QIAGEN Multiplex PCR mix. Primers were used at final concentrations of 0.2 µM (*M. haemolytica*, *B. trehalosi*, *P. multocida,* and *M. ovipneumoniae*) or 0.5 µM (leukotoxin A). Each reaction included an initial activation and denaturation step (95°C, 15 min) and a final 72°C extension step (10 min for Mhgcp-2, lktA, lktA set-1, and LM primers; 9 min for KMT primers; 5 min for Btsod and Mhgcp primers). Cycling conditions were as follows: *M. ovipneumoniae*, 30 cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 30 s; *B. trehalosi* and *M. haemolytica* (Mhgcp and Btsod primers), 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 40 s; *P. multocida* and *lktA* (lktA primers), 30 cycles of 95°C for 60 s, 55°C for 60 s, 72°C for 60 s; *M. haemolytica* (Mhgcp-2 primers), 40 cycles of 95°C for 30 s, 54°C for 30 s, 72°C for 30 s; *lktA* (lktA set-1 primers), 40 cycles of 95°C for 30 s, 52°C for 30 s, 72°C for 40 s. Leukotoxin expression was detected in Pasteurellaceae isolates by MTT dye reduction cytotoxicity assay as described previously [22].

The 16S–23S ribosomal operon intergenic spacer (IGS) regions of *M. ovipneumoniae* recovered from animals in these studies were PCR amplified (Table 1) and sequenced as previously described [23].

16S rDNA analyses to identify the predominant bacterial flora in pneumonic lung tissues. In previous studies, culture-independent evaluation of the microbial flora of lung tissues in naturally occurring bighorn sheep pneumonia revealed a polymicrobial flora late in the disease course [13,23]. For comparison, we applied the same methods to lung tissues of the experimentally challenged animals in this study. Note that more sensitive detection of specific respiratory pathogens was provided by the PCR assays described earlier, whereas these 16S studies were designed instead to identify the numerically predominant bacteria in affected lungs. The library size used was based on the binary distribution to provide a 95% chance of detection of each taxon comprising 10% or more of the ribosomal operon frequency in the source tissue. Two 1 g samples of pneumonic lung tissues were aseptically collected from sites at least 10 cm apart, homogenized by stomaching, and DNA was extracted (DNeasy tissue kit; Qiagen, Valencia, CA) from 100 uL aliquots of each homogenate. 16S rDNA segments were PCR amplified and cloned as described [13]. Insert DNA was sequenced from 16 clones derived from each of the two homogenates from each animal, and each sequence was attributed to species (≥99% identity) or genus (≥97% identity) based on BLAST GenBank similarity [24].

Results

Experiment 1

M. ovipneumoniae infection of DS #00, introduced into pen 1 to start the experiment, was confirmed by positive nasal swab samples obtained on days 1, 4, and 7 after inoculation prior to its introduction into pen #1, and on days 1, 2, 4, 7, 14, 21, 28, 60 and 90 after its introduction into pen #1, confirming that the experimental colonization had been successful and maintained throughout experiment 1. *M. ovipneumoniae* was first detected in the bighorn sheep (BHS #82) commingled with DS #00 in pen #1 on day 28, and subsequent tests on days 60 and 90 were also positive. BHS #82 developed signs of respiratory disease including nasal discharge (onset day 37); coughing and fever (onset day 42); and lethargy and ear paresis (onset day 61) (Figure 1a). Signs of respiratory disease were observed in the bighorn sheep in pens #2 (BHS #89) and #3 (BHS #07) beginning on days 62 and 67, respectively; these signs also included fever, lethargy, paroxysmal coughing, nasal discharge, head shaking, and drooping ears. No signs of respiratory disease were observed in the commingled domestic sheep at any time during the experiment. *M.*

Table 1. Primers and PCR reaction targets used in these experiments.

Pathogen/Virulence gene	Target	Primer Name	Sequence (5′ → 3′)	Size (bp)	Reference
M. haemolytica	*gcp*	MhgcpF	AGA GGC CAA TCT GCA AAC CTC G	267	[33]
		MhgcpR	GTT CGT ATT GCC CAA CGC CG		
M. haemolytica	*gcp*	MhgcpF2	TGG GCA ATA CGA ACT ACT CGG G	227	[34]
		MhgcpR2	CTT TAA TCG TAT TCG CAG		
B. trehalosi	*sodA*	BtsodAF	GCC TGC GGA CAA ACG TGT TG	144	[33]
		BtsodAR	TTT CAA CAG AAC CAA AAT CAC GAA TG		
P. multocida	*kmt1*	KMT1T7	ATC CGC TAT TTA CCC AGT GG	460	[35]
		KMT1SP6	GCT GTA AAC GAA CTC GCC AC		
Pasteurellaceae leukotoxin	*lktA*	lktAF	TGT GGA TGC GTT TGA AGA AGG	1,145	[36]
		lktAR	ACT TGC TTT GAG GTG ATC CG		
M. haemolytica leukotoxin	*lktA*	lktAF set-1	CTT ACA TTT TAG CCC AAC GTG	497	[34]
		lktAR set-1	TAA ATT CGC AAG ATA ACG GG		
Mycoplasma ovipneumoniae	16s rDNA	LMF	TGA ACG GAA TAT GTT AGC TT	361	[20,21]
		LMR	GAC TTC ATC CTG CAC TCT GT		
Mycoplasma ovipneumoniae	16S–23S IGS	MoIGSF	GGA ACA CCT CCT TTC TAC GG	Variable~490	[23]
		MoIGSR	CCA AGG CAT CCA CCA AAT AC		

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ovipneumoniae was detected in nasal swab samples from all bighorn and domestic sheep in pens #2 and #3 when sampled on day 70. The bighorn sheep were euthanized for necropsy on days 93 (BHS #89) and 99 (BHS #82 and #07). At necropsy, significant abnormal findings were limited to the respiratory tract. Bronchopneumonia affecting 25–50% of the lung volume was observed in all three bighorn sheep (Figure 2). Histopathological examination revealed peribronchiolitis with large lymphoid cuffs, bronchiectasis with purulent exudates, pulmonary atelectasis, and hyperplastic bronchial epithelia lacking visible cilia (Figure 2).

Experiment 2

On day 21 following release of the inoculated bighorn into pen #1, *M. ovipneumoniae* was detected in the inoculated animal and one pen mate (BHS #38 and #39); the third animal (BHS #40) evaded capture and sampling on that day. The first signs of respiratory disease were observed in pen #1 animals on day 21 during drive net capture for sampling, apparently triggered by exertion (Figure 2a). On day 34, inoculated BHS #39 died in pen #1. On day 49, signs of respiratory disease were first observed in the bighorn sheep in pen #2 (Figure 2b). On days 65 and 109, #41, and #42 in pen #2 died or were euthanized *in extremis*. The surviving three bighorn sheep exhibited varying degrees of respiratory disease: BHS #38 showed persistent respiratory disease, while BHS #40 and #C showed decreasing respiratory disease over time, which became minimal after days 161 and 154, respectively. On day 204, the three surviving bighorn sheep were euthanized for necropsy. At necropsy, significant abnormal findings were limited to the respiratory tract. All six bighorn sheep had bronchopneumonia, with consolidation of lung tissue volumes ranging from an estimated 5% (BHS #40) to 80–100% (BHS #41) (Figure 2). Histopathological examination revealed severe peribronchiolitis with large lymphoid cuffs as seen in experiment 1. Animals that died or were euthanized in extremis had an overlying necrotizing bronchiolitis (#39) or abscessing bronchiolitis with bronchiectasis (BHS #41, #42) (Figure 2).

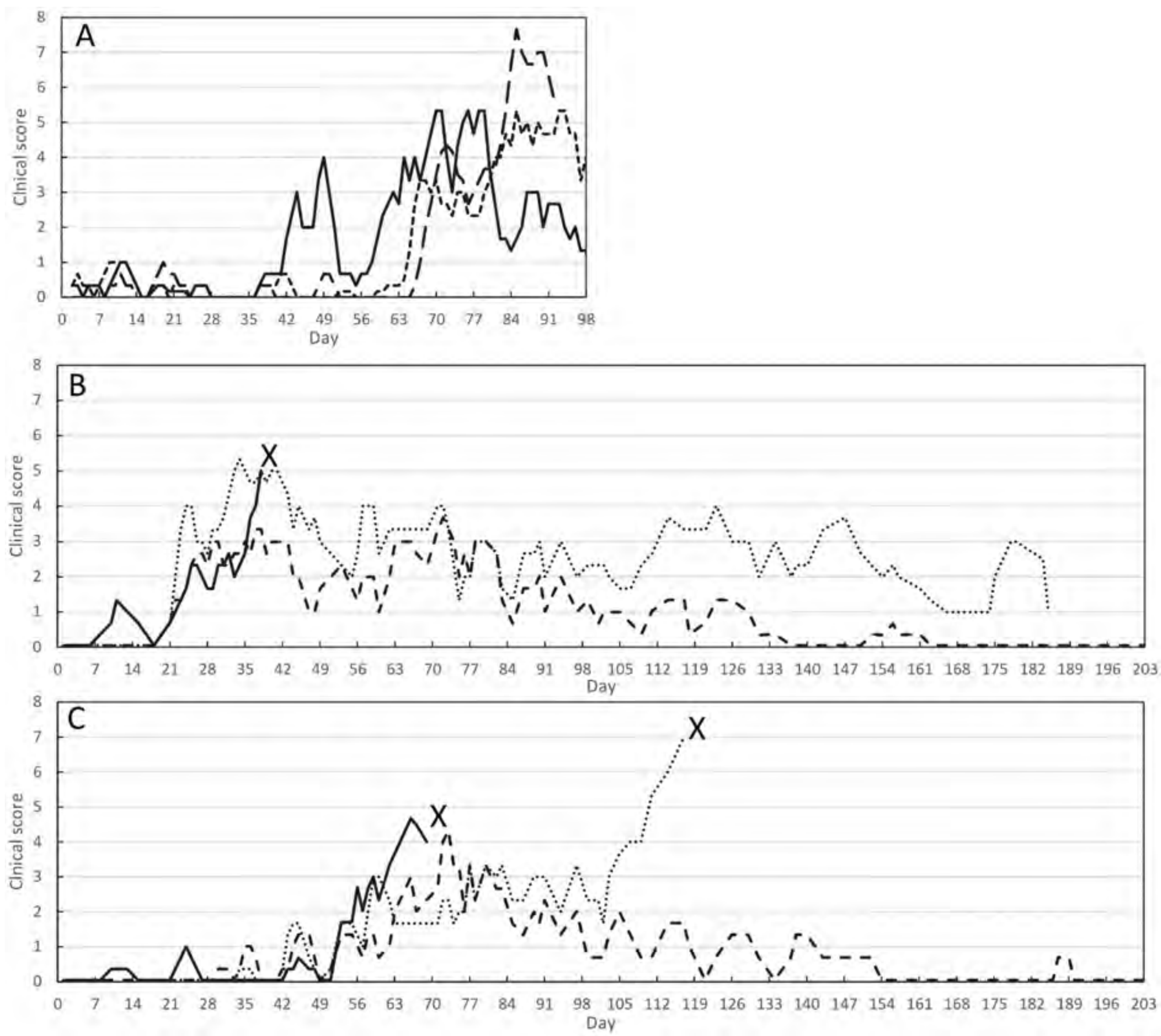

Figure 1. Clinical signs exhibited by *M. ovipneumoniae* infected bighorn sheep. Clinical scores (3-day moving averages) of bighorn sheep following introduction of *M. ovipneumoniae*: A) Experiment 1, 3 separate pens; solid line, Pen 1, BHS #82; dashed line, Pen 2, BHS #89; dotted line, Pen 3, BHS #07; B) Experiment 2, Pen 1: solid line, BHS #39 (died day 34); dashed line, BHS #40; dotted line; BHS #38.; C) Experiment 2, Pen 2: solid line, BHS #42 (euthanized day 109); dotted line, BHS #41 (died day 65); dashed line, BHS #C.
doi:10.1371/journal.pone.0110039.g001

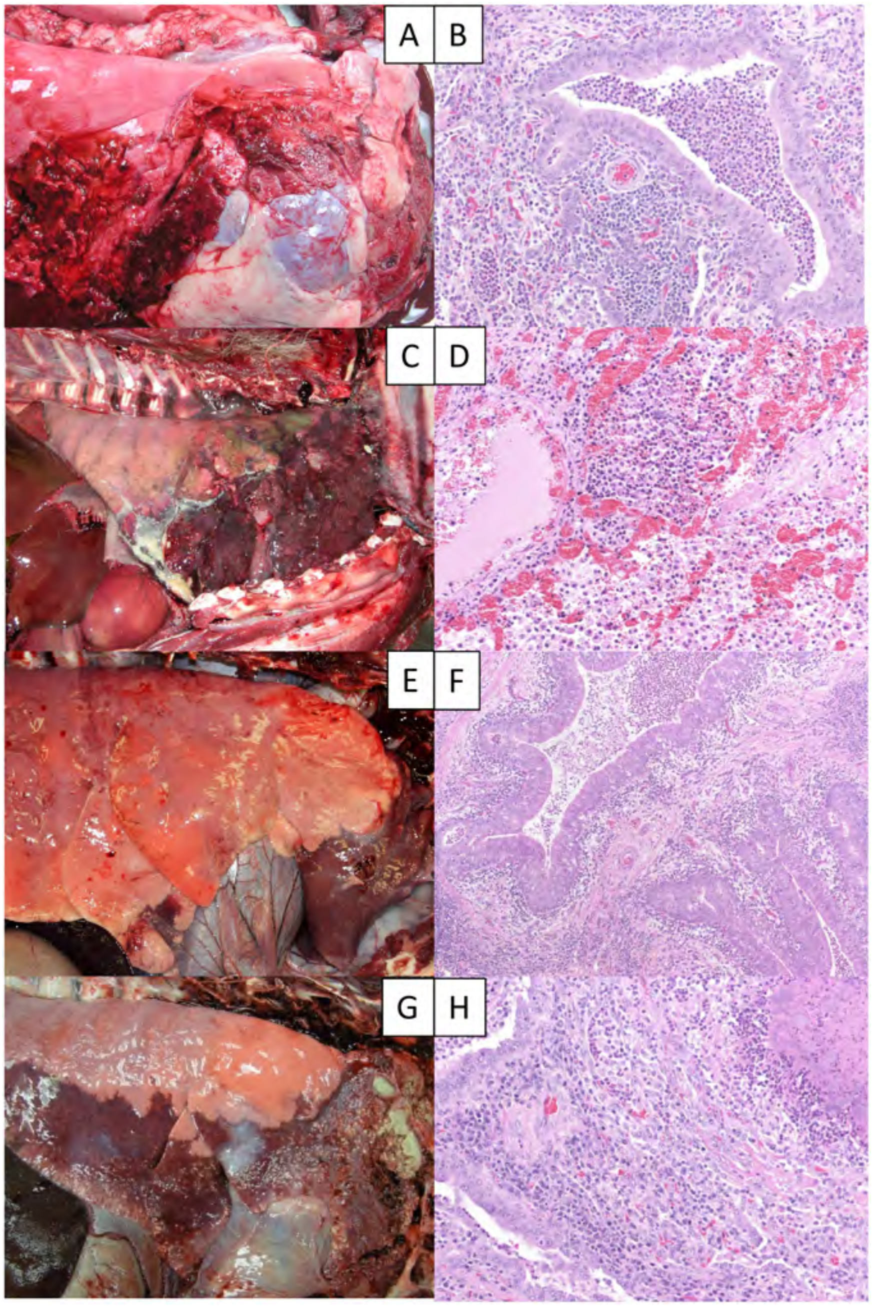

Figure 2. Gross and histologic lesions in lungs of bighorn sheep experimentally infected with *M. ovipneumoniae*. Images of BHS #82 (A, B), BHS #39 (C, D), BHS #C (E, F) and BHS #42 (G, H). Original magnification of histologic images was 200X (B, D, H) or 100X (F).
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Microbiology

All bighorn sheep in both experiments seroconverted to *M. ovipneumoniae* (Table 2). Most experimental animals had neutralizing antibody to parainfluenza-3 virus, but no significant changes in antibody titers were observed during the experimental period. Detectable antibody to other ovine respiratory viruses, including border disease virus, ovine progressive pneumonia virus, and respiratory syncytial virus was occasionally observed in single samples.

M. ovipneumoniae was detected at necropsy in both upper and lower respiratory tracts of all bighorn sheep except BHS #40 whose lung tissues were PCR negative and whose upper respiratory samples were PCR indeterminate (Table 3). Aerobic cultures and/or PCR tests identified *B. trehalosi* from pneumonic lung tissues from all bighorn sheep in both experiments (Table 3). *B. trehalosi* isolates from BHS #82 and #07 carried *lktA* and expressed leukotoxin activity (Table 3). *P. multocida* and *M. haemolytica* were not detected in these animals by either aerobic culture or PCR.

Culture independent survey of bacteria in pneumonic bighorn sheep lung tissues

DNA sequences of cloned 16S rDNA revealed that the predominant bacterial species in pneumonic sections of lung were

Table 2. Antibody responses to *M. ovipneumoniae* and parainfluenza-3 (PI-3) virus.

			M. ovipneumoniae[1]		PI-3 virus[2]	
Experiment	ID	Pen	Pre[3]	Post[3]	Pre[3]	Post[3]
1	82	1	−8%	93%	512	512
1	89	2	−7%	88%	128	128
1	07	3	−1%	92%	256	512
2	38	1	−6%	74%	Neg	64
2	39	1	−13%	67%	Neg	<32
2	40	1	−23%	75%	64	512
2	41	2	−19%	82%	512	NT
2	42	2	−11%	82%	256	NT
2	C	2	−4%	66%	256	512

[1]*M. ovipneumoniae* antibody detected by cELISA, expressed as percentage inhibition of the binding of an agent-specific monoclonal antibody [14,18].
[2]PI-3 virus neutralizing antibody detected by virus neutralization [37].
[3]Pre samples in experiment 1 were obtained on the day that the *M. ovipneumoniae* colonized domestic sheep was introduced to pen 1 and in experiment 2 were obtained on the day that BHS #39 was inoculated with *M. ovipneumoniae*. 'Post' samples in both experiments were obtained at necropsy. Neg = No titer detected. NT = Not tested, due to inadequate specimen volume.
doi:10.1371/journal.pone.0110039.t002

diverse (Table 4). In experiment 1, *M. ovipneumoniae* was detected in the lung tissues of all animals. *B. trehalosi* also comprised substantial proportions of the pneumonic lung flora in two animals (BHS #82 and #07), while obligate anaerobic species, primarily Fusobacterium spp., predominated in the third animal (BHS #89). The flora identified in the pneumonic lungs of the animals in experiment 2 was also substantially comprised of mixed obligate anaerobes especially *Fusobacterium* spp. (Table 4).

Molecular epidemiology of respiratory pathogens. Consistent with epidemic transmission, *M. ovipneumoniae* strains recovered from all experimental sheep within each experiment shared identical IGS DNA sequences with the respective challenge inoculum (GenBank HQ615162 in experiment 1; KJ551511 in experiment 2).

Discussion

The most striking finding of these experiments was the high transmissibility of *M. ovipneumoniae* and the consistent development of pneumonia that followed infection of bighorn sheep. The bacterium was naturally transmitted from single experimentally inoculated animals (a domestic sheep in experiment 1 and a bighorn sheep in experiment 2) to all animals within and between pens up to 12 m distant. Eight of nine bighorn sheep exposed to *M. ovipneumoniae* developed severe bronchopneumonia and three died, while all the domestic sheep remained healthy.

Previous experimental challenge studies conducted with *M. haemolytica or B. trehalosi* in the absence of *M. ovipneumoniae* have not documented transmission. For example, Foreyt et al. [8]

Table 3. Microbiologic findings from pneumonic lung tissues, based on aerobic culture and species specific PCR.

Expt.	ID	Bacterial pathogens identified in pneumonic lung tissues				
		B. trehalosi	*M. haemolytica*	*lktA*	*M. ovipneumoniae*	Other[5]
1	82	Cult, *sodA*[1]	Neg[2]	Pos[3]	16S[4]	None
1	89	Cult, *sodA*	Neg	Neg[3]	16S	*Pasteurella* sp.[5]
1	07	Cult, *sodA*	Neg	Pos	16S	*Pasteurella* sp.
2	38	Cult, *sodA*	Neg	Neg	16S	*Pasteurella* sp.
2	39	NT, *sodA*	NT, Neg[2]	Neg	16S	NT[5]
2	40	Cult	Neg	Neg	Neg[4]	*Trueperella pyogenes*[5]
2	41	Cult, *sodA*	Neg	Neg	16S	None
2	42	Cult	Neg	Neg	16S	None
2	C	Cult	Neg	Neg	16S	*Pasteurella* sp.

[1]Cult = *B. trehalosi* detected by bacterial culture; *sodA* = *B. trehalosi* detected by *sodA* species-specific PCR (Table 1); NT = Unable to test by bacterial culture (overgrowth by *Proteus* sp.).
[2]Neg = *M. haemolytica* not detected by either bacterial culture or by PCR with either *gcp* primer set (Table 1); NT = Unable to test by bacterial culture (overgrowth by *Proteus* sp.).
[3]Neg = Pasteurellaceae *lktA* not detected in DNA extracts from pneumonic lung tissues by two different *lktA* PCRs (Table 1) [34,36]. Pos = *lktA* detected in *B. trehalosi* isolates obtained from BHS #82 and #07 [36].
[4]16S = *M. ovipneumoniae* detected by PCR (Table 1) [20]; Neg = *M. ovipneumoniae* not detected by PCR.
[5]*Pasteurella* sp., *Trueperella pyogenes* = Bacteria isolated and identified by aerobic culture; *Pasteurella* sp. were determined not to be *B. trehalosi, M. haemolytica,* or *P. multocida*; NT = Unable to test by bacterial culture due to overgrowth by *Proteus* sp.
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Table 4. Microbiologic findings by 16S clone library (culture independent) method.

Expt.	ID	Bacterial species identified in pneumonic lung tissues					
		Btre[1]	Movi[1]	Fuso[1]	Prev[1]	Porphyro[1]	Other[1]
1	82	20 (62.5)[2]	8 (25)	0	3 (9.4)	0	1 (3.1)
1	89	1 (3.1)	7 (21.9)	21 (65.6)	1 (3.1)	0	2 (6.3)
1	07	16 (50.0)	12 (37.5)	0	0	0	4 (12.5)
2	38	4 (7.1)	2 (3.6)	8 (14.3)	20 (35.7)	9 (16.1)	13 (23.2)
2	C	0	0	17 (30.4)	5 (8.9)	19 (33.9)	15 (26.8)
2	39	2 (6.3)	0	24 (75.0)	0	0	6 (18.8)
2	40	0	0	0	0	0	56 (100.0)
2	41	1 (3.1)	0	21 (65.6)	5 (15.6)	0	5 (15.6)
2	42	0	0	31 (96.9)	0	0	1 (3.1)

[1]Btre = *B. trehalosi;* Movi = *M. ovipneumoniae;* Fuso = *Fusobacterium* sp.; Prev = *Prevotella* sp.; Porphyro = *Porphyromonas* sp.; Other = taxa other than those previously listed, each comprising <5% of sequenced clones.
[2]N (%) of the sequenced 16S clones from each animal whose DNA sequences were identical to those of the tabulated bacterial species in each column.
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reported a series of three experiments in which commingled bighorn sheep were either challenged with intra-tracheal *M. haemolytica* or given sterile BHI as controls. Four of the five control bighorn sheep survived without evidence of disease while commingled with eight *M. haemolytica*-challenged bighorn sheep, of which seven died of pneumonia [8]. Commingled bighorn sheep also remained healthy in several other studies where individual bighorn sheep died with apparent *M. haemolytica* bronchopneumonia (confirmed by isolation of this bacterium from lung tissues) [15,25,26].

In addition to high transmissibility, the time course of disease development and the predominant microbiology of the pneumonic lung tissues following experimental introduction of *M. ovipneumoniae* differed from that seen in previous bighorn sheep challenge experiments with other respiratory pathogens. Bighorn sheep directly challenged with leukotoxin positive *M. haemolytica* or *B. trehalosi* develop peracute bronchopneumonia and >90% die within a week of challenges with 10^5 cfu or more [16,27–30]. In contrast, disease following experimental *M. ovipneumoniae* exposures was considerably slower in onset (14–21 days post infection) and development (deaths occurring 34 to 109 days post infection; respiratory disease persisted up to 6 months post-infection); this slow time course closely resembles that documented previously in bighorn lamb pneumonia outbreaks [13]. After lethal *M. haemolytica* challenge, the agent is typically isolated from lung tissues in high numbers and pure cultures [15,25]; in contrast in naturally occurring pneumonia outbreaks *M. ovipneumoniae* may be predominant early in the disease course but 16S library analyses have been used to document its overgrowth by diverse other bacteria later in the disease course [14,23]. Although the numbers of animals in the experimental *M. ovipneumoniae* infection studies reported here are small, the results are consistent with the trend for early predominance of *M. ovipneumoniae* followed by overgrowth by diverse other bacterial later in the disease course (Tables 3 and 4) [13,14,23].

Our results also differ from our previous attempt to experimentally reproduce respiratory disease by challenge inoculation of 1-week-old bighorn lambs with *M. ovipneumoniae*, which produced minor lesions and seroconversion but no clinically significant respiratory disease [13]. However, laboratory passage of *M. ovipneumoniae* (as was performed in that experiment) has been reported to attenuate virulence in *M. ovipneumoniae* [31]. Challenge of bighorn sheep with un-passaged *M. ovipneumoniae* produced different results, as observed here in experiment #2. In another study [16], nasal washings from domestic sheep naturally colonized with *M. ovipneumoniae* or lung homogenates from a *M. ovipneumoniae*-infected bighorn sheep were used for challenge of bighorn sheep after ceftiofur treatment to eliminate detectable Pasteurellaceae. Consistent with increased virulence of un-passaged *M. ovipneumoniae*, infection and respiratory disease signs were observed in all four bighorn sheep, one of which died 19 days following challenge. The three surviving animals continued to exhibit respiratory disease signs for 42 days, at which time the experiment was terminated by challenge with *M. haemolytica* (using a dose documented to be rapidly fatal to bighorn sheep even in the absence of *M. ovipneumoniae*) [16]. As a result, the longer term effects of the mycoplasma infection were not determined in that study. Therefore, the experiments reported here are the first in which naïve bighorn sheep were exposed to un-passaged *M. ovipneumoniae* and then followed over a time period comparable with the naturally occurring disease course.

The possibility of viral agents contributing to the disease observed in this study cannot be completely ruled out, since the inoculum was derived from nasal washings from domestic sheep and no virucidal treatments were applied. However, a previous study using ultrafiltrates of bighorn sheep pneumonic lung tissues or nasal washings from domestic sheep failed to reproduce any respiratory disease in inoculated susceptible bighorn sheep [16]. In addition, serologic monitoring for the predominant domestic sheep respiratory viruses did not demonstrate seroconversion of the experimental animals in this study, as described in the Results and in Table 2. Therefore, the most parsimonious interpretation of the data presented here is that the disease observed resulted from *M. ovipneumoniae* infection and the sequelae of that infection.

The transmission of *M. ovipneumoniae* from pen-to-pen in these experiments strongly suggests that direct contact is not necessary for epizootic spread of pneumonia in bighorn sheep. Feeding, watering and other procedures involving animal care or research staff were designed to minimize the risk of human or fomite-mediated transmission of the pathogen from pen to pen, although we recognize it is impossible to completely rule out this possibility. On the other hand, since aerosolized droplet transmission is recognized as a transmission route for the closely related bacterium, *Mycoplasma hyopneumoniae* (the cause of atypical pneumonia of swine) [32], it is plausible that a similar transmission mode occurs with *M. ovipneumoniae*. Infectious aerosols generated by coughing animals would likely contribute to the explosive nature of the pneumonia outbreaks observed following initial introduction of *M. ovipneumoniae* into naïve bighorn sheep populations.

In conclusion, we demonstrated that experimental *M. ovipneumoniae* infection of naïve bighorn sheep induces chronic, severe bronchopneumonia associated with multiple secondary bacterial infections and that this infection spread rapidly to animals both within the same pen and to animals in nearby pens. The significance of these findings would be clarified by parallel experiments specifically designed to determine transmissibility and associated disease outcomes in other agents associated with bighorn sheep pneumonia, particularly *M. haemolytica,* in the absence of *M. ovipneumoniae*. Furthermore, the case-fatality rates of *M. ovipneumoniae* infected animals described here contrasts with the nearly 100% mortality that follows experimental commingling of bighorn sheep with presumptively or documented *M. ovipneumoniae*-positive domestic sheep and suggests an important role for polymicrobial secondary infections in determining mortality rates, which could be investigated in future studies. Finally, *M. ovipneumoniae* was still detected in nasal swab samples of several surviving bighorn sheep that were euthanized at the completion of these studies, suggesting that survivors of naturally occurring pneumonia outbreaks may continue to carry and shed this agent in nasal secretions. Such carriage may provide a mechanism for the post-invasion disease epizootics in lambs described in free-ranging populations. If so, this presumptive carrier state requires further study to characterize the factors that determine its occurrence and persistence, as these may be critical for the development of effective management control measures for this devastating disease.

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Author Contributions

Conceived and designed the experiments: TEB EFC JLO S. Srikumaran WJF. Performed the experiments: TEB EFC JLO KAP KL S. Shanthalingam. Analyzed the data: TEB EFC KAP KL. Contributed reagents/materials/analysis tools: TEB EFC KAP KL S. Shanthalingam S. Srikumaran. Contributed to the writing of the manuscript: TEB EFC KAP S. Shanthalingam S. Srikumaran WJF.

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Fence Marking to Reduce Greater Sage-grouse (*Centrocercus urophasianus*) Collisions and Mortality near Farson, Wyoming – Summary of Interim Results

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Background: Fence collisions have been anecdotally reported to cause sage-grouse injury and mortality but few efforts have been made to quantify this concern and publish results. Our study was initiated after two falconers independently reported numerous sage-grouse mortalities on range fences in Sublette and Sweetwater Counties in Wyoming. One of these falconers subsequently began marking such fences with aluminum beverage cans in a volunteer effort to reduce these mortalities. Our study seeks to quantify the level of sage-grouse fence strikes and mortalities and test whether marking devices can effectively reduce collisions in a cost effective manner that is not visually intrusive. Our interim results are summarized below.

Study Area: Approximately 12 miles northeast of Farson, WY adjacent to Little Sandy Creek on the Sweetwater-Sublette County Line. Greater sage-grouse use the area in large numbers year-round. Two large leks (100+ males) are located within 2 miles of the fence. The creek and associated riparian area serve as late brood-rearing habitat and the fence bisects winter habitat for, at least, several hundred grouse.

Study Dates: These results are for the April 15, 2005 – May 14, 2009 period. The study is on-going.

Study Fence: 3-wire BLM range fence that is approximately 7.6 km (4.7 mi) long. The fence generally runs from southwest to northeast but does so in a zigzag manner.

Pretreatment data: From April 15, 2005 through Nov. 16, 2007 pretreatment data were collected during 9 surveys where 1-3 observers documented evidence of wildlife fence strikes and mortality while driving 2-3 mph immediately adjacent to the fence. These surveys resulted in evidence of 170 bird strikes/mortalities and 2 pronghorn mortalities. Confirmed greater sage-grouse accounted for 146 (86%) of the 170 strikes/mortalities documented. The other 22 observations were of waterfowl (n=4; 2%), raptors (n=5; 3%), passerines (n=2; 1%), shorebirds (n=1; <1%), and unknown birds (n=12; 7%).

Treatment/Control data: From Nov. 16, 2007 through May 14, 2009 approximately 1.54 miles (2.5 km) of the fence was marked in approximately .26 mi (416 m) sections with either FireFly™ bird diverters (donated by FireFly Diverters LLC for this study) or homemade markers patterned after those developed and used by the University of Oklahoma's Sutton Avian Research Center http://www.suttoncenter.org/fence_marking.html to reduce lesser prairie-chicken fence mortality. The later were modified with reflective tape to increase visibility in snow cover conditions. The fence was unmarked (control) for 3.2 miles (5 km). Marked sections were bounded on either side by unmarked sections. Only the top wire was marked since very few collisions were documented on the lower two wires during pretreatment monitoring.

During the Nov. 16, 2007 through May 14, 2009 period, 6 surveys were conducted in the same manner as those conducted in the pretreatment phase of the study. Results suggest markers (all types combined) reduced bird fence collisions by 70% over unmarked sections. Seven (7) bird strikes, all sage-grouse, were documented in marked sections (4.55 strikes/mile) while 47 bird strikes (15.31 strikes/mile) were recorded in the unmarked sections. Thirty-six (36) of these were confirmed sage-grouse (11.73 strikes/mile). If only confirmed sage-grouse data are compared, the markers appear to have reduced grouse mortality by 61%.

On-going/Future Efforts: On May 14, 2009 the treatment sections were changed to control sections, the types of markers were changed, and more treatment sections were added. Half of the fence is now marked, alternating between sections of treatment and control. All of the markers are now based on the Sutton design. The FireFly I design has been eliminated from the study. Although it was highly effective (0 strikes), the price, maintenance and visibility of the device was not appropriate for wide scale use. With this information, the company, FireFly Diverters LLC, has applied their unique system of reflective/glow in the dark tape to the Sutton model and now markets a FireFly III Grouse Diverter (see attached) which we are currently testing along with other versions of the Sutton device to which different reflective tapes have been applied. Early indications suggest all of these markers will succeed and likely further decrease avian fence collisions beyond the 70% level suggested by our initial efforts reported above. We intend to attempt to publish our results after the next phase of the study is complete.

Interim Management Recommendations: Not every fence is a problem; those that tend to cause problems typically include one or more of the following characteristics: 1) constructed with steel t-posts, 2) are constructed near leks, 3) bisect winter concentration areas, and/or 4) border riparian areas. Areas of greater topographic relief (roughness) appear to have lower incidence of collisions apparently because the birds have to fly higher to avoid the ground. Avoid building fences within at least ¼ mile (preferably 0.6 mile) of leks. New and existing fences in these areas should be surveyed for evidence of grouse fence strikes before installing permanent fence markers. In brief, surveys can be conducted by walking, driving or riding slowly (2-3 mph) along the fence looking for carcasses or concentrations of feathers on the ground and individual feathers caught on top wire barbs. Evidence of fence strikes does not last long due to weather and scavengers. The discovery of fence strikes is therefore cause for mitigation. Where the decision has been made to mark a fence we currently recommend the top wire be marked with at least 2 markers of the Sutton design modified with high quality reflective tape. While we have yet to substantiate the need for reflective tape, untaped markers become essentially invisible with snow cover. Arrangements are being made to make markers available to ranchers at no cost. Contact the author for further information.

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Short communication

Mycoplasma ovipneumoniae can predispose bighorn sheep to fatal *Mannheimia haemolytica* pneumonia

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A B S T R A C T

Mycoplasma ovipneumoniae has been isolated from the lungs of pneumonic bighorn sheep (BHS). However experimental reproduction of fatal pneumonia in BHS with *M. ovipneumoniae* was not successful. Therefore the specific role, if any, of *M. ovipneumoniae* in BHS pneumonia is unclear. The objective of this study was to determine whether *M. ovipneumoniae* alone causes fatal pneumonia in BHS, or predisposes them to infection by *Mannheimia haemolytica*. We chose *M. haemolytica* for this study because of its isolation from pneumonic BHS, and its consistent ability to cause fatal pneumonia under experimental conditions. Since *in vitro* culture could attenuate virulence of *M. ovipneumoniae*, we used ceftiofur-treated lung homogenates from pneumonic BHS lambs or nasopharyngeal washings from *M. ovipneumoniae*-positive domestic sheep (DS) as the source of *M. ovipneumoniae.* Two adult BHS were inoculated intranasally with lung homogenates while two others received nasopharyngeal washings from DS. All BHS developed clinical signs of respiratory infection, but only one BHS died. The dead BHS had carried leukotoxin-positive *M. haemolytica* in the nasopharynx before the onset of this study. It is likely that *M. ovipneumoniae* colonization predisposed this BHS to fatal infection with the *M. haemolytica* already present in this animal. The remaining three BHS developed pneumonia and died 1–5 days following intranasal inoculation with *M. haemolytica*. On necropsy, lungs of all four BHS showed lesions characteristic of bronchopneumonia. *M. haemolytica* and *M. ovipneumoniae* were isolated from the lungs. These results suggest that *M. ovipneumoniae* alone may not cause fatal pneumonia in BHS, but can predispose them to fatal pneumonia due to *M. haemolytica* infection.

1. Introduction

Mannheimia (*Pasteurella*) *haemolytica* has been isolated from the lungs of bighorn sheep (BHS, *Ovis canadensis*) that died of pneumonia (Miller, 2001). Experimental infection with this organism has confirmed its ability to cause fatal bronchopneumonia in BHS (Foreyt et al., 1994; Dassanayake et al., 2009). *M. haemolytica* has long been identified as a commensal bacterium of the upper respiratory tract of ruminants (Dunbar et al., 1990; Weiser et al., 2009). Active viral infection and stress factors have been identified as predisposing factors for pneumonia caused by *M. haemolytica* in cattle (Rehmtulla and Thompson, 1981). However,

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the role of predisposing factors in *M. haemolytica*-caused pneumonia in BHS has not been investigated.

Mycoplasma ovipneumoniae and *Mycoplasma arginini* have been isolated from the upper respiratory tract of young and adult domestic sheep (DS, *Ovis aries*; Brogden et al., 1988). *M. ovipneumoniae* (but not *M. arginini*) causes atypical pneumonia especially in DS lambs; however, experimental reproduction of pneumonia with *M. ovipneumoniae* in DS lambs has been inconsistent (Buddle et al., 1984; Ruffin, 2001). Unlike in DS, only non-pathogenic *M. arginini* has been isolated from healthy BHS (Woolf et al., 1970). In our recent study (Besser et al., 2008), *M. ovipneumoniae* was detected in the bronchoalveolar lavage fluid from pneumonic BHS lambs by culture and 16S rRNA species-specific PCR. *M. ovipneumoniae*-specific 16S sequences and antibodies were detected in lung tissues and serum respectively, of bronchopneumonic BHS, but not from BHS dying of other causes. However, experimental inoculation of *M. ovipneumoniae* failed to cause fatal pneumonia in BHS lambs (Besser et al., 2008). Therefore, the objective of this study was to elucidate the role of *M. ovipneumoniae* in BHS pneumonia.

2. Materials and methods

2.1. Bacterial strains and growth conditions

M. haemolytica serotype A1 strain 89010807N (Murphy et al., 1995) and serotype A2 strain WSU-1 (Foreyt et al., 1994) were grown in BHI agar supplemented with 5% sheep blood and BHI broth as previously described (Dassanayake et al., 2009).

2.2. Bighorn sheep challenge studies

Nasal and pharyngeal swabs and blood samples were collected from all the animals before bacterial inoculation, and were submitted to Washington Animal Disease Diagnostic Laboratory (WADDL) at Washington State University for detection of *M. ovipneumoniae*, *M. haemolytica* or other *Pasteurella* sp, and any known respiratory viruses. Serology was performed to detect antibodies against respiratory viruses, *M. ovipneumoniae* and *M. haemolytica* leukotoxin (LktA).

The animals were inoculated as detailed in Table 1. The inoculum was administered intranasally using an atomizer. *M. ovipneumoniae* inoculum for Groups I and II were obtained from two sources: (1) lung homogenates from pneumonic BHS (three lambs and one adult; Besser et al., 2008); (2) nasopharyngeal washings from DS (four ewes) that were *M. ovipneumoniae*-positive by culture and PCR assay. These preparations were filtered using a 0.22 μm filter (to remove any bacteria), and the filtrates were submitted to WADDL to detect respiratory viruses. The unfiltered nasopharyngeal washings and lung homogenates were treated with the antibiotic ceftiofur (64 μg/ml, 37 °C for 1 h), expected to reduce or eliminate *M. haemolytica* and other ceftiofur-susceptible bacteria, but not *M. ovipneumoniae*. BHS were inoculated either with the filtrate, ceftiofur-treated *M. ovipneumoniae*-positive lung homogenates, nasopharyngeal washings or *M. haemolytica* as detailed in Table 1.

The animals in each group were observed daily and scored for the signs of pneumonia including anorexia, lethargy, cough, dyspnoea and nasal discharge. The animals that died before the end of the observation period were necropsied immediately, and appropriate tissues were collected for bacteriological, viral and histopathological examinations. The animals in the control Group (III) were euthanized 3 weeks post-challenge, necropsied and tissue samples collected. The lungs were carefully examined for pneumonic lesions. The degree of involvement of right and left lungs was noted as percent pneumonic scores. Pleuritis was noted as present or absent. Bacterial and viral isolations were attempted using routine methods at WADDL. *M. haemolytica* isolates were serotyped by agglutination test using anti-serotype A1 and A2 specific sera.

2.3. PCR detection of M. ovipneumoniae and M. haemolytica leukotoxin

M. ovipneumoniae-specific 16S rRNA PCR was performed as previously described (McAuliffe et al., 2003; Besser et al., 2008). Leukotoxin A gene (*lktA*) of *M. haemolytica* and *Bibersteinia trehalosi* was amplified by PCR using *lktA*F (5′-TCAAGAAGAGCTGGCAAC-3′) and *lktA*R (5′-AGTGAGGGCAACTAAACC-3′) primers in a final volume

Table 1
Inoculation timeline of *M. ovipneumoniae* and *M. haemolytica* to BHS.

Group	BHS	Day	Inoculum[a]
I	OR26	0	Filtrate (0.22 μm) from *M. ovipneumoniae*-positive pneumonic BHS (three lambs and one adult) lung homogenates in PBS (10 ml)
	R124		
		28	*M. ovipneumoniae*-positive, pneumonic BHS (three lambs and one adult) lung homogenates in PBS, unfiltered but treated with ceftiofur (10 ml)
		42	Same treatment as on day 28
		70	*M. haemolytica* serotype A1 (1×10^6 CFU in 5 ml RPMI)
II	Y45	0	Filtrate (0.22 μm) from nasal washings (PBS) from four *M. ovipneumoniae*-positive DS (10 ml)
	R123		
		28	Nasal washings (PBS) from four *M. ovipneumoniae*-positive DS, unfiltered, but treated with ceftiofur (10 ml)
		42	Same treatment as on day 28
		70	*M. haemolytica* serotype A2 (1×10^6 CFU in 5 ml RPMI)
III	Y30	0	RPMI (5 ml)
	Y39		

[a] Administered intranasally using an atomizer.

of 50 μl with GoTaq® PCR SuperMix (Promega Inc., Madison, WI) under standard conditions.

2.4. Serology

Anti-LktA-neutralizing antibodies from BHS serum samples were detected by MTT dye reduction cytotoxicity assay as previously described (Gentry and Srikumaran, 1991). Indirect hemagglutination assay for *M. ovipneumoniae* was performed by WADDL using *M. ovipneumoniae* antigen-sensitized and non-sensitized erythrocytes with serially diluted serum samples as described previously (Besser et al., 2008). Serum neutralization assays were performed by WADDL to determine antibody titers for respiratory viruses including BRSV, BVDV, BHV-1, and PI-3.

2.5. Histopathology

Histopathology was performed by WADDL. Lung lesions were described by noting the character of the inflammatory infiltrate, degree of necrosis, presence or absence of abscessation and bacterial colonies (Besser et al., 2008; Dassanayake et al., 2009).

3. Results

3.1. The microbial profile of the nasopharynx of the BHS

All the animals were culture- and PCR-negative for *M. ovipneumoniae*, although all BHS were positive for non-pathogenic *M. arginini* (Table 2). As expected, none of the animals had demonstrable *M. ovipneumoniae* antibody titers (Table 3). All the animals were culture-positive for *B. trehalosi* but were negative for *lktA* by PCR (Table 2). Several animals had antibody titers to RSV (R123, R124, Y45) and PI-3 (OR26, Y45, R123, R124) (Table 3). However, all were culture-negative for respiratory viruses (Table 2). All the animals except two (R123, R124) were negative for *M. haemolytica* (Table 2). Of the two that were positive, one had *lktA*-positive *M. haemolytica* (R124) while the other one had *lktA*-negative *M. haemolytica* (R123; Table 2). All the animals had insignificant levels of anti-LktA antibodies (Table 3). We could not perform serological assays for the control animals' sera due to the poor quality of the sera.

3.2. *M. ovipneumoniae* fails to induce fatal pneumonia in BHS

Lung homogenates from pneumonic BHS lambs and nasopharyngeal washings from *M. ovipneumoniae*-positive DS ewes were used as the source of *M. ovipneumoniae* for inoculation of four BHS (Table 1). When animals were inoculated with the filtrates, none of the BHS developed any signs of respiratory viral infection, during the 4-week observation period, demonstrating the absence of any BHS respiratory viral pathogens in the inoculum. These preparations were negative for any viruses by culture as well. None of the animals developed any signs of pneumonia following intranasal

Table 2
Bacterial and viral pathogens isolated from or detected in nasopharynx and lungs of bighorn sheep before and after *M. ovipneumoniae* and *M. haemolytica* challenges.

Groups	animal	M.h		M.ovi		M.arg		B.t		BRSV		PI-3		BVDV		BHV-1	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
I	OR26	–	+[a,c]	–	+	+	+	+[b]	+[b]	–	+[e]	–	–	–	–	–	–
	R124	+[a]	+[a]	–	+	+	+	+[b]	+[b]	–	–	–	–	–	–	–	–
II	R123	+[b]	+[a,d]	–	+	+	+	+[b]	+[b]	–	+[e]	–	–	–	–	–	–
	Y45	–	+[a,d]	–	+	+	+	+[b]	+[b]	–	–	–	–	–	–	–	–
III	Y30	–	–	–	–	+	+	+[b]	+[b]	–	–	–	–	–	–	–	–
	Y39	–	–	–	–	+	+	+[b]	+[b]	–	–	–	–	–	–	–	–

M.h – *M. haemolytica*; M.ovi – *M. ovipneumoniae*; M.arg – *M. arginini*; B.t – *B. trehalosi*; BRSV – bovine respiratory syncytial virus; BVDV – bovine viral diarrhea virus; BHV-1 – bovine herpes virus-1; PI-3 – parainfluenza-3; Pre – before the challenge; Post – at necropsy.
[a] *lktA*-positive.
[b] *lktA*-negative.
[c] *M. haemolytica* serotype A1.
[d] *M. haemolytica* serotype A2.
[e] Detected by ELISA.

Table 3
Serum antibody titers for *Pasteurellaceae* leukotoxin A, *M. ovipneumoniae* and respiratory viruses before and after *M. ovipneumoniae* exposures and *M. haemolytica* challenges.

Groups	Animal	LktA		M.ovi		BRSV		PI-3	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post
I	OR26	10	10	0	>2560	0	0	128	128
	R124	80	500	0	1280	32	128	32	64
II	R123	160	10	0	>2560	4	0	128	128
	Y45	40	100	0	>2560	0	8	64	32

LktA – *Pasteurellaceae* leukotoxin A (*M. haemolytica* and *B. trehalosi*). M.ovi – *M. ovipneumoniae*. BRSV – bovine respiratory syncytial virus. PI-3 – parainfluenza-3. Pre – before the challenge. Post – at necropsy.

challenge with ceftiofur-treated BHS pneumonic lung homogenates or DS nasopharyngeal washings although all four BHS became culture- and PCR-positive for *M. ovipneumoniae* 2 weeks post-challenge. Therefore, the animals were re-challenged with the same inoculum (Table 1). During the next few days, all the BHS developed signs of *M. ovipneumoniae* infection including lethargy, reduced appetite, intermittent cough, nasal discharge, and head shaking. One animal from Group I (R124) died of pneumonia on day 47. However, it should be noted that this animal had been consistently positive for *lktA*-positive *M. haemolytica* since day 1 of the experiment. Anti-LktA, anti-*M. ovipneumoniae* and BRSV titers gradually increased (Table 3). However, BRSV was not isolated by culture, from any of the samples collected. Post-mortem examination of R124 revealed acute bronchopneumonia in the right lung with severe consolidation over 50% of the ventral portion of all lobes. Histologically, the lungs had severe filling of alveoli and many bronchioles with neutrophils and variable amounts of fibrin and edema residue. All the samples taken from the lesional tissue were heavily positive for *M. haemolytica*, *B. trehalosi* and *M. ovipneumoniae*. All the isolates of *M. haemolytica* but not *B. trehalosi* were PCR-positive for *lktA*. R124 also showed neutralizing titers for BRSV and PI-3, but not for any other viruses (Table 3).

3.3. Inoculation of M. haemolytica causes pneumonia and death of M. ovipneumoniae-inoculated BHS

M. haemolytica serotype A1 and A2 were intranasally inoculated into the remaining BHS in Group I and two BHS in Group II, respectively, on day 70 as shown in Table 1. Both R123 and Y45 died 1 day post-inoculation with *M. haemolytica*. The gross- and histopathology of the lungs of both these animals were similar. The right and left lungs showed 30–50% consolidation (Fig. 1A). There was some fibrin deposition on the pleural surface of the left cranial lobe, bronchi and pericardium suggesting that the death of the animals was due to acute broncho/pleuropneumonia characteristic of *M. haemolytica* infection. The lungs had regional filling of alveoli with neutrophils, fibrin, and erythrocytes (Fig. 1C). All the samples collected from the animals, including the middle ears, were positive for *M. haemolytica* serotype A2, *B. trehalosi*, *Pasteurella multocida* (*toxA*-negative) and *M. ovipneumoniae*. All the isolates of *M. haemolytica*, but not *B. trehalosi*, were positive for *lktA* by PCR (Table 2). LktA-neutralizing titers of R123 changed only slightly over time except at the time of death when the titers became low (Table 3). Lkt neutralization titers of Y45 remained unchanged. *M. ovipneumoniae* titers of both R123 and Y45 increased from undetectable titers prior to experimental challenge to high titers (>1:2560) after the second *M. ovipneumoniae* challenge until the time of death.

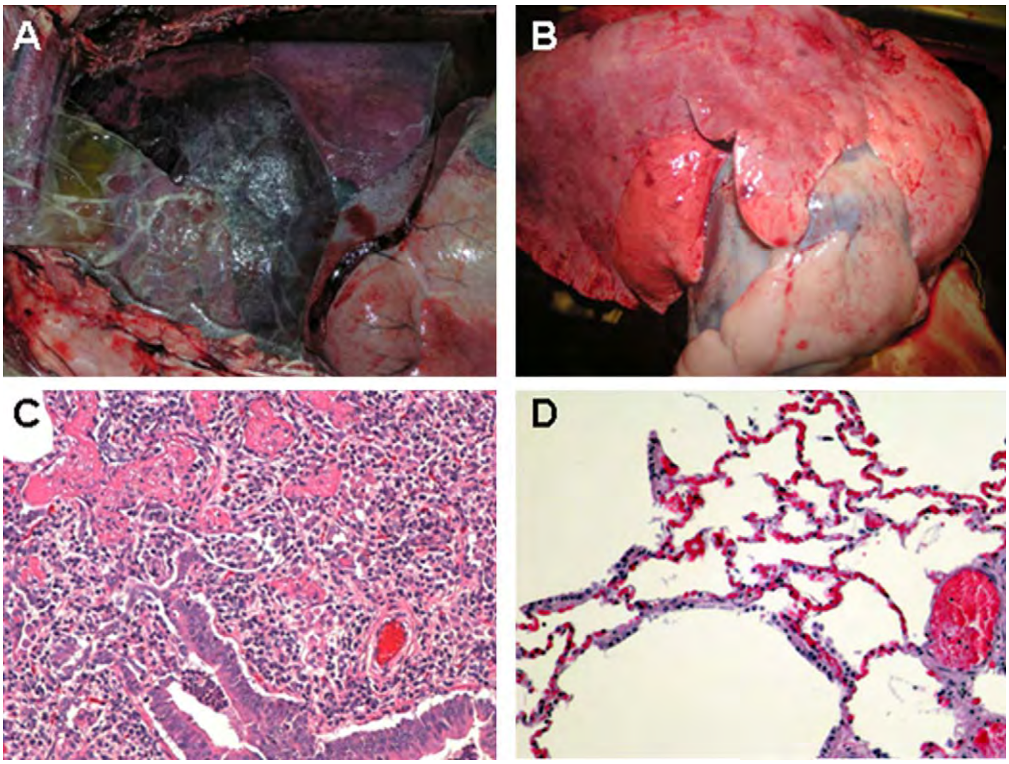

Fig. 1. Lung lesions of BHS inoculated with *M. ovipneumoniae* followed by *M. haemolytica*. BHS were inoculated with two doses of *M. ovipneumoniae* (2 weeks apart) followed by one dose of *M. haemolytica* 4 weeks after the second *M. ovipneumoniae* inoculation. (A) Gross pathology of the lungs of BHS (R123) inoculated with *M. ovipneumoniae* followed by *M. haemolytica* A2. (B) Gross pathology of the lungs of a control BHS (Y39) administered sterile RPMI. (C) Histopathology of lungs of R123. (D) Histopathology of lungs of Y39. (C, D: hematoxylin and eosin staining, original magnification = 100×).

Except for low neutralizing titers (1:4) for BRSV on two occasions and stable PI-3 titers (1:128), R123 did not show neutralization titers against other respiratory viruses tested (Table 3). Y45 had a BRSV positive titer of 1:8 at the time of death and fairly consistent PI-3 positive titers (1:32–64) during the study period. Although nasal swabs and lung tissues were positive for BRSV antigen in R123 by ELISA, we could not isolate any viruses in cell cultures (Table 2).

The animal OR26 died 5 days following *M. haemolytica* serotype A1 inoculation. The gross- and histopathologic lesions in this animal were similar to those of R123 and Y45. As expected, *M. haemolytica* A1, *M. ovipneumoniae*, *B. trehalosi* (*lktA*-negative), and *P. multocida* (*toxA*-negative) were isolated from numerous samples. This animal showed very low LktA-neutralizing antibody titer during the course of the study. *M. ovipneumoniae* titers increased from undetectable titers prior to challenge, to high titers (>1:2560) following second *M. ovipneumoniae* inoculation. Anti-PI-3 titer remained unchanged at 1:128, but no antibodies against other respiratory viruses were detected throughout the study period. OR26 also showed positive BRSV results by ELISA on lung tissues. No viruses were isolated in cell cultures. As expected, the lungs of the two BHS in the control group (Y30 and Y39) showed no evidence of pneumonia (Fig. 1B and D).

4. Discussion

M. ovipneumoniae has been isolated from wild BHS lambs and adults in naturally occurring pneumonia in previous studies (Besser et al., 2008). However, *M. ovipneumoniae* isolated from these animals failed to induce sustained clinical illness in two BHS lambs given multiple intranasal inoculations despite successful oropharyngeal colonization. Virulence attenuation of *M. ovipneumoniae* occurs during laboratory culture (Gilmour et al., 1979; Jones et al., 1982) which could be responsible for the failure to reproduce clinical disease. Therefore we reasoned that the use of lung homogenates from pneumonic BHS or nasopharyngeal washings from *M. ovipneumoniae*-positive DS should avoid this possibility of attenuation and better assess the etiologic role of this agent.

Although all BHS inoculated with unfiltered, but ceftiofur-treated, lung homogenates and nasopharyngeal washings developed clinical signs of *M. ovipneumoniae* infection, only one died prior to the time of intranasal *M. haemolytica* challenge. Therefore, based on this study, we propose that *M. ovipneumoniae* alone is not adequate for the induction of fatal bronchopneumonia in BHS, which is in agreement with the findings of our previous *M. ovipneumoniae* challenge studies with BHS lambs (Besser et al., 2008). Our finding that three out of three BHS developed bronchopneumonia and died 1–5 days post-inoculation with *M. haemolytica* clearly indicates that *M. haemolytica* is the pathogen that causes fatal pneumonia in BHS challenged under our experimental protocol. The difference in the interval between the inoculation and death of the BHS in Group II (1 day) and Group I (5 days) is very likely due to the difference in virulence between *M. haemolytica* serotype A2 and serotype A1 that was used to inoculate the BHS. Our earlier studies have indicated that the serotype A2 is more virulent than serotype A1 (unpublished observation). The death of one BHS in Group I after inoculation with *M. ovipneumoniae*, but before inoculation with *M. haemolytica*, was very likely due to the presence of *lktA*-positive *M. haemolytica* in the nasopharynx of this BHS right from the onset of this study. The pneumonic lesions of the lungs were indicative of *M. haemolytica*-caused pneumonia. The death of all three BHS following intranasal inoculation with *M. haemolytica* suggests that *M. ovipneumoniae* acted as a primary pathogen, reducing the resistance of BHS to the *M. haemolytica* challenges predisposing these animals to relatively rapid development of fatal pneumonia due to *M. haemolytica* infection. It is likely that *M. ovipneumoniae*-induced loss of mucociliary defense of the respiratory tract (Niang et al., 1998) facilitated rapid proliferation and descent of *M. haemolytica* into the lower respiratory tract and induction of fatal bronchopneumonia. However, in a previous study by us intra-tracheal inoculation of *M. haemolytica* (1×10^9 CFU of a serotype A1 strain) resulted in the death of all four BHS within 48 h (Dassanayake et al., 2009). Furthermore, in a recent experimental challenge study by us, intranasal inoculation of a strain of *M. haemolytica* (1×10^6 CFU of serotype A2) caused the death of three out of four BHS within 48 h (unpublished observation), which questions the necessity for a predisposing agent such as *M. ovipneumoniae* to render the mucociliary apparatus dysfunctional in order for *M. haemolytica* to cause fatal bronchopneumonia, at least in that experimental challenge model.

Antibodies specific for RSV and PI-3 have been detected in several BHS herds (Elliott et al., 1994; Spraker et al., 1986). Although RSV was not isolated by culture from any of the BHS in this study, lung tissue from two of them (R123 and OR26) were positive for RSV by ELISA. The RSV titers of two animals (R124 and Y45) increased (from 1:32 to 1:128, and from undetectable to 1:8, respectively) during the experiment. Therefore, we cannot rule out the possibility that RSV also was involved in the induction of pneumonia in these animals. Studies are currently underway to elucidate the role of RSV and PI-3 in the etiology of pneumonia in BHS.

In summary, our findings indicate that *M. ovipneumoniae* by itself did not cause fatal pneumonia in BHS used in this study. However, it did predispose them to fatal pneumonia caused by *M. haemolytica*. We propose that low virulent strains, but not high virulent strains, of *M. haemolytica* may require a predisposing agent such as *M. ovipneumoniae* for the induction of fatal bronchopneumonia in BHS.

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Linking conservation actions to demography: grass height explains variation in greater sage-grouse nest survival

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Linking conservation actions to demography: grass height explains variation in greater sage-grouse nest survival

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Conservation success often hinges on our ability to link demography with implementable management actions to influence population growth (λ). Nest success is demonstrated to be important to λ in greater sage-grouse *Centrocercus urophasianus*, an imperiled species in the North American sagebrush-steppe. Enhancing this vital rate through management represents an opportunity to increase bird numbers inside population strongholds. We identified management for grass height as an action that can improve nest success in an analysis of sage-grouse nests (n = 529) from a long-term study (2003–2007) in the Powder River Basin, southeast Montana and northeast Wyoming, USA. Average grass height by study area and year varied (11.4–29.2 cm) but its positive effects on nest survival were consistent among study years and study areas that differed in absolute rates of nest success. We tested the predictive ability of models by grouping output from log-link analyses (2004–2006) into two bins with nest success probabilities < 0.45 and > 0.55, and validated the relationship with additional data from 2003 and 2007. Nests with probabilities > 0.55 were 1.64 (2004–2006) to 3.11 (2007) times more likely to hatch than those < 0.45, except in 2003 when an early wet spring resulted in universally high grass height at nest sites (29.2 cm) and high predicted nest success (64%). The high predictive power of grass height illustrates its utility as a management tool to increase nest success within priority landscapes. Relationships suggest that managing grass height during drought may benefit sage-grouse populations.

Achieving desired conservation outcomes requires planning at scales that match the biological needs of wide-ranging focal species (Nicholson et al. 2013). Inherent in conservation success is our ability to link demography to implementable management actions that influence population growth (λ; Mills 2012). Implementing locally beneficial conservation practices inside intact ecosystems maximally benefits species for which landscape context matters (Wilson et al. 2007, Schultz 2010). Advances in spatial ecology make landscape prioritization more feasible (Millspaugh and Thompson 2009), but identifying intact targets is only a first step (Knight et al. 2008). Still missing in most plans is a demographic link between a conservation action and its ability to influence demographic traits influencing λ (Wisdom et al. 2000, Caswell 2001).

Greater sage-grouse *Centrocercus urophasianus* (hereafter sage-grouse) are native only to western arid and semiarid sagebrush *Artemisia* spp. landscapes (Schroeder et al. 1999), and extirpated from half their range (Schroeder et al. 2004), the species is a candidate for listing under the federal Endangered Species Act (US Fish and Wildlife Service 2010). Major fragmenting threats include energy development (Naugle 2012), wildfire (Bukowski and Baker 2013, Murphy et al. 2013), cultivation for row crop production (Foley et al. 2011) and others (Knick et al. 2013). The current sage-grouse distribution encompasses 76 million hectares, yet population densities are highly clumped across their range (Doherty et al. 2010a). In efforts to focus conservation actions, the US Fish and Wildlife Service identified "Priority Areas for Conservation" (PACs; US Fish and Wildlife Service 2013) by consulting US states to incorporate the best available population and habitat data into site delineation. Research has focused on reducing threats to populations within PACs (Baruch-Mordo et al. 2013, Copeland et al. 2013), yet management actions that aim to bolster populations within priority areas will be critical for a species with declining distribution.

The purpose of our paper is to increase conservation effectiveness by exploring linkages between demography and implementable actions to benefit populations. Nest success is demonstrably important to λ, and enhancing this vital rate through management may benefit populations (Taylor et al. 2012). Variation in nest survival may in part be explained by grass height (DeLong et al. 1995), a feature influenced by grazing (Rickard et al. 1975), and a preeminent landuse in sagebrush systems. We used generalized linear models to estimate the influence of vegetation and nest characteristics on sage-grouse nest survival within a landscape context (Dinsmore et al. 2002, Rotella et al. 2004). Findings will help guide the US Dept of Agriculture's Sage Grouse Initiative (SGI) in implementing rotational grazing systems designed to increase hiding cover for nesting grouse inside PACs on 847 000 ha of privately-owned rangelands (<www.sagegrouseinitiative.com/our-work/proactive-conservation/> under Grazing Systems).

Material and methods

Study area

We sampled sage-grouse in two distinct study areas in Johnson and Sheridan Counties in northeast Wyoming (southern region), and Bighorn, Rosebud, and Powder River Counties in southeast Montana (northern region), USA. Northern study areas were dominated by sagebrush, with conifer encroachment in more rugged landscapes and overall larger grassland areas. Southern study areas were also dominated by sagebrush, but had no conifers and exhibited smaller grassland areas. Shrub–steppe habitats were dominated by Wyoming big sagebrush *A. tridentata wyomingensis* with an understory of native and non-native grasses. Land use in both study areas was dominated by cattle ranching and land tenure was a mix of federal, state and private. Doherty et al. (2008) provides detailed descriptions of study areas. Because of the differences in landscape context, study area was included as a categorical blocking variable.

Capture, radio-tracking and predictor variables

We captured sage-grouse in rocket-nets and walk-in traps (Giesen et al. 1982) and by spotlighting (Wakkinen et al. 1992) March–April and July–October in 2003–2007. We aged females, fitted them with necklace style VHF radio collars, and relocated sage-grouse to monitor nests by ground based radio-tracking throughout the breeding season. We used established protocols (Connelly et al. 2003) to quantify local vegetative features known to influence habitat selection within ≤15 m of nests (Connelly et al. 2000, Hagen et al. 2007; Table 1). Doherty et al. (2010b) provides a full description of nest monitoring.

Statistical analyses and model selection

We used generalized linear models with a binomial likelihood and a log-link to estimate the influence nest age, study area and grass height on the daily survival rates (DSR) of nests (Dinsmore et al. 2002, Rotella et al. 2004). We derived nest survival rates by multiplying DSR together over the 28 day predicted incubation time for sage-grouse. We divided samples into nests used to build the model (n = 383 nests in 2004–2006) and those used to test model stability and predictive capability (n = 146 in 2003 and 2007).

We followed an iterative system for model selection. We first included a variable that controlled for the known effect of a spring snow storm in 2005 on DSR in all variable screenings and final model selection (Walker 2008).

Table 1. List of variables used in model selection explaining sage-grouse nest survival, Powder River Basin, Montana and Wyoming, USA, 2004–2006.

Candidate variables	Description
Local scale habitat variables	
Shrub canopy cover	using the line-intercept method along two 30 m perpendicular transects centered at nest or random locations (Canfield 1941)
Shrub density	all shrubs > 15 cm within 1 m of transect line were counted, total /120 m^2
Quadratic shrub canopy cover	shrub canopy cover + (shrub canopy cover × shrub canopy cover)
Nearest shrub height	height of nearest shrub to Daubenmire quadrant location. There were 10 Daubenmire quads on each of the two 30 m transects for a total of 20 Daubenmire quads. They were spaced 3 m apart and started at 0 m
Visual obstruction at nest	height density readings at 0, 1, 3 and 5 m from nest or available shrub in each cardinal direction (Robel et al. 1970)
Nearest grass height	average of the vegetative droop height for the nearest grass from the 20 Daubenmire quadrants
Tallest grass height	average of the vegetative droop height for the tallest grass from the 20 Daubenmire quadrants
Average grass height	(nearest grass height + tallest grass height)/2
Nest characteristic variables	
Hen age	yearling or adult (Walker 2008)
Nest age	(nest age in days + nest age in $days^2$) (Walker 2008)
Snowstormmarker	grouped 7 nests that were abandoned following major snow event in May 2005
Abiotic site variables	
Study area	north or south Powder River Basin
Year	year of observation

We assigned predictor variables into 1 of 3 model categories: 1) habitat, 2) nest characteristic, and 3) site variables (Table 1). We first examined univariate selection for study area and the 8 habitat variables, and removed variables if 95% confidence intervals overlapped zero. If predictor variables were highly correlated ($r \geq |0.7|$), only the variable with the greatest biological merit was included in the model (Chatfield 1995). When variables were moderately correlated (i.e. $|0.3| \leq r < |0.7|$), we checked for stability and consistency of parameter estimates as predictor variables were added.

We allowed each variable that made it past variable screening to compete with all other combinations of variables to identify the most parsimonious model for habitat and study area. If variables made it past screening we determined if their addition improved model fit via Akaike's information criterion with a small sample size correction factor (AIC_c; Burnham and Anderson 2002). After obtaining the best habitat model using AIC_c values, we then tested if inclusion of nest characteristic variables (Table 1) and an additional abiotic site variable (year effect) documented in Walker (2008) were still important predictor variables when included with habitat covariates. We followed the exact variable screening and AIC methods described above to test if these variables improved model fit.

We tested the predictive strength of the final habitat model by grouping predicted nest survival probability from log-link analyses (2004–2006) into two bins with probabilities of nest survival, < 0.45 and > 0.55, generically representing low and high nest survival probabilities, respectively. We then compared observed nest success from independent data sets (2003 and 2007) between low and high validation bins, and calculated the ratio of observed nest success between the high and low bins. We reasoned that observed nest success should be higher in the top validation bin if the final model predicted nest success well across years, demonstrated by a ratio of observed nest success > 1 between bins. We further evaluated the predictive model by comparing predicted nest success from our top model to observed nest success by year. Average grass height around nesting sage-grouse in a given year (Table 1) was the only continuous predictor variable included in our top model, thus we evaluated how well one variable served as an indicator of nest success. Statistical analyses were performed in program SAS ver. 8.0 (SAS Inst. <http://v8doc.sascom/sashtml/>).

We performed a bootstrap analysis to quantify precision and the effect size of grass height on nest survival, using beta coefficients from the best approximating model (Burnham and Anderson 2002). We used the logistic exposure equation (Rotella et al. 2004) to generate the predicted probability of successfully hatching a nest for each bootstrap dataset ($n = 5000$) by systematically varying grass height within the observed range of variation. We computed at each percentage the probability of successfully hatching a nest for each of 5000 simulations. We ordered these probabilities and used a rankit adjustment (Chambers et al. 1983) to estimate upper and lower 95% confidence intervals.

Results

Nearest, tallest and average grass height were the only variables with significant coefficients when tested univariately. Nearest, tallest and average grass height were all positively associated with nest success, but were highly correlated and could not be included in the same model. Average and nearest grass height had virtually identical univariate coefficient estimates, however average grass height showed less variation around the estimate (average grass height $\beta = 0.034$, $SE = 0.013$, 95% $CI = 0.008–0.060$ vs nearest grass height $\beta = 0.039$, $SE = 0.019$, 95% $CI = 0.001–0.076$). Further, average grass height outcompeted nearest and tallest grass measures based on AIC_c values, thus it was retained for additional modeling.

The addition of study area increased model fit, while hen age and year effects were removed from the model because they explained no additional variation in nest survival when included with habitat variables and confidence intervals around effect estimates overlapped zero. The inclusion of nest age increased model fit ($w_i = 0.974$; Table 2). Our final model included average grass height, nest age, study area and the variable that controlled for the known effect of a spring snow storm in 2005 on DSR.

Table 2. Comparisons of grass height, study area and nest age variables to identify the AICc best model explaining sage-grouse nest survival, Powder River Basin, Montana and Wyoming, 2004–2006[a].

Model	K	AIC_c	ΔAIC_c	w_i
Average grass height + study area + nest age	6	834.418	0.000	0.974
Average grass height + study area	4	841.634	7.216	0.026
Average grass height	3	866.099	31.681	0.000
Study area	3	927.881	93.463	0.000

[a]all models included a categorical blocking variable which controlled for nests abandoned in a heavy spring storm in 2005 (Walker 2008).

Estimates of average grass height tracked annual trends in nest success (Fig. 1; northern region 2003–2007, beta estimate $= 0.036$, $p = 0.023$; southern region 2004–2007, beta estimate $= 0.079$, $p = 0.001$). Bootstrap analyses showed the positive relationship between average grass height and nest success (Fig. 2). Our final model including grass height and study area demonstrated large effect sizes (Fig. 2). Nests with probabilities > 0.55 were 1.64 (2004–2006) to 3.11 (2007) times more likely to hatch than those < 0.45 (Table 3), except in 2003 when average grass height (29.2 cm) and apparent nest success reached their highest recorded levels (68%, Fig. 1).

Discussion

High predictive power of grass height illustrates its utility as a management tool to benefit sage-grouse populations. Findings show grass height is a strong predictor of nest survival inside intact landscapes, and increasing hiding cover can increase nest success, a demographic rate that explains a

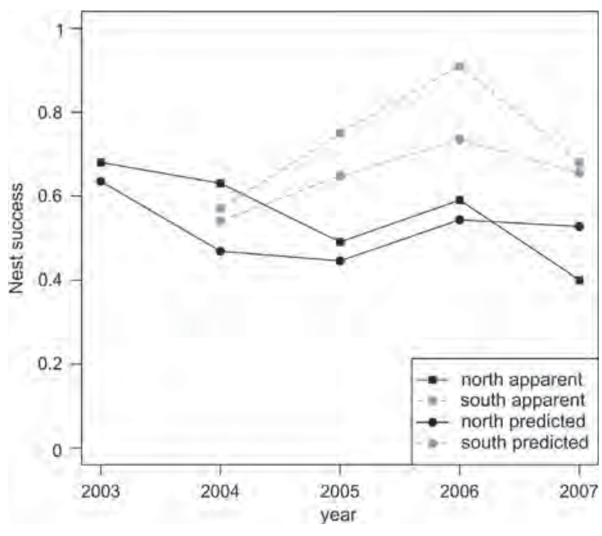

Figure 1. Apparent and predicted annual nest survival by year for sage-grouse in the Powder River Basin, Montana and Wyoming, US, 2003–2007. The final model included the effects of grass height, nest age, study area, and 2005 spring snow storm. Grass height measurements were averaged across nests within years to make annual predictions.

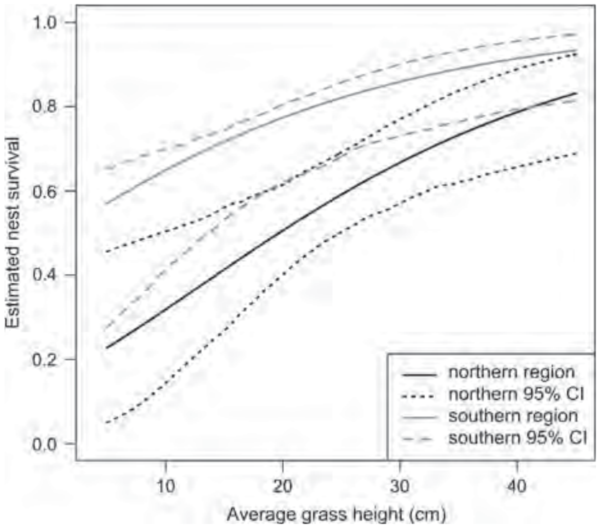

Figure 2. Relationship between average grass height and sage-grouse nest survival, Powder River Basin, Montana and Wyoming, USA, 2004–2006. Estimates of nest survival (95% confidence intervals [CIs]) in both study areas are based on 5000 bootstrap samples.

third of variation in λ (Taylor et al. 2012). Moreover, grass height is a reliable management tool because it explained variation (Fig. 2) despite variability in absolute rates of nest success between study areas. Positive effects of grass height should be evaluated on other important demographic rates including adult female and chick survival (Taylor et al. 2012) to see if benefits extend beyond what is now known.

Managing grass height in large and intact landscapes with grazing is a tool that may benefit populations in eastern Montana and northeast Wyoming. Positive effects of grass height in our study areas explained variation in nest success between years with large and precise effect sizes. Differing intercepts prohibit extrapolating of results to novel sagebrush systems because absolute effects likely depend upon regional conditions that influence grass and shrub composition. South and west of our study areas where sagebrush rather than grass provides most hiding cover, grass height had only a weak effect on nest success, and nest fates were dominated by year and site effects (Holloran et al. 2005). Grass height is positively related to nest success for other prairie grouse species

Table 3. Validation of grass height as a predictor for sage-grouse nest success, Powder River Basin, Montana and Wyoming, 2003–2007. We tested the AICc best model (Table 2) by calculating the predicted probability of hatching for each nest by applying grass height and region coefficients from log-link analysis (2004–2006) to observed grass heights at nests. We used the predicted probability (n is number of nests in each category) of hatching to group nests with probabilities of <0.45 and >0.55 and then compared apparent nest success ratios. We also validated the relationship with independent data sets (2003 and 2007). Nest age was excluded because we exponentiated daily survival rate for nests across the 28-day incubation period.

Predicted probability	Observed nest success		
	2003	2004–2006	2007
p < 0.45 (low)	0.714 (n = 7)	0.486 (n = 70)	0.200 (n = 5)
p > 0.55 (high)	0.667 (n = 30)	0.796 (n = 184)	0.623 (n = 52)
Ratio (high/low)	0.93	1.64	3.11

and subspecies (Attwater's prairie-chickens *Tympanuchus cupido attwateri*, Lehmann 1941; plains sharp-tailed grouse *T. phasianellus jamesi*, Hillman and Jackson 1973; greater prairie-chicken *T. cupido pinnatus*, McKee et al. 1998).

Findings suggest that maintaining grass height during drought may provide the greatest benefits to populations. Average grass height and predicted nest success in this study is within the range of published literature (Schroeder et al. 1999, Connelly et al. 2000). Benefits may be negligible in years resembling 2003 when spring rains provided abundant grass and the correspondingly highest predicted nest success for the northern study area. High variation in pooled grass height by study area and years (11.4–29.2 cm) also suggested that modifying grazing practices to maintain nesting cover could improve a habitat feature that otherwise limits λ. We have identified a strong corollary of nest success in the Powder River Basin (PRB). If this relationship is validated in new study areas across different parts of the sage-grouse range, and if the relationship between grass height and nest success can be calibrated within these new areas, grass height may be useful as a surrogate to monitor nest success.

Findings emphasize the importance of an indirect effect of grazing on sage-grouse nest success. Results have broad implications because livestock grazing is the most widespread land use in the world (Holechek et al. 2003), affecting 70% of land area in the western US (Fleischner 1994). Effects of grazing on sage-grouse habitat may be wide-ranging depending upon current and historic timing and intensity of grazing, soil conditions, precipitation, plant communities and habitat features under consideration (Beck and Mitchell 2000, Connelly et al. 2000, 2004, Crawford et al. 2004). However, adjustments to duration and timing of grazing also may increase residual cover with the added benefit of increasing long-term rangeland health on which birds depend. For example, reducing the short-term stocking rate of sheep increased black grouse *Tetrao tetrix* numbers by 6% annually in Europe by increasing residual cover (Calladine et al. 2002). Replicated experiments to document sage-grouse response to different grazing systems are needed to help guide land managers to practices that are beneficial to sage-grouse and economically viable to producers (Krausman et al. 2011).

Habitat management within a PAC-based conservation strategy may benefit populations, but sage-grouse are a wildland species, and grass height is of little consequence if sagebrush systems continue to be replaced by anthropogenic land uses (Knick et al. 2013). Viability of ranching as a predominant land use may in part determine the future of sage-grouse conservation in the West. The SGI has increased by four-fold their implementation of rotational grazing systems by resting for up to 17 months the pastures used by nesting sage-grouse grouse within 488 000 ha inside Montana's PACs (J. Siddoway pers. comm.). Our findings suggest that these types of grazing systems that promote nest success may provide one mechanism to offset population losses by increasing bird numbers.

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NEVADA DIVISION OF WILDLIFE'S

BIGHORN SHEEP MANAGEMENT PLAN

OCTOBER 2001


WILDLIFE RESTORATION
Federal Aid Project

STATE OF NEVADA
Kenny C. Guinn, Governor

NEVADA DEPARTMENT OF CONSERVATION AND NATURAL RESOURCES
R. Michael Turnipseed, P.E., Director

DIVISION OF WILDLIFE
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GAME BUREAU
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BOARD OF WILDLIFE COMMISSIONERS

THE VALUE OF BIGHORN SHEEP

The sight of bighorn sheep leaping nimbly across rugged slopes elicits emotions that impress and inspire viewers. From primitive inhabitants to civilized peoples, a recurring theme in records kept on bighorn sheep is the strong sentiment elicited by this animal.

One of the most difficult tasks in wildlife management is to place value on wildlife. Economics alone do not even come close to describing the values of wildlife to the people of the State of Nevada. Other values, which are nearly impossible to quantify, must be considered when evaluating what an animal is worth. Activities such as wildlife viewing and photography are examples of the use of the bighorn sheep resource that are not well documented but no doubt account for thousands of recreational days annually. Even people that have no expectations of seeing bighorn sheep in the wild want to know they are present and will be into the future.

The interest and enthusiasm expressed in bighorn sheep through conservation organizations such as Nevada Bighorns Unlimited, the Fraternity of the Desert Bighorn and the Foundation for North American Wild Sheep attests to the tremendous respect and admiration that sportsmen and the general public have for the State's bighorn sheep. Through political and financial support, construction of water developments, and other habitat improvement projects, these bighorn-support groups have benefited many wildlife species. The Nevada Division of Wildlife recognizes these immeasurable values of bighorn sheep and has the responsibility to ensure that they are managed for the enjoyment and use by both present and future generations.

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ACKNOWLEDGEMENTS

This plan was developed in a collaborative process by the bighorn sheep management team. The team was formed from sportsmen and bighorn sheep enthusiasts selected by the Nevada Board of Wildlife Commissioners and Nevada Division of Wildlife (NDOW) biologists selected by the agency. Team members included:

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- **Jack Robb** representing the Nevada Bighorns Unlimited – Reno
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- **Craig Stevenson**, NDOW southern Nevada water development/habitat biologist
- **Tony Wasley**, NDOW eastern Nevada bighorn sheep biologist

The photographs on the plan's cover were taken by Craig Stevenson. Tony Wasley developed the maps within the plan. Special thanks go to the many NDOW biologists and staff involved in the review of the initial draft plan who provided valuable comments to improve the plan and manuscript. Appreciation is also extended to other agencies and organizations and to the general public who took the time to review the document and provide valuable comments. Funding for this project was from tag and license revenues and financial assistance provided by the Federal Aid in Wildlife Restoration program.

EXECUTIVE SUMMARY

The bighorn sheep management plan is a guiding document for the Nevada Board of Wildlife Commissioners (Commission) and the Nevada Division of Wildlife (Division) efforts in the conservation and management of bighorn sheep populations and their habitat. The plan includes Commission policies that are overall goals that guide the Division. The majority of the plan is a framework that outlines the actions and strategies that Division employees will follow in planning and conducting bighorn sheep management and conservation.

Bighorn sheep have been shown to be one of the more numerous and most widely distributed large ungulates throughout historic Nevada. But by the late 19th century, several factors caused the decline of Nevada's bighorn populations.

The quality and quantity of suitable habitat will ultimately determine the number of bighorn sheep that the State of Nevada will support. Continued collaboration with land management agencies, government entities, private landowners, and sportsmen is imperative when protecting and enhancing bighorn sheep habitat. All occupied and potential bighorn sheep habitat will be delineated and limiting factors will be identified for each. Information gathered through this activity will be the basis for protection and enhancement activities. The purchase of conservation easements, property and associated grazing privileges, conversions of Animal Unit Months (AUM's) from domestic sheep to cattle or water rights, will be done to protect or enhance important bighorn sheep habitat. The Division will actively pursue a program to provide water for bighorn sheep as a means to increase population levels and distribution in water deficient habitats.

From a population management perspective, the underlying goal of this plan is to restore and maintain bighorn herds at optimal population levels based on a multitude of demographic and ecological parameters. Bighorn sheep will be reintroduced into suitable but unoccupied habitats. Bighorn herds below optimal levels will be augmented to bolster populations. Comprehensive planning, coordination, and follow up will be conducted in the capture and release of bighorn sheep. All future releases of bighorn subspecies will be within their identified delineation area, with the largest portion of Nevada being delineated for desert bighorn sheep. Bighorn populations will be adequately monitored to assess trends and detect significant demographic changes and/or home range/movement changes. The Division will investigate and address all disease related problems in a timely fashion.

Bighorn sheep hunting is a legitimate and desirable use of the bighorn resource. The Division will develop quota recommendations with the expectation of obtaining a statewide average age of 6 years for harvested rams. Since bighorn sheep are a highly regarded and sought after big-game species, the Division will continue to protect bighorn sheep populations through education and appropriate enforcement of pertinent wildlife laws and regulations

The desert bighorn sheep is Nevada's state animal; yet, the general public has very little knowledge about bighorn sheep. Therefore, the Division is challenged to increase public awareness and appreciation for bighorn sheep and their habitats in order to facilitate decisions favorable to their long-term well being.

BIGHORN SHEEP MANAGEMENT PLAN'S

WILDLIFE COMMISSION POLICIES

- The Division will work to protect all bighorn sheep habitat that is currently in good condition.

- In order to expand numbers and distribution of bighorn sheep, limiting factors, such as lack of water and poor forage conditions, need to be addressed. Management actions to enhance these deficiencies will be aggressively pursued.

- The Division will increase bighorn populations of all subspecies statewide to a level where all habitats are occupied and each herd is self-sustaining.

- Bighorn sheep hunting is a legitimate and desirable use of the bighorn resource.

- The Division will increase public awareness and appreciation for bighorn sheep and their habitats in order to facilitate decisions favorable to their long-term well being.

HISTORY

The earliest archaeological record of bighorns in Nevada are remains from Pintwater Cave, northwest of Las Vegas, dated at 28,000 years before the present (Buck et al. 1997). Archeological investigations based on bones and petroglyphs have shown bighorns to be one of the more numerous and most widely distributed large ungulates throughout historic Nevada (Harrington 1933; Jennings 1957; Gruhn 1976). John C. Fremont wrote on January 11, 1834 during his travels through Nevada's Lake Range, "On our road down, the next day, we saw herds of mountain sheep....." (Smith 1909). But by the beginning of the late 19th century, commercial and illegal hunting, competition with livestock, and the effects of livestock diseases all appear to have caused the decline of Nevada's bighorn populations.

The earliest effort at bighorn management in Nevada appeared as an 1861 law closing sheep harvest between January 1st and July 1st. Other laws were enacted, varying the hunting season dates, but in 1901, the legislature closed bighorn hunting and it continued to be closed until 1952. As more laws and attention were brought on bighorn sheep management, indications were that illegal, subsistence-based hunting in the state began to decline during the 1940's (Jonez 1957).

The Nevada Division of Wildlife (Division), formerly known as the Department of Fish and Game, began bighorn sheep management in the late 1940's. In 1936, the U.S. Fish and Wildlife Service created the Desert National Wildlife Range for the protection of several desert bighorn sheep herds in southern Nevada. However, despite conservation efforts, Nevada's bighorn numbers continued to decline until the middle part of the century.

Figure 1 depicts the estimated bighorn sheep distribution in 1860, 1960, and 2001. The 1860 distribution is based on historic accounts and archeological evidence of bighorn sheep and biological judgment of areas that had adequate bighorn habitat. Using this distribution and a conservative density value for bighorn sheep, it was calculated by the bighorn sheep management team that Nevada's bighorn population in 1860 exceeded 30,000. But by 1960, it was estimated to have declined to a level between 2,000 and 3,000 bighorn. By the 1980's, bighorn sheep management intensified and restored animals to many of their historic ranges through habitat improvement and transplant programs. The 2001 statewide estimate was 6,500 bighorn sheep in 74 mountain ranges.

The continued existence of bighorn sheep in Nevada will rely on a mixture of science, sentiment and proper management decisions. This plan is a part of an effort to continue a course of action to ensure that this species will endure.

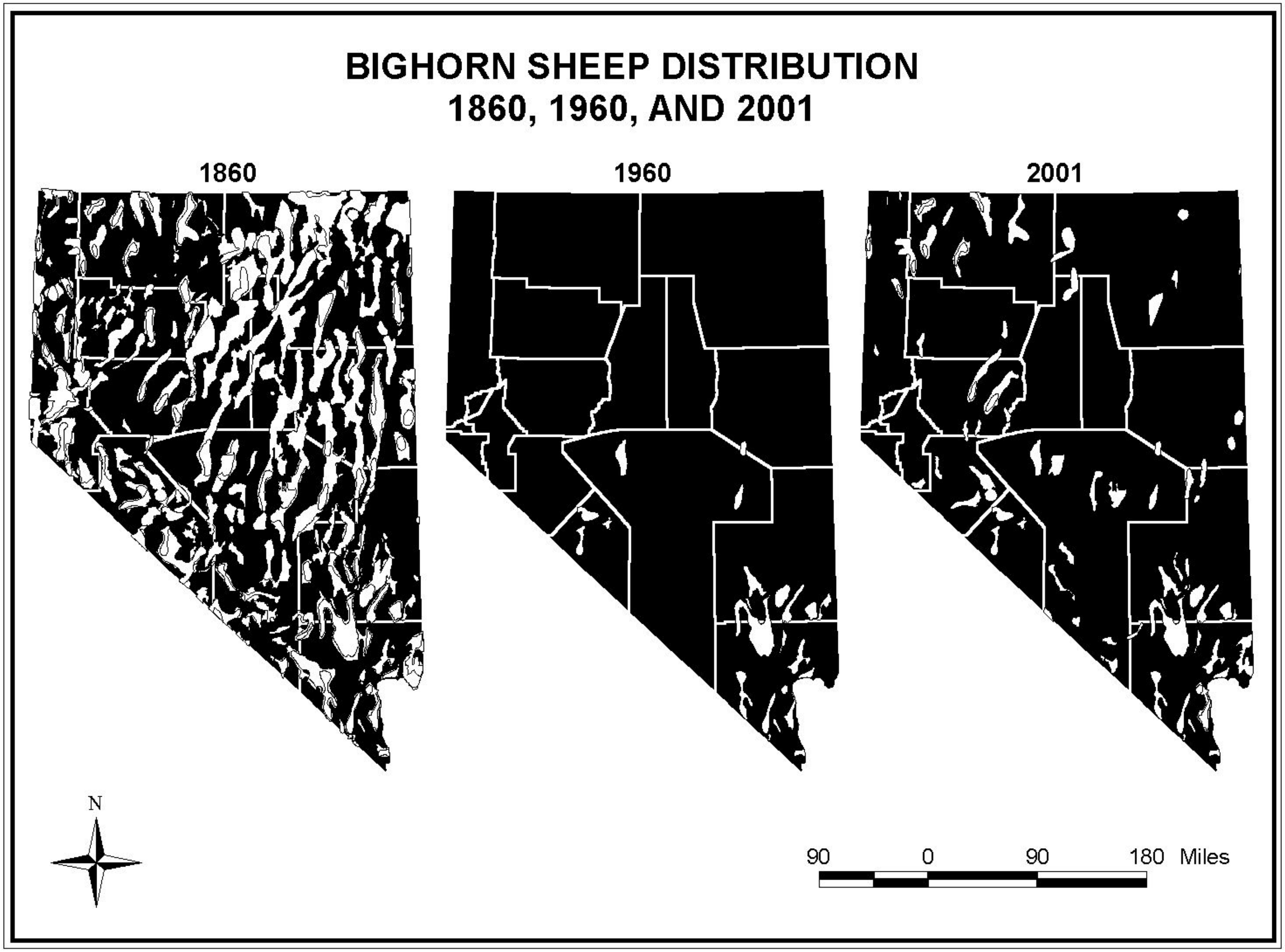

Figure 1. Bighorn Sheep Distribution in Nevada in 1860, 1960, and 2001.

HABITAT MANAGEMENT

The quality and quantity of suitable habitat will ultimately determine the number of bighorn sheep that the State of Nevada will support. Since most of the bighorn sheep habitat is managed by the Bureau of Land Management, the U.S. Forest Service, the U.S. Fish and Wildlife Service, the National Park Service, military installations, Indian Tribes, and private landowners, it is imperative that the Division always strive for cooperation and collaboration with these entities. State, County, and Local Governments also make decisions that have the potential to impact bighorn habitat. It is important that the Division provides input for all decisions affecting bighorn sheep habitat since the loss of habitat, or reduction in the habitat quality, will reduce the number of sheep that an area can support. The Division supports land use and habitat designations (i.e., wilderness, ACEC's, etc) as long as wildlife management activities that are used to manage bighorn populations and their habitat are allowed to continue.

Conservation organizations, such as Nevada Bighorns Unlimited (NBU), the Fraternity of the Desert Bighorn Sheep (Fraternity), The Foundation for North America Wild Sheep (FNAWS) and others, play an extremely important role in habitat protection and enhancement. The Division will continue to foster excellent working relationships with these groups in order to maximize habitat protection and habitat enhancement efforts.

POLICY STATEMENTS

- **The Division will work to protect all bighorn sheep habitat that is currently in good condition.**
- **In order to expand numbers and distribution of bighorn sheep, limiting factors, such as lack of water and poor forage conditions, need to be addressed. Management actions to enhance these deficiencies will be aggressively pursued.**

Habitat Delineation

Management Action: All occupied and potential bighorn sheep habitat will be delineated and limiting factors will be identified for each. Information gathered through this activity will then be used as a major tool to identify protection and enhancement activities.

Strategy: Biologists will identify all occupied and potential bighorn habitat within their area of responsibility (Figure 2). Factors that limit an area's ability to provide optimal habitat for bighorn sheep will be identified.

Strategy: The habitat information that depicts current distribution at optimal and less than optimal levels, potential habitat, and limiting factors will be incorporated into the Geographic Information System (GIS) database.

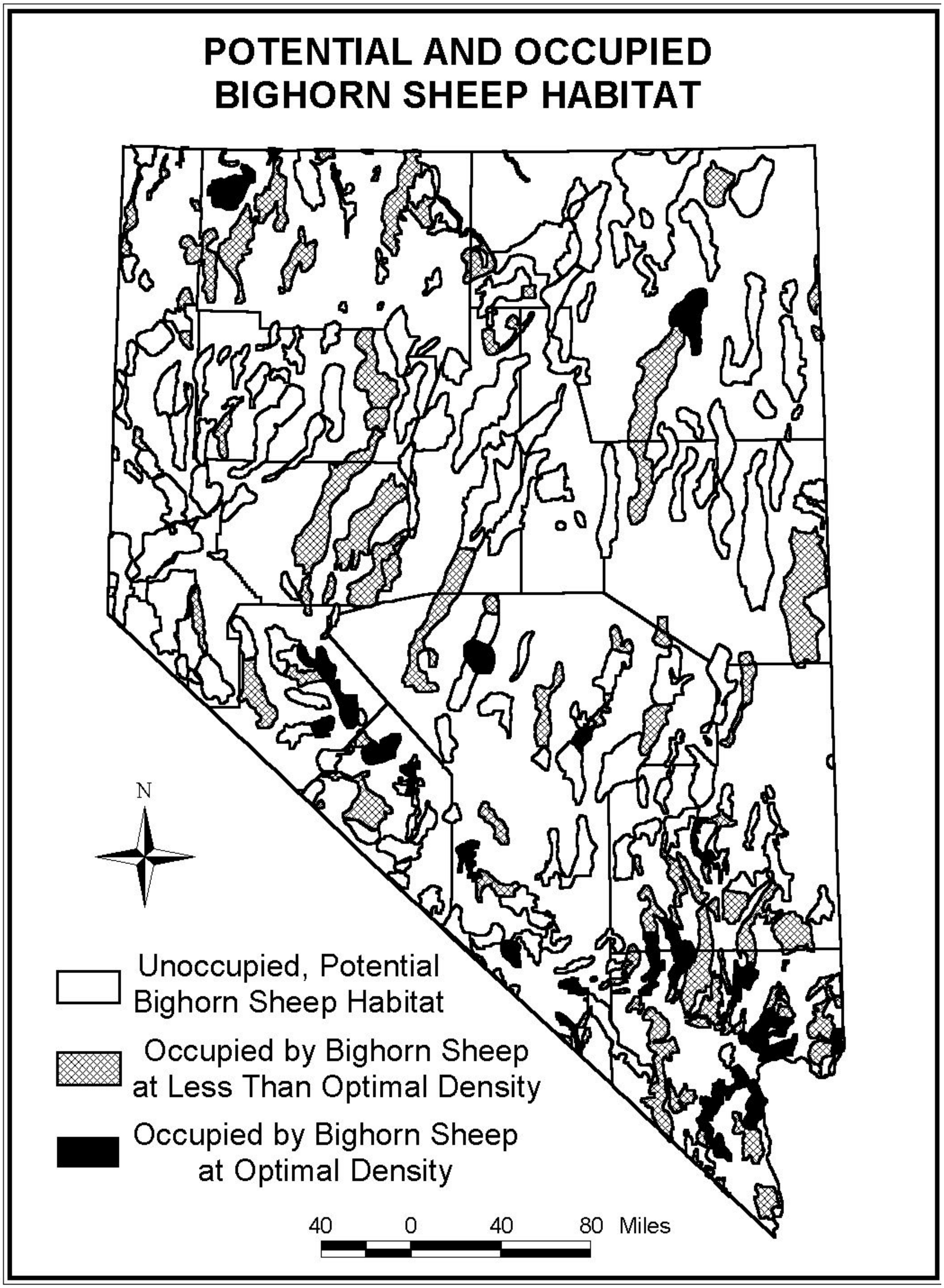

Figure 2. Occupied and unoccupied potential bighorn sheep habitat in Nevada as of 2001.

Strategy: The maps and information will be provided to the land management agencies for incorporation into land use planning documents, and will be used to help facilitate habitat protection and enhancement activities.

Habitat Acquisition

Thousands of acres of bighorn sheep habitat have been lost in recent years to urbanization in southern Nevada. Thousands of acres of bighorn sheep habitat have been traded from public ownership through land exchanges. None of these land exchanges have acquired additional bighorn habitat to compensate for this loss. In addition, human activity such as highways and reservoirs has fragmented huge expanses of historic bighorn sheep habitat.

Domestic sheep operations pose the largest obstacle to the further expansion of bighorn sheep populations in the State of Nevada due to continued concerns over disease transmission. For example, out of 12 mountain ranges identified in southern Nevada that contain suitable bighorn sheep habitat, but are currently unoccupied, 8 have domestic sheep associated with them. In the past, willing sellers have approached both the Division and conservation organizations with a desire to sell their domestic sheep grazing operations. However, no process has been established to evaluate these offers and therefore, opportunities to secure wildlife habitat for the long-term have been lost.

As directed by Commission Policy (P-62), it is imperative that the Division does everything possible to prevent the loss of habitat. In situations when the loss of habitat is inevitable, replacement or compensatory mitigation is a viable option. Habitat acquisition is one avenue that the Division will pursue to compensate for the loss of habitat. Habitat acquisition, through willing sellers, is also consistent with the Division's strategic plan

Management Action: The purchase of conservation easements, property and associated grazing privileges, conversions of Animal Unit Months (AUM's) from domestic sheep to cattle, or acquisition of water rights, will be pursued in order to protect or enhance important sheep habitat.

Strategy: Any AUM conversion, acquisition of private land, grazing privileges or easements will only be accomplished through a willing seller. The purchase of conservation easements and AUM conversions would be preferred over the purchase of property.

Strategy: The Division will develop guidelines and criteria in order to evaluate potential habitat acquisitions in a timely fashion.

Strategy: Potential funding sources and partners will be identified so that when opportunities do arise, they can be acted on in a timely fashion. Funding sources could include mitigation from urban sprawl (such as Southern Nevada Public Lands Management Act), conservation organization partnerships, heritage account, bond revenues and federal aid.

Strategy: The Commission's mitigation policy (P-62), will be used to direct Division activities associated with the potential loss of habitat and the associated mitigation alternatives including habitat acquisition.

Special Habitat Designation

The objective of special habitat designations would be to ensure that large blocks of existing high quality public habitat would be managed and protected, with an emphasis on bighorn sheep for the long-term. An example of an area that could be designated a special bighorn habitat area is the Arrow Canyon, Meadow Valley, Delamar, South Hiko and S. Pahroc Ranges. This is a large, continuous block of sheep habitat that is threatened by development. Maintaining not only the bighorn habitat, but also the migration corridors between these ranges, is essential to the long-term future of bighorn sheep in these areas.

Management Action: The Division will work with land management agencies and conservation organizations to designate critical bighorn sheep habitats with the goal of providing long-term protection to these areas.

Strategy: Through the use of GIS, evaluate potential threats to bighorn sheep habitat, and other biological and political/social issues to determine and prioritize areas suited for designation.

Strategy: Coordinate with land management agencies to determine what designation options would be best suited to protect large, continuous blocks of sheep habitat.

Strategy: Form partnerships with conservation organizations and land management agencies and actively pursue designations in top priority areas.

Movement Corridor Protection

Bighorn sheep movement can be categorized into two general types. The first is daily movement where bighorns move between watering areas, foraging areas and resting areas. These movements normally do not exceed more than a few miles in a day. The second is seasonal movements where bighorn move to other parts of a range or to other mountain ranges in response to changes in vegetation quality, water availability or weather. These movements can include several thousand feet in elevation and a 20- or 30-mile movement to another range. The impediment of either of these movements can be devastating to a bighorn sheep population.

Management Action: The Division will work to maintain bighorn sheep movement corridors.

Strategy: The GIS will be used to delineate important movement corridors. This information will be provided to land management agencies and the Department of Transportation.

Strategy: The Division will follow Commission Policy 62 (mitigation policy) when reviewing and commenting on movement impediments.

Strategy: The Division's first priority will be to minimize fences, roads, ditches and other movement impediments in bighorn sheep habitat. The Division will work with land management agencies and private landowners to consider alternatives to impediments, or to relocate the activity to an area with less impact to bighorn sheep.

Strategy: The Division realizes that some fences will be constructed within bighorn habitat. In these instances, the following fence specification should be used: A 39-inch high, three-strand fence with a smooth bottom wire. The wire spacing from ground up would be 20", 15" and 4" (BLM Handbook).

Strategy: Any roads built in bighorn sheep habitat or movement corridors must be constructed in such a way as to allow continued bighorn movement. Some strategies could include under or over passes, ramps cut into steep side slopes, alternatives to continuous guard rails and/or fence specifications along roads that allow sheep movement.

Water Development

Nevada is the driest state in the nation. The southern half of the state is extremely dry, especially in habitats capable of supporting bighorn sheep. To compound this problem, many of the natural water sources have been degraded or eliminated from a wildlife standpoint by human development, livestock use or have been eliminated by the pumping of the ground water for either agriculture or urban development.

The Division has evaluated dozens of Nevada's mountain ranges as to their suitability to support bighorn sheep. Many ranges have the topography and the vegetative resources to support bighorn sheep but lack adequate, available water. The protection and development of water is one of the management activities that can be used to expand both bighorn sheep distribution and population size.

Through December 2000, approximately 240 water developments had been constructed within bighorn sheep habitat. Not only are bighorn sheep dependent on these units, but a whole host of other wildlife species regularly use these waters. It is imperative that these existing developments be regularly maintained and kept in working order. In some years these developments do go dry.

Management Action: The Division will actively pursue a program to provide water for bighorn sheep as a means to increase population levels and distribution in water deficient habitats.

Strategy: The protection and development of natural water sources will be a high priority. The Division will work with other agencies to protect riparian areas. Conservation easements will be pursued in order to protect important water sources for wildlife. The acquisition of water rights will be pursued as identified in Commission Policy 61 (Water Rights) including the development of guidelines and procedures for water right filings.

Strategy: The Division will aggressively pursue protection of existing water developments against actions or activities that intend to remove or eliminate any water development that is used by bighorn sheep.

Strategy: The Division will pursue water developments in water deficient habitats to mitigate for habitat losses in other areas. Consideration must be given for multiple water sources in summer range to moderate impacts from failed water developments and focused predation. When determining water development sites, consideration should be given to provide for winter range or dry areas.

Strategy: The maintenance of existing water developments will be a high priority. A combination of approaches may need to be employed to ensure that all waters are maintained. Strategies could include the establishment of a permanent fund whereby the interest from the account would be used to fund a long-term annual maintenance program. Other approaches could include the use of conservation groups, volunteer labor, area biologists and agency fire crews.

Strategy: The Division will, where feasible, augment water in those water developments that are deficient in available water. Conservation groups, volunteer labor, area biologists and agency fire crews may be utilized.

Strategy: The Division will work cooperatively with federal land management agencies, conservation organizations and private landowners to develop adequate water distribution for bighorn sheep throughout the state.

Strategy: The Division will use the best development design for a given site in order to provide adequate water in the most cost efficient and maintenance-free manner. Other factors will be considered when designing developments such as the merits of using one large development in an area verses several smaller units.

Strategy: The Division in cooperation with land management agencies will use employees, private contractors, conservation organizations and volunteers for the installation of water developments in order to achieve water development objectives.

Grazing Input

Livestock, feral horses and feral burros are associated with most of the bighorn sheep habitat within the State. In many instances, livestock, horses, and burros compete directly with bighorns for forage, water, and space. It is important that bighorn sheep habitats are managed to ensure land use objectives are achieved and that habitats are maintained in good to excellent ecological condition.

Management Action: The Division will encourage and support land management decisions and resource management techniques that result in the attainment of good to excellent ecological condition on public and private rangelands.

Strategy: The Division will encourage and support the management of livestock when such management results in the attainment of land use goals and objectives consistent with wildlife needs. The Division should take appropriate action, including litigation, when these goals and objectives are not obtained.

Strategy: The Division will encourage and support the management of feral horses and burros when such management results in the attainment of land use goals and objectives consistent with wildlife needs. The Division should take appropriate action, including litigation, when these goals and objectives are not obtained.

Strategy: The Division will encourage and support sound monitoring procedures as the basis to determine the condition of ranges and to assess the amount of use by class of animal. The Division should take appropriate action, including litigation, when these goals and objectives are not obtained.

Strategy: The Division will provide comments or take other appropriate action through the land use planning process when poor range conditions exist and are in need of improvement for the benefit of wildlife including bighorns. The Division should take appropriate action, including litigation, when these goals and objectives are not obtained.

Fire

The effects of fire on bighorn sheep habitat vary depending on the vegetative community impacted. In some of the lower elevation sagebrush habitats, cheatgrass readily establishes after a fire and prohibits the reestablishment of native vegetation. In other areas, primarily dominated by pinyon and juniper trees, fires can be a major benefit to sheep habitat by increasing the productivity of the site through reduction in tree cover and increasing grasses and forbs.

Management Action: The Division will evaluate the effects of fire on bighorn sheep habitat on a case-by-case basis. In areas where fire is determined to be detrimental, the Division will work with land management agencies to reduce fire intensity and frequency. In areas where fire may benefit bighorn habitat, the Division will support the burning of some habitats when tiered to a plan which has definable objectives established through a collaborative process.

Strategy: The biologist will determine the effects of fire on the bighorn sheep resources and habitats within their areas of responsibility, and the information will be incorporated into GIS.

Strategy: The information will be provided to land management agencies to be used in fire suppression decisions. Areas of critical concern will be emphasized.

Strategy: In areas where fire will benefit bighorn habitat, the Division may support prescribed fire tiered to a burn plan.

Strategy: The Division will maintain a high level of interaction with land management agencies following wildfire in order to develop seed mixes to enhance bighorn forage and cover values. The Division will also encourage and support good grazing management practices following fire.

Strategy: The Division will work with the land management agencies to develop green-stripping in strategic locations in order to reduce the frequency and intensity of fires in crucial bighorn sheep habitat.

Roads, Off Road Vehicle Use

Off-road races will continue to increase throughout Nevada. Land management agencies field numerous requests for new races and route locations each year. Bighorn sheep habitat will be impacted both by the race participants and by the spectators to the event.

The development of new roads, improvement of existing roads, and use of all terrain vehicles (ATVs) will bring more people into bighorn sheep habitat. Often, bighorn sheep will move away from otherwise suitable habitat due to increased human activity.

Management Action: The Division will support the development and maintenance of reasonable access to all public lands. In areas where roads and off-road use pose serious impacts to the well being of bighorn sheep, the Division will work with land management agencies and private landowners to reduce these conflicts.

Strategy: The Division will monitor the proposed racecourses and will actively work with land management agencies and private landowners to locate races away from bighorn habitat. Bighorn habitat GIS maps will be distributed to various land management agencies in order to assist them in their decision making process. The Division should seek cooperator status with the BLM through a statewide MOU on review of applications for off-road races. Land management agencies should be encouraged to map existing roads designated for off-road races.

Strategy: The Division will maintain a high level of interaction with land management agencies regarding the building or maintenance of roads within bighorn sheep habitat. In areas where potential conflict exists, the Division may recommend alternative locations or recommend downgrading the quality of the road. The rehabilitation of roads used for fire suppression, off-road races or mining should be considered.

Strategy: The Division will continue to monitor impacts of ATV use on bighorn sheep habitat and bighorn behavior and ATV-related hunter complaints. If significant conflicts arise, the Division will work with appropriate land management agencies to address these conflicts.

Mining

Mining occurs in several mountain ranges occupied by bighorn sheep. Issues associated with mining include direct habitat loss, indirect habitat loss such as habitat fragmentation from roads, increased disturbances, potential contact with lethal chemicals such as cyanide, and animal entrapment.

The mining industry, for the most part, has demonstrated successful reclamation practices on dumps and roads. In some instances, opportunities may exist to rehabilitate a mine area in order to enhance the area for bighorn sheep. (In Alberta, Canada for example, bighorn sheep inhabit the high walls and the dumps of a coalmine where grass was used to rehabilitate the disturbances).

Management Action: The Division will continue working closely with the mining industry and land management agencies in regards to wildlife and wildlife habitat issues associated with mining activity.

Strategy: The Division will follow Commission Policy 62 (mitigation policy) when reviewing and commenting on mining activities within bighorn sheep habitat.

Strategy: The Division will continue to foster a good working relationship with the mining industry to mitigate the affects of mining on bighorn sheep habitat.

Strategy: The Division will, through its mining program, take a pro-active approach to ensure that needs of bighorn sheep are addressed in operation, mitigation and reclamation plans.

POPULATION MANAGEMENT

Population management involves surveying bighorn numbers and distribution, delineating subspecies distribution boundaries, capturing and transplanting bighorns, disease detection and control, and evaluating and controlling predators. The primary factor involved in the management of bighorns is ensuring the proper balance between bighorn numbers and habitat quality and quantity. The underlying goal of this plan is to maintain bighorn herds at optimal population levels. Division biologists will use habitat condition, lamb recruitment, herd health, and past herd history in determining optimal population levels. Though animal density is a common parameter in referencing the proper balance of numbers and habitat, it is highly variable for bighorn sheep throughout Nevada. Because of differences that occur among habitat types, season of use, subspecies, and water availability for a given amount of surface area, density alone is inadequate as a parameter to determine proper bighorn numbers. Optimal population levels based on a multitude of demographic and ecological parameters allows for bighorn numbers and distribution to be managed at the appropriate level for a given herd and area.

POLICY STATEMENT

The Division will increase bighorn populations of all subspecies statewide to a level where all habitats are occupied and each herd is self-sustaining.

Bighorn Sheep Capture and Transplanting

Reintroductions of bighorn sheep into unoccupied bighorn habitat will largely depend upon the resolution of current limitations and conflicts such as domestic sheep grazing and trailing routes, habitat deficiencies, and the revision of land management agencies' land use plans. The Division supports the release of bighorns from Nevada to bighorn sheep habitats beyond the boundaries of this state. This supports the overall goal of bighorn sheep restoration throughout North America. Conservation organizations, such as NBU, Fraternity, FNAWS, and others, play an extremely important role in the capture and transplant program. The Division will continue to foster excellent working relationships with these groups to increase bighorn sheep populations.

Reintroductions

Management Action: Establish bighorn sheep populations in suitable but unoccupied habitat.

Strategy: Select reintroduction sites as identified by biologists through the habitat delineation process (see Habitat Delineation section) that have been enhanced through Habitat Management actions and strategies.

Strategy: Evaluate the degree of risk involved with transplanting bighorn sheep adjacent to occupied domestic sheep grazing allotments and trailing routes. Consult with the land management agencies and concerned publics to determine the overall long-term implications of a bighorn release with consideration for other multiple uses and potential recreational and scientific values.

Strategy: Obtain release site clearance in coordination with the appropriate land management agencies. Conservation groups and outside interests may be solicited to help obtain clearance.

Strategy: Incorporate bighorn sheep reintroduction sites into the Big Game Release Plan. The intent of listing sites in the release plan is to provide an adequate number of optional sites for possible reintroductions at any one time.

Strategy: Coordinate at both the biologist and staff levels to annually prioritize reintroduction sites. In-state reintroductions will take priority over out-of-state releases.

Strategy: Biologists with predator management expertise will evaluate possible predation on bighorn sheep release. If it is determined that predation is a limiting factor, predator management will be instituted until the population shows an increasing annual trend. If predator control does not result in an increasing annual trend, then other limiting factors will be examined. Commission Policy 25, 'Wildlife Damage Management' will be followed.

Strategy: Coordination and notification with land management agencies and other interested parties will occur prior to a reintroduction.

Strategy: The preferred number for a release complement will be between 20 and 50 bighorn sheep dependent upon capture stock availability. Some sites may require subsequent reintroduction efforts to attain a viable reintroduction.

Augmentations

Management Action: Augment bighorn sheep populations to bolster populations that are below optimal levels and in some cases increase genetic diversity.

Strategy: Identify augmentations sites through the habitat delineation process (see Habitat Delineation section) where existing populations are below optimal levels or could benefit from increasing genetic diversity or improving herd health. See Reintroduction strategy regarding augmenting bighorn herds adjacent to occupied domestic sheep grazing allotments and trailing routes.

Strategy: Incorporate bighorn sheep augmentation sites into the Big Game Release Plan. The intent of listing sites in the release plan is to provide an adequate number of optional augmentation sites at any one time.

Strategy: Coordinate at both the biologist and staff levels to annually prioritize reintroduction sites. High priority in-state augmentations will take priority over out-of-state releases.

Strategy: Biologists with predator management expertise will evaluate possible predation on bighorn sheep release. If it is determined that predation is a limiting factor, predator management will be instituted until the population shows an increasing annual trend. If predator control does not result in an increasing annual trend, then other limiting factors will be examined. Commission Policy 25, 'Wildlife Damage Management' will be followed.

Strategy: Coordination and notification with land management agencies and other interested parties will occur prior to an augmentation.

Capture

Management Action: Capture bighorn to reintroduce into suitable habitat and augment existing populations.

Strategy: Annually determine suitable capture stock from both in-state and out-of-state sources. The big game staff biologist will facilitate and coordinate with regional biologists in securing out-of-state capture sources.

Strategy: The Division will use bighorn sheep from existing populations that are approaching or exceeding optimal levels. Bighorn sheep may be captured from populations that are below optimal levels if the herd has been surveyed within 12 months of the capture operation and the regional staff recommends that the population is capable of supporting the deficit.

Strategy: The Division will consider the potential of disease transmission from a particular capture stock to the release site and adjacent bighorn populations.

Strategy: The Division will consider potential capture problems such as bighorn lambing period and conflicts with ongoing hunting seasons.

Strategy: The Division will finalize a protocol that identifies recommend procedures for capturing, transporting and transplanting bighorns.

Population Monitoring

It is essential to maintain an effective monitoring program for bighorn populations that are relatively low in number and are subject to catastrophic events. Bighorn populations are highly sensitive to changes due to the harsh environments they inhabit. Without knowledge of population status and distribution, the Division is unable to make good sound management decisions regarding harvest, augmentations, habitat conservation and enhancement, and incompatible activities in bighorn habitat.

Management Action: Bighorn populations will be adequately monitored to assess trends and detect significant demographic changes and/or home range/movement changes.

Strategy: Aerially survey bighorn populations a minimum of every two years. Populations that serve as capture stock will be flown on an annual basis. Populations may be flown more often if downward trend exists. Bighorn rams will be classified as follows: yearlings, 2-3 year-old age, 4-5 year-old age, and 6 year-old and older age group.

Strategy: The Division will obtain the necessary Global Positioning System (GPS) and Geographic Information System (GIS) technology and equipment to enable the Division to efficiently collect, display and analyze data.

Strategy: Satellite and radio telemetry and GIS technology will be used when necessary to meet monitoring objectives.

Strategy: Biologist will document bighorn locations on standardized field forms. GPS technology will be the preferred method.

Strategy: The Division will institute hunter logbooks for all tagholders to maintain field observations during scouting and hunting trips. Volunteers may be used to conduct data entry and to plot bighorn sheep observations to assist in determining current bighorn distribution patterns and densities.

Strategy: Division biologists while surveying for a certain species or conducting a specific work assignment should take advantage of opportunities to survey and document bighorn sheep while in the same general area.

Strategy: Bighorn population modeling will be standardized and used to develop annual estimates of population size, structure, and trend.

Subspecies Delineation

Bighorn sheep subspecies boundaries in Nevada were originally based on analysis of skull characteristics by Cowan (1940). Recent genetic and morphometric analysis (Ramey 1993, 2000; Wehausen 2000) suggests that the desert bighorn was distributed throughout Nevada and California bighorns that originated in British Columbia are a branch of the Rocky Mountain subspecies. Based on past management action that released California and Rocky Mountain bighorns and the desires of sportsmen, the Division of Wildlife will continue to manage them, but certainly, a strong emphasis will be placed on expanding desert bighorn sheep distribution into currently unoccupied habitats.

California bighorns, now considered a race of Rocky Mountain bighorns, have adapted well to northern Nevada habitats and climate. California bighorn herds in Nevada from the year they were released to 2001 showed a remarkable 14% average annual rate of increase. This fact reveals that contrary to the historic genetic race of desert bighorns, the management decision to restore northern Nevada with California bighorns was a success, because of similar habitat and climate. Strong consideration was made to continue this management philosophy in north central Nevada to reintroduce bighorn sheep that are best suited for the habitat and climate. Based on the overall goal of desert bighorn sheep conservation throughout North America and recognizing their historic distribution, efforts will be made to expand desert bighorn distribution in Nevada, acknowledging previous subspecies management decisions and development of manmade barriers across once contiguous bighorn habitat.

The boundary delineation for future bighorn sheep releases is depicted in Figure 3.

Desert bighorn sheep releases will be restricted to south and west of a line formed by Interstate 80 from the California line to Elko, south along Highway 228/892 to Highway 50, east to Highway 93, south to the Lake Valley Summit and east to the Utah line along the Atlanta Mine/Trough Springs/Big Springs Roads. Rocky Mountain subspecies releases will occur north and east of this line including the line formed by Highway 225/226 north from Elko.

Though the Division acknowledges the scientific determination that California bighorns in Nevada are not a distinct subspecies, for purposes of management, the Division will continue to recognize existing California bighorn herds as a separate subspecies. California bighorns will be released north of the desert bighorn boundary and west of the Rocky Mountain bighorn boundary. The northeastern portion of the state in Elko County excluding Units 101 – 104 and 121 would be where either California or Rocky Mountain bighorns could be released depending on habitat suitability, sheep availability, or the political and social atmosphere at the time (see Figure 3).

It should be noted that this geographic delineation is for the purpose of future releases. Management units will still be used for the purpose of harvest management.

Management Action: The Division will follow the revised bighorn sheep subspecies delineation map as a guide in determining which areas receive which subspecies for future re-introductions and augmentations (Figure 3).

Strategy: The Division will reference the subspecies delineation map in the development of the biennial big game release plan.

Strategy: Desert bighorn herds from mountain ranges with similar topography, habitat, and climate will be the preferred capture stock for releases to mountain ranges in the northern half of the desert bighorn subspecies delineation area.

Strategy: Once an area has been established as a particular subspecies management unit, it will remain an area for that particular subspecies regardless of the amount of mixing that has occurred, unless compelling scientific information exists to the contrary.

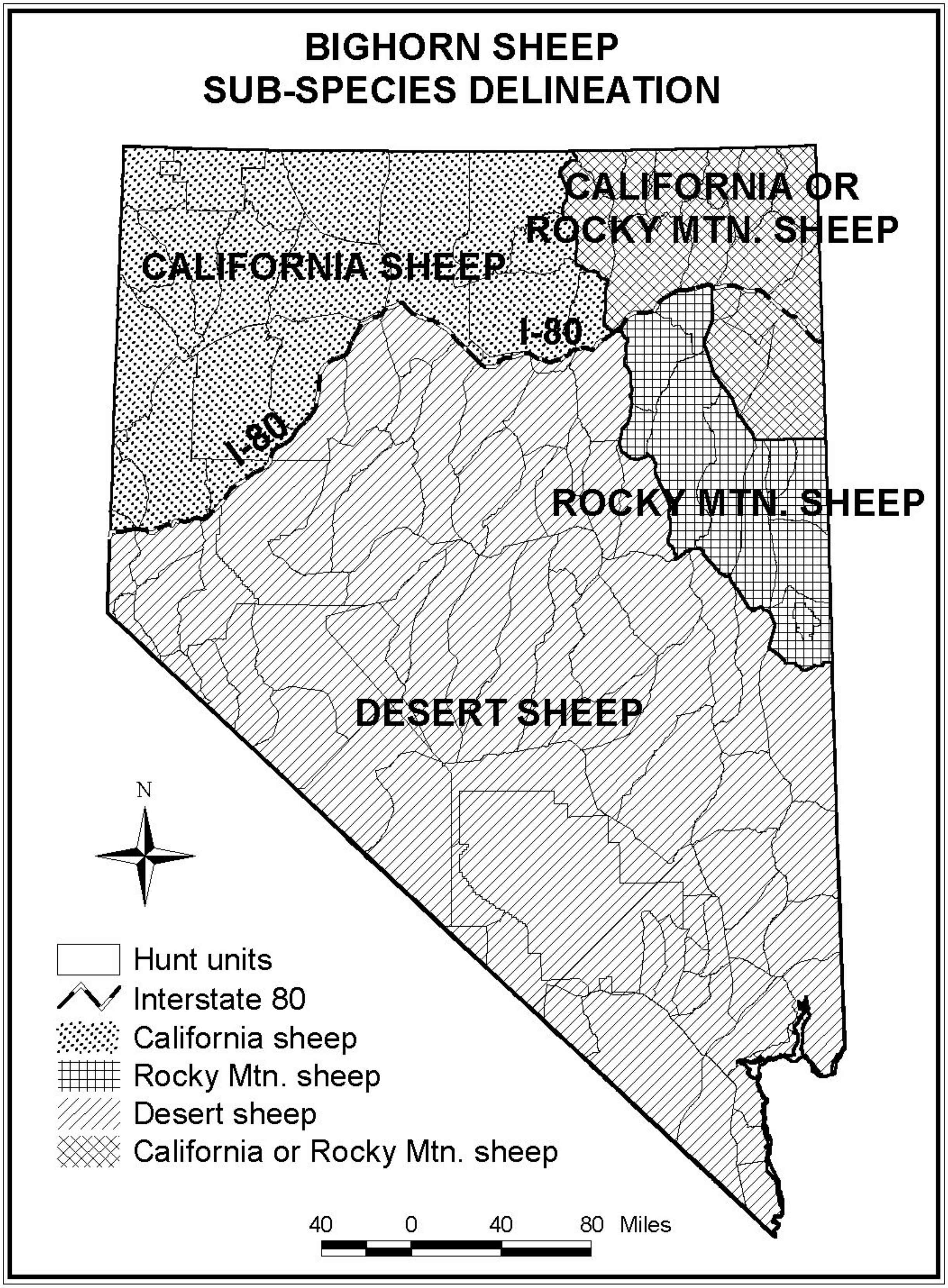

Figure 3. Bighorn sheep subspecies delineation boundaries for future transplants of desert, California, and Rocky Mountain bighorn sheep.

Disease

Bighorn sheep have been known to experience periodic epizootics resulting in wide fluctuations in population levels (Buechner 1960). Recently, these epizootics have been diagnosed as pneumonia-related epidemics (Onderka and Wishart 1984; Coggins 1988; Festa_Bianchet 1988; Cassirer et al. 1996; Ward et al. 1997). The Division recognizes the inherent susceptibility of bighorns to certain disease agents such as *Pasteurella*. Attempts to vaccinate bighorn sheep to combat this disease have been unsuccessful (Cassirer et al. 2001).

Management Action: The Division will investigate and address all disease related problems in a timely fashion.

Strategy: The Division will develop a protocol for disease sampling and testing and adapt it each year to incorporate the most up-to-date methods and information available.

Strategy: The Division will provide each bighorn sheep biologist in addition to each region, a sufficient number of sampling kits and instructional video in preparation of potential disease events.

Strategy: If an unusually high number of mortalities occur during a capture event and the consensus is that it may be disease related, any living bighorn already captured will not be transported to another site. One live sheep should be taken to a wildlife diagnostic laboratory for surveillance.

Strategy: Following the discovery of a disease event, either a ground or aerial survey will be initiated to investigate the potential impact to the rest of the population.

Strategy: The Bighorn Sheep Interaction With Domestic Sheep and Disease and Health Assessment protocols will be followed.

Strategy: The Division may initiate a disease prevention or health enhancement program for a particular population if the costs and benefits are justified.

Strategy: The Division will minimize domestic farm flock sheep/wild sheep interactions through all possible means. This could include entering into cooperative agreements with willing landowners, education, and cooperating with Department of Agriculture.

Strategy: The Division will encourage and support disease research when objectives are clearly outlined and results can be applied directly to management activities.

Predator Management

<u>Management Action:</u> The Division will evaluate and if necessary conduct science-based (treatment-control study design, monitoring and documentation of results) predator management to enhance survival of bighorn sheep.

Strategy: For existing herds, the Division will use criteria to determine if predator management should be initiated. Criteria include but are not limited to the following:

- Continued low recruitment or population trend (stagnant or below maintenance levels)
- Predator-caused bighorn sheep mortalities are located.
- Evidence suggests that a predator has targeted a certain segment of the bighorn herd.
- Hunter/Public observations
- Benefits of a predator control program can be measured and successfully implemented.
- Environmental conditions (i.e., reduction in alternative prey or water sources) that may cause added vulnerability to predation.

Strategy: The Division will monitor and document the effectiveness of predator management.

Strategy: Biologists will evaluate possible predation on bighorn sheep release. If it is determined that predation is a limiting factor, predator management will be instituted until the population shows an increasing annual trend. Commission Policy 25, 'Wildlife Damage Management' will be followed

Strategy: The Division will use the most appropriate and effective agency or individual to conduct predator management. (i.e., designated Division employee, Wildlife Services, private individual, etc.)

HARVEST MANAGEMENT

POLICY STATEMENT

Bighorn sheep hunting is a legitimate and desirable use of the bighorn resource.

Quota Criteria and Tag Requirements

Hunting bighorn sheep in Nevada is a rare privilege. The average odds of drawing a resident or nonresident tag for the 2001 sheep season were 68 to 1. The first regulated desert bighorn sheep hunting season was held in the spring of 1952. In 1966, a significant change in desert bighorn hunting regulations occurred with the passage of the trophy ram regulation. This regulation replaced the three-quarter-curl law and required hunters to harvest a ram at least 7 years of age or with a Nevada horn score of 144 points. In 1996, the trophy ram regulation was replaced on a statewide basis with the any ram regulation allowing hunters to harvest any male bighorn. The first California bighorn sheep hunting season was in 1984 and has been under the any ram regulation since its inception. Figure 4 shows that the average age of harvested rams has declined only slightly since the implementation of the any ram regulation but has averaged between 5 and 7 years of age. Therefore, it would seem a reasonable strategy for the Division to manage for an average age of harvested rams. With input from the public, this target age could be easily measured and met with adjustments in quotas and season structure.

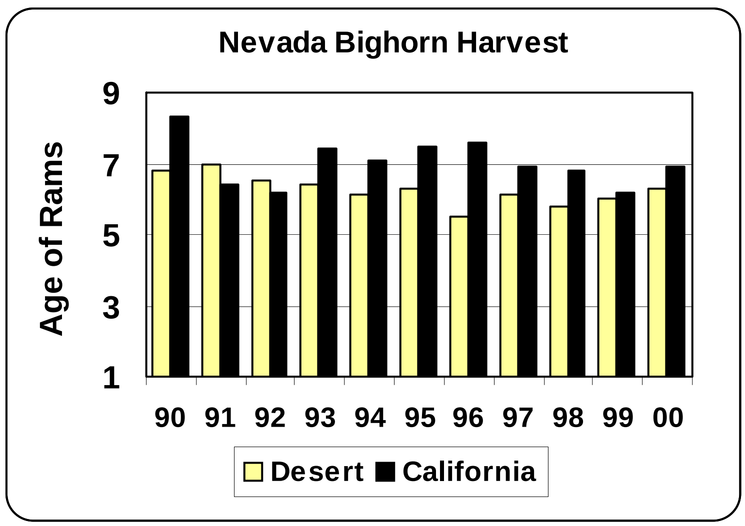

Figure 4. Average age of harvested desert and California bighorn sheep rams in Nevada from 1990 – 2000.

<u>Management Action</u>: Division biologists will develop annual quota recommendations for review by the public. The majority of Nevada's sheep hunters would like to have an opportunity to harvest a mature bighorn ram. Quota recommendations will reflect this expectation by striving to obtain a statewide average age of harvested rams of 6 years.

Strategy: Quota criteria for tag numbers will be based on 8% of the total rams not to exceed 50% of the estimated number of mature rams 6 years of age or older from each unit group's population model. Hunter success rates will not be used to generate quotas.

Strategy: Eligibility restrictions for applying for a bighorn sheep tag (subspecies specific) will be a 5-year wait after receiving a tag and 10-year wait after harvesting a bighorn sheep of that subspecies.

Strategy: Hunters must attend a mandatory indoctrination course provided by the Division as a requirement of receiving their tag. Guides must attend once every 5 years. Guides will be able to attend indoctrination for client.

Strategy: Maintain the any ram regulation.

Strategy: Maintain mandatory checkout of harvested sheep to estimate ram age and horn score.

Strategy: Bighorn sheep populations are susceptible to a large-scale die-off. The Division cannot be accountable to tagholders for this occurrence.

Strategy: Nonresident hunters will be allowed up to 10 percent of annual tag numbers. Distribution of these tags will be based on a fair and equitable cross section of bighorn hunting opportunity within the state.

Season Structure

Nevada is a large state diverse in both topography and weather patterns. Sheep seasons have been conducted during almost every month of the year, with the majority held during the late fall and early winter period. There has been considerable experimentation with season lengths, with the trend in recent years toward longer seasons. Lengths have varied since 1952 from a 4-day to 60-day seasons. With the success of bighorn reestablishment program in northern Nevada, season timing and lengths have become more diverse. A bighorn-hunting season designed for desert bighorn in the southern part of the state may be less desirable for bighorn hunting in the northern portion of the state.

Management Action:. Sheep seasons will remain flexible to take into account the biological needs of the animal and to allow for a quality hunting experience***.***

Strategy: Split seasons or extended seasons may be used to reduce the number of hunters in the field when hunter congestion becomes an issue.

Strategy: General seasons will not occur during the peak of the rut.

Strategy: Hunting seasons will not be structured to reduce hunter success.

Strategy: Season lengths will not be shorter than 21 days. Season length may be less in units controlled by Department of Defense.

Strategy: Any legal weapon will remain as a means of harvesting bighorn sheep during all seasons.

Strategy: The harvest of ewes may be considered as a population management tool if all other options for population control have been exhausted. Harvest and eligibility regulations for ewe hunts will be developed prior to 2003.

Strategy: The initial hunt on a reintroduced population or rebounding population will be based on survey observations of rams that meet the quota criteria.

LAW ENFORCEMENT

Bighorn sheep are a highly regarded and sought-after big game species. Within the big game hunting community, bighorn sheep have an additional, unique value associated with a hunter's recognition for harvesting a "grand slam". A "grand slam" refers to harvesting all races of North American thin-horn and bighorn sheep: Dall, Stone, Rocky Mountain (including California), and Desert. There is a need to protect them from a small segment of society that will go to extremes to harvest a bighorn sheep.

In addition, the desert bighorn holds the distinction of being Nevada's state animal. Whether for the protection of bighorns for future harvest or simply for their intrinsic values, the Nevada Division of Wildlife has the responsibility to protect bighorn sheep for all to enjoy.

Management Action: The Division will continue to protect and ensure enhancement of bighorn sheep populations by gaining awareness and compliance of the public through education and appropriate enforcement of pertinent wildlife laws and regulations.

Strategy: Game wardens will participate in bighorn sheep indoctrination classes for the purposes of promoting the safe and lawful pursuit of bighorns and enhancing the sportsmen's knowledge of pertinent hunting laws and regulations.

Strategy: Conduct special investigations whenever sufficient grounds or evidence exists which indicates that a bighorn sheep has been unlawfully taken or possessed.

Strategy: Conduct frequent field patrols during bighorn sheep hunting seasons, thereby increasing contact with bighorn sheep hunters and hunting guides.

Strategy: Conduct frequent field patrols in areas where bighorn are particularly vulnerable to opportunistic poaching.

ECONOMICS

Hunter Expenditures

The Nevada Division published a “Survey of the Economic Value of Trophy Big Game and Deer Harvest” in 1986, which is the only known attempt at assigning dollar values to Nevada’s bighorn sheep resource (Fenton Kay 1988). This study queried sheep hunters about the amount of money they spent on their sheep hunts during 1984 and 1985. Costs included in this survey were guide fees, license and tag fees, fuel, equipment, lodging, food, taxidermy and miscellaneous costs such as phone calls and broken equipment. The current consumer price index was used to convert dollar values from 1986 to 2000. Based on this study and the current average days hunted, it was assumed that a total of 11 days were expended on travel, scouting, and hunting bighorn sheep. Based on these inputs, resident and nonresident hunters expended an average of $2,924 and $10,077 per hunt, respectively in 2000. Expanding these figures to all the 2000 bighorn sheep hunters, 159 resident hunters expended $465,000 and 20 nonresident hunters expended $201,000 for a total of $666,000

A complete evaluation of the economic values of bighorn should also include consideration of nonconsumptive values. Nonconsumptive values would include the value of the resource to the non-hunting public. These values could include just knowing the resource existed even if the person had no expectation of using the resource and knowing the resource will exist into the future. No data exists to estimate these values. The dollar value of bighorn sheep to the nonconsumptive users of the state of Nevada may be higher than that of the hunting public.

Division Revenue

Division revenue to manage bighorn sheep is derived from a number of sources. These sources include tag and license revenue, federal aid derived from the Pittman and Robertson or Wildlife Restoration Act (Congressional mandate that apportions proceeds of an excise tax on firearm and ammunition to each state wildlife agency) and funding from sportsmen and conservation groups.

Figure 5 displays funds generated from resident and nonresident tags, heritage tags, and the potential federal aid match for the last 20 years. Since the first sheep season in 1952, sheep hunters have spent $2,232,332 on tag fees to hunt bighorn in Nevada. Bighorn sheep heritage tag hunters have contributed the lion’s share of this figure spending $1,730,202 for the privilege of pursuing bighorn (Figure 5). A new program named Partnership In Wildlife (PIW) allows hunters to donate part of their tag fee for a second chance at drawing a sheep tag if unsuccessful in the first drawing. Since 1996 this program has generated $108,151 that has been deposited into the

Heritage Account to fund special projects.

Sportsmen and conservation groups have contributed a significant amount of funds to bighorn sheep management in Nevada. For example, through 2000, FNAWS has donated $144,000, and the Fraternity of the Desert Bighorn has donated $1,200,000 since 1984 to the Division and to land management agencies for bighorn sheep population and habitat management. Other organizations such as the Nevada Bighorns Unlimited chapters have also contributed a significant amount toward bighorn sheep management.

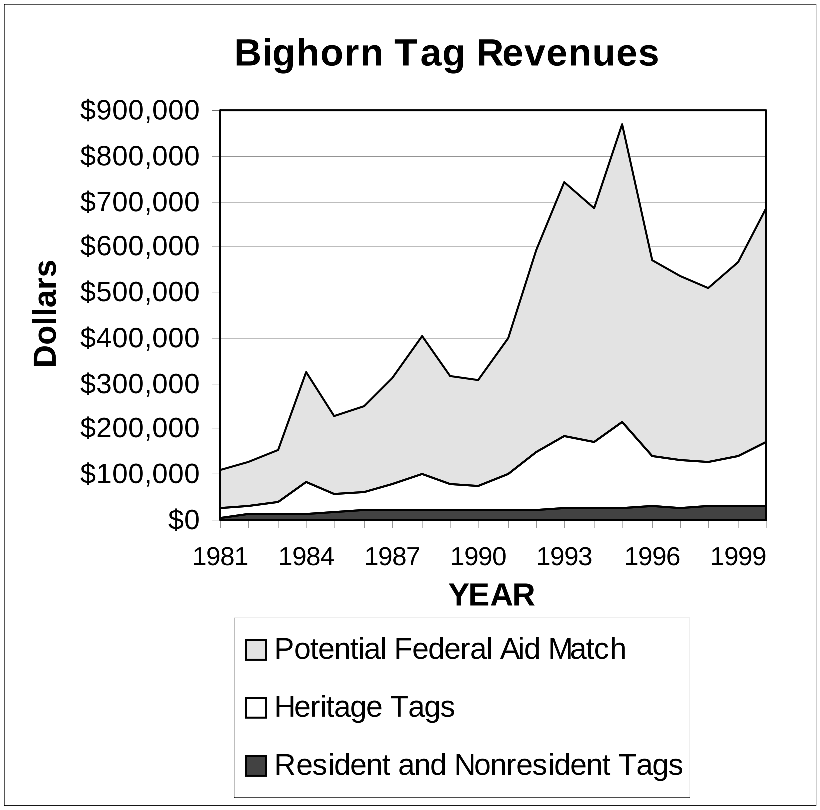

Figure 5. Nevada bighorn sheep tag sales revenue and its potential federal aid match from 1981 – 2000.

In addition to the monetary contributions, these organizations have also donated endless number of volunteer hours during habitat improvement and capture projects. The Fraternity of the Desert Bighorn has estimated their members to work 52,000 hours worth $800,000.

Division Expenditures

The expenditure of money by the Division to manage bighorn sheep includes salaries for personnel, flight charges for aerial composition surveys and telemetry work on newly introduced populations and operating costs including travel and mileage. Table 2 shows these costs by region for fiscal year 2000.

Table 1. Annual Division bighorn sheep management expenditures for FY2000.

Region	Salaries	Flight Charges	Operating – Travel- Mileage	Total Cost
Western	$45,931	$1,978	$3,128	$51,037
Eastern	$9,097	$1,617	$676	$11,390
Southern	$53,616	$23,244	$2,895	$79,755
Capture Costs*				$35,797
Total Cost	**$108,644**	**$26,839**	**$6,695**	**$177,979**

*Includes netgun company and veterinarian contract costs only.

Trapping and Transplanting Costs

Since the late 1960's, a total of 1,293 Desert Bighorn, 587 California Bighorn and 265 Rocky Mountain Bighorn have been released into 58 different mountain ranges within the state. Based on the best available records the Division has expended just over $930,000 dollars on this program. This cost can be broken down by subspecies totaling $520,000 for Desert bighorn, $288,000 for California bighorn and $124,000 for Rocky Mountain bighorn. This program has been a huge success in terms of both public support and the establishment of new and viable sheep populations.

CONSERVATION EDUCATION

The desert bighorn sheep is Nevada's state animal; yet, the general public has very little knowledge about bighorn sheep. The hunting public has more knowledge about bighorn sheep but lacks an understanding of the threats to bighorn sheep habitat.

Most sportsmen do not know the process for involvement in population and habitat management decisions. Support for bighorn sheep is lacking in significant decisions affecting bighorn sheep habitat. It is believed that an increased awareness and educational program could enhance the support for bighorn sheep in land management, legislative, and local government decisions.

POLICY STATEMENT

The Division will increase public awareness and appreciation for bighorn sheep and their habitats in order to facilitate decisions favorable to their long-term well being.

Educating Nevada's Youth

Nevada's youth is the key to the future well being of the State's wildlife. Extensive efforts are already being implemented in many of Nevada's schools to educate students in basic ecological principals. The Division, in conjunction with conservation organizations, should provide support materials for this program that will enhance the understanding and appreciation of bighorn sheep and their habitat. An effort should also be made to teach kids the role that sportsmen play in the conservation of Nevada's wildlife. The conservation of bighorn sheep habitat is the most important element of this public awareness program

Management Action: The Division will continue to support wildlife education in the school system and will provide material that will teach kids about bighorn sheep and their habitat.

Strategy: Develop a compact disc (CD) program about bighorn sheep and their habitat to be used in schools similar to the BLM produced program "The Magnificent Ram".

Strategy: Build portable boxes or "wildlife trunks" that contain bighorn and other wildlife furs, horns and hoofs to be used in schools and other youth group events for hands-on interactions. Eventually, every community would have one of these boxes.

Strategy: Develop a video/CD that tells the story of bighorn sheep extirpation from Nevada's mountain ranges and the efforts of sportsmen and Division to bring them back.

Strategy: Encourage sportsmen groups to provide educational materials (books, brochures, posters, etc.) to youth and schools.

Educating the General Public

Nevada's general public, for the most part, is indifferent towards Nevada's wildlife. It is believed that a major contributor towards this attitude is the lack of a consistent medium needed to bring wildlife issues to the forefront of the public. A combination of strategies will need to be implemented over a long period of time in order to bring greater awareness to Nevada's wildlife. The conservation of bighorn sheep habitat is the most important element of this public awareness program.

Management Action: Continue to use all of the means available to educate the general public on issues pertaining to bighorn sheep and other wildlife.

Strategy: Support and participate, where appropriate, with conservation organizations in habitat improvement projects that are within view of the general public.

Strategy: Construct kiosks with interpretive materials along roadsides adjacent to bighorn sheep habitat and bighorn sheep viewing opportunities.

Strategy: Develop additional bighorn sheep dioramas and interpretive displays in public facilities such as airports.

Strategy: Encourage sportsmen groups to advertise in newspapers and other media to portray bighorn sheep conservation efforts and solicit involvement in such efforts.

Strategy: Conduct “ride alongs” with influential individuals during aerial surveys to gain support of the bighorn sheep conservation efforts.

Strategy: Pursue Department of Tourism for sponsoring advertisements and stories about bighorn sheep viewing and conservation.

Educating Hunters

Educating hunters on issues relating to wildlife is probably the easiest because we have mediums that consistently reach them. These sources include the Sportsmen Almanac, the Division’s web page and hunter indoctrinations. Unfortunately, very few hunters realize the importance of habitat and even fewer get directly involved in the decision-making processes that impact wildlife and habitat.

Management Action: Continue to use all available sources to educate hunters on issues relating to bighorn sheep. Emphasis should be placed on the importance of habitat and the decision-making processes that affect bighorn sheep and their habitat.

Strategy: Develop a video of bighorn sheep (ecology and conservation) to be used in the hunter indoctrination classes. This video could be produced in such a way as to be used in schools and civic presentations.

Strategy: Update and improve the “Hunting the Desert Bighorn Sheep” pamphlet. Funding for this could include conservation organization partnerships or advertisements.

Strategy: Have the bighorn sheep conservation groups sponsor articles in the Almanac and other Division publications dedicated to bighorn sheep and their habitat.

PLAN EVALUATION

Original team members will meet August 2004 to evaluate the plan’s implementation. A written report will be developed and presented to the Commission.

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Appendix A
Laws and Regulations pertinent to Bighorn Sheep Management

Appropriate Federal Laws, Policies and Agreements Pertinent to Bighorn Sheep Management in Nevada

Taylor Grazing Act, 1934. As amended, provides for wildlife management on public lands.

Executive Order 7373. 1936. Created the Desert National Wildlife Range for the protection of resident desert bighorn sheep.

50 CFR . Code of Federal Regulations pertaining to wildlife

Fish and Wildlife Coordination Act of 1956. Encourages the development of cooperative agreements for a variety of fish and wildlife programs on Federal lands.

National Wildlife Refuge System Administration Act. 1966.

National Environmental Policy Act, 1968 (1981). - 42 U.S.C. 4321-4347. Requires that actions taken or permitted by Federal agencies be analyzed to determine their effects on the environment.

Master Memorandum of Understanding Between the Nevada Department of Fish and Game and the Bureau of Sport Fisheries and Wildlife, Department of the Interior, 1970.

Master Memorandum of Understanding Between the Nevada Department of Fish and Game and the Bureau of Land Management, Department of the Interior, 1970.

Master Memorandum of Understanding Between the Nevada Department of Fish and Game and the National Park Service, Department of the Interior, 1971.

Wild Free-Roaming Horse and Burro Act, 1971. Sec.3.(a) "... All management activities shall be at the minimal feasible level and shall be carried out in consultation with the wildlife agency of the State wherein such lands are located to protect the natural ecological balance of all wildlife species which inhabit such lands, particularly endangered wildlife species. Any adjustments in forage allocations on any such lands shall take into consideration the needs of other wildlife species which inhabit such lands. ...." and (b) in determining the number of horses and burros on the public lands and appropriate management levels ...”the Secretary shall consult with the United States Fish and Wildlife Service, wildlife agencies of the State or States wherein wild free-roaming horses and burros are located ....”

Endangered Species Act, 1973.

Sikes Act, 1974. “Section 201. (a) The Secretary of the Interior and the Secretary of Agriculture shall each, in cooperation with the State agencies and in accordance with comprehensive plans developed pursuant to section 202 of this title, plan, develop, maintain, and coordinate programs for the conservation and rehabilitation of wildlife, fish, and game. ...”

Master Memorandum of Understanding Between the Nevada Department of Fish and Game and the U.S. Department of the Agriculture, Forest Service, Region 4, 1971.

Federal Land Policy and Management Act, 1976 - Sec.102. (a) “The Congress declares that it is the policy of the United States that .... (8) the public lands be managed in a manner that will protect the quality of scientific, scenic, historical, ecological, environmental, air and atmospheric, water resource, and archeological values; that, where appropriate, will preserve and protect certain lands in their natural condition; that will provide food and habitat for fish and wildlife and domestic animals;.... “, “(11) regulations and plans for the protection of public land areas of critical environmental concern be promptly developed; . . . .”,

Sec. 103. (j) “The term “withdrawal” means withholding an area of Federal land from settlement, sale, location, or entry, under some or all of the general land laws, for the purpose of limiting activities under those laws in order to maintain other public values in the area or reserving the area for a particular public purpose or program;

Five Party Cooperative Agreement. 1977. U.S. Department of Defense (Air Force), U.S. Department of Energy (Nevada Test Site), U.S. Department of the Interior (Fish and Wildlife Service and Bureau of Land Management) and Nevada Department of Fish and Game. Provides for cooperative management of the Nellis Air Force Range and the Nevada Test Site.

Public Rangelands Improvement Act, 1978. Directs that the condition of the public rangelands be improved so that they become as productive as feasible for wildlife habitat and other rangeland values. The Act provides for on-the-ground funding of wildlife habitat protection, improvement and maintenance projects.

The Fish and Wildlife Conservation Act of 1980.

43 CFR 24.3. General jurisdictional principles. “(a) In general the States possess broad trustee and police powers over fish and wildlife within their borders.....” (b) “.... Congress has, in fact, reaffirmed the basic responsibility and authority of the States to manage fish and resident wildlife on Federal lands."

43 CFR 1610.3-1 Coordination of planning efforts. "(b) State Directors and District Managers shall provide other Federal agencies, State and local governments, and Indian tribes opportunity for review, advise and suggestion on issues and topics which may affect or influence other agency or other government programs."

Rangewide Plan for Managing Habitat of Desert Bighorn Sheep on Public Lands. 1988.
BLM plan.

Grazing Guidelines for Management of Domestic Sheep in Bighorn Sheep Habitats. 1992. Revised 1998.
Recognizes the need for spatial separation of domestic sheep and bighorns, and continued cooperation between all affected interests and agencies.

Mountain Sheep Ecosystem Management Strategy in the 11 Western States and Alaska. 1995. BLM Plan.

Nevada Revised Statutes Pertinent to Bighorn Sheep Management

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1952. Commission authorizes first hunt.

Nevada Legislature designates desert bighorn sheep as official state animal. 1973.

NRS 501.182. The Commission may enter into cooperative agreements with adjacent states for the management of interstate wildlife populations......

NRS 503.584. "1. The legislature finds that: (a) The economic growth of the State of Nevada has been attended with some serious and unfortunate consequences. Nevada has experienced the extermination or extirpation of some of her native species . . . . .
2. The purpose of NRS 503.584 to 503.589, inclusive, is to provide a program for the: (a) Conservation, protection, restoration and propagation of selected species of native fish and other vertebrate wildlife, including migratory birds; and (b) Perpetuation of the populations and habitats of such species."

NRS 503.587. "The commission shall use its authority to manage land to carry out a program for conserving, protecting, restoring and propagating selected species of native fish, wildlife and other vertebrates and their habitats which are threatened with extinction and destruction."

NRS 533.023. As used in this chapter, "wildlife purposes" includes the watering of wildlife and the establishment and maintenance of wetlands, fisheries and other wildlife habitats.

NRS 533.367. Before a person may obtain a right to the use of water from a spring or water that has seeped to the surface of the ground, he must ensure that wildlife which customarily uses the water will have access to it. The state engineer may waive this requirement for a domestic use of water.

Nevada Administrative Code Pertinent To Bighorn Sheep Management

Season dates set under the authority of sections 501.181, 502.140, 502.250, 503.120 and 503.140 of NRS. Includes indoctrination requirements, Wildlife Heritage tags and Partners in Wildlife tags.

NAC 502.403.

NAC 503.020. Game mammals.

9.	Sheep	Bighorn.........................	*Ovis canadensis canadensis*
			Ovis canadensis nelsoni
			Ovis canadensis californiana

NAC 503.094. Scientific permit for collection or shipping of wildlife: Application; contents; term or permit; reporting requirement; conditions and restrictions.
NAC 503.101. Factors for classification of wildlife as game.

NAC 503.110. Restrictions on importation, transportation and possession of certain species.
1. Except as otherwise provided in this section and NAC 504.486, the importation, transportation or possession of the following species of live wildlife or hybrids thereof, including viable embryos or gametes, is prohibited:
(d) Mammals

(30)	Barbary (Aoudad) Sheep..................................	*Ammotragus lervia*
(31)	Mouflon sheep, Urial, Bighorn and Argali…….	All species of the genus Ovis, except domestic sheep, Ovis aries.

NAC 503.173. Cape and horns or antlers or wildlife must be maintained with carcass.

Commission Policies Pertinent To Bighorn Sheep Management

Commission Policy Number 22. Establishes direction for the introduction, transplant, release and re-establishment of fish and wildlife into the State and exportation of the same out of the State as guided by NRS 501.181.

Commission Policy Number 25. To inform the public and guide the Division in actions relating to mammalian predator management.

Commission Policy Number 60. Water application guidelines.

Commission Policy Number 61. Guides the Division in securing water for the preservation, maintenance and enhancement of wildlife and their habitats.

Commission Policy Number 62. Guides the Division in mitigation activities which have the potential to adversely impact fish and wildlife resources in Nevada.

Department of Agriculture Regulations on Lost Or Trespass Domestic Sheep And Goats

Definitions: "Estray" means any livestock running at large upon public or private lands in the State of Nevada, whose owner is unknown in the section where the animal is found. *(NRS 569.005)*
"Livestock" means: (d) All goats or animals of the caprine species; (e) All sheep or animals of the ovine species;... *(NRS 569.005)*

All estrays are the property of the Department of Agriculture (NRS 569.010).
NDA is not responsible for any trespass or damage caused by those estrays.

A written notice must immediately be sent to NDA by . . . any individual who impounds any livestock (NRS 569.020).

NDA or its authorized agent (usually the brand inspector) will attempt to determine ownership by following NRS 569.060-.070.

. . . NDA may dispose of the estray (usually through sale to defer expenses incurred (NRS 569.080).

NDA may destroy livestock infected with or exposed to disease: Procedure; owner's compensation (NRS 571.190)

43 CFR (BLM)

SUBCHAPTER B - LAND RESOURCE MANAGEMENT (2000)

Group 2000–Land Resource Management; General

PART 2070–DESIGNATION OF AREAS AND SITES

Subpart 2070–Designation of Areas and Sites

S 2070.0-1 Purpose.
This subpart defines the circumstances and procedures under which specific areas of public and other Federal lands exclusively administered by the Secretary of the

Interior through the Bureau of Land Management may be designated and identified.

S 2070.0-3 Authority.

(a) Section 1 (b) (1) of the Classification and Multiple Use Act of September 19, 1964 (78 Stat. 986, 43 U.S.C. 1411)

(b) Section 2478 of the Revised Statute (43 U.S.C. 1201)

Subpart 2071–Type and Effect of Designations

S 2071.1 Areas or sites that may be designated.

(a) No lands may be designated under the regulations in this subpart unless they are either (1) classified for retention for multiple uses management under the regulations and criteria in Group 2400 of this chapter, or (2) withdrawn or reserved under the regulations in Group 2300 of this chapter o r other appropriate authority, or (3) given special status by act of Congress ....

(b) The following types of areas and sites may be designated under the regulations in this subpart:

(1) *Recreation lands*. . . . .Scenic areas of natural beauty . . .

Recreation lands will contain one or more of the six classes adopted by the Bureau of Outdoor Recreation. . . .

(i) Class I - High density recreation areas: ....
(ii) Class II - General outdoor recreation areas: ....
(iii) Class III - Natural environment areas: . . . .
(iv) Class IV - Outstanding natural areas: . . . .
(v) Class V - Primitive areas: . . . . .
(vi) Class VI - Historic and cultural sites: ....

(2) *Recreation sites*. Small tracts, intensive recreation, facilities.

(3) *Resource conservation areas*. These are relatively small areas of land which include a variety of resource management activities demonstrating multiple use and sustained yield conservation action.

(4) *Natural resources experiment and research areas*. These are relatively small areas of land which are used for research and experimental purposes.

(5) *National resource lands.* Large areas, multiple use management, emphasis on products (minerals, timber, etc.)

Isolation and Serologic Evidence of a Respiratory Syncytial Virus in Bighorn Sheep from Colorado

Author(s): Terry R. Spraker and James K. CollinsWilliam J. Adrian and James H. Otterman
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Earle's salt base supplemented with 10% heat-inactivated FBS. Gentamicin was added to the medium at a final concentration at 50 μg/ml. The microtiter plates were incubated at 37 C for 5 days. Antibody titers were expressed as the highest dilution of serum that prevented 50% RSV cytopathogenic effect.

Neutralizing antibodies to RSV were detected in 29 (42%) of the 69 mountain goats, including kids (25%), yearlings (28%), 2–4-yr-olds (43%), 5–7-yr-olds (75%), and 8–10-yr-olds (25%). Fifty-six percent of the males and 35% of the females were seropositive for RSV. Antibody titers ranged from 1:5 to 1:20 (median = 1:5). This is the first report on the occurrence of RSV antibodies in mountain goats and indicates enzootic transmission in the population. The importance of RSV infection in the epizootiology of respiratory disease in mountain goats is unknown.

Journal of Wildlife Diseases, 22(3), 1986, pp. 416–418

Isolation and Serologic Evidence of a Respiratory Syncytial Virus in Bighorn Sheep from Colorado

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In December 1984, personnel of the Colorado Division of Wildlife began baiting Rocky Mountain bighorn sheep (*Ovis c. canadensis* Shaw) near Ouray, Colorado in order to treat them for *Protostrongylus stilesi* Dikmans. When the sheep began to visit the bait station, it was observed that approximately 50% of the herd were coughing and about 20% had a nasal discharge. Since a bighorn lamb had been found dead on the bait station the previous week, it was decided to collect and necropsy sick animals from this herd in order to investigate this possible respiratory problem.

On 4 January 1985, two clinically ill sheep exhibiting signs of coughing, slightly dull rough hair coat, and nasal discharge were collected and necropsied. One animal was an adult ewe and the other animal was an 8-mo-old ewe lamb. Gross necropsy findings were similar in both animals and included a moderate suppurative rhinitis/tracheitis and subacute suppurative bronchopneumonia. Approximately 5% of lung parenchyma was consolidated in both animals. The thymus was totally atrophied in the lamb. Gross lesions of the respiratory system were similar to those in bighorn sheep with early cases of bronchopneumonia observed during previous die-offs in Colorado (Spraker et al., 1984, J. Wildl. Dis. 20: 319–327). Tissue samples from posterior nasal septum lymphoid tissue, trachea, consolidated and normal lung parenchyma, and lungworm nodules were placed in viral transport media and transported on ice to the Diagnostic Laboratory, Colorado State University, Fort Collins, Colorado. These tissues were also cultured for bacteria.

A respiratory syncytial virus (RSV) was isolated from posterior nasal septum lymphoid tissue, trachea, and a lungworm nodule from the 8-mo-old lamb. The virus was identified by induction of characteristic syncytial cytopathic effect in fetal

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TABLE 1. Results of serological testing for bovine respiratory syncytial virus and parainfluenza type-3 virus in bighorn sheep from Ouray, Colorado during the winters of 1983 and 1985.

	1983			1985		
Virus	No. sheep tested	Antibody titer	No. positive	No. sheep tested	Antibody titer	No. positive
BRSV[a]	16	1:8[c]	4 (25%)	40	1:8[c]	1 (2%)
PI-3[b]	16	1:8[d]	7 (44%)	40	1:8[c]	9 (23%)
		1:16	7 (44%)		1:16	10 (25%)
		1:32	2 (12%)			

[a] Bovine respiratory syncytial virus.
[b] Parainfluenza type-3 virus.
[c] Neutralizing antibody titer against BRSV.
[d] Hemagglutination-inhibition antibody against PI-3.

lamb lung cell cultures and by fluorescent antibody (FA) testing on infected cells with a FA reagent specific for bovine respiratory syncytial virus (BRSV) (supplied by Dr. Merwin Frey, Virus Research Laboratories, University of Nebraska, Lincoln, Nebraska 68583, USA). Cytopathic effect developed within 3–5 days after inoculation of the specimens. No virus was isolated from the adult ewe. Routine FA tests were negative for both BRSV and parainfluenza type-3 (PI-3) virus on lung tissues from both animals. Sera from these two sheep were checked for antibody titers to PI-3 virus using a hemagglutination inhibition (HI) test, to infectious rhinotracheitis virus (IBRV) using the serum neutralization test, and to bluetongue virus (BTV) and ovine progressive pneumonia virus (OPPV) using the agar immunodiffusion test. Reagents were obtained from the National Veterinary Services Laboratories, Ames, Iowa 50010, USA. The adult ewe had a titer of 1:8 to BRSV and 1:32 to PI-3 virus. Antibody titers of <1:8 for BRSV and 1:16 for PI-3 virus were found in the lamb. All other serological tests were negative. *Pasteurella haemolytica* biotype T was isolated from nasal cavities, tonsils, and lungs of both sheep.

During the last week of January 1985, the Ouray herd was trapped with a drop net (Schmidt et al., 1978, Wildl. Soc. Bull. 6: 159–163) and blood samples were collected from the sheep for serology and nasal swabs were taken for bacterial culture. Sera had been collected previously from this herd in February 1983, and at that time the herd appeared to be healthy. Sera collected during the winters of 1983 and 1985 were tested for antibody to BRSV using the serum neutralization (SN) test (reagents obtained from Dr. Merwin Frey) and PI-3 virus using the HI test. Results of the serological survey for these two viruses demonstrated higher prevalences of antibodies to both BRSV and PI-3 virus in 1983 when compared to 1985 (Table 1). *Pasteurella haemolytica* biotype T was isolated from nasal swabs from 17 of 40 animals during the trapping of January 1985.

Results of this investigation document the presence of a bighorn sheep respiratory syncytial virus within the Ouray herd. The serological results can be interpreted in at least two ways. First, it is evident from the prevalences that viral activity was higher for BRSV and PI-3 virus in the winter of 1983 than during the winter of 1985. Since more of the observed animals were sick during the winter of 1985 than in 1983, the seropositives could suggest that these viruses did not play a role in the pathogenesis of the respiratory problem in 1985. Alternatively, the antibody titers may have decreased and animals may have lost detectable antibody due to natural decline or to chronic stress occurring during the last several years. The sheep could have then become susceptible to infections with these agents, predisposing them to bacterial (*Pasteurella*) pneumonia. The exact role of this respiratory syncytial virus or of PI-3 virus in the pathogenesis of illness of sheep of the Ouray herd could not be elucidated, but further serologic testing should help to clarify their roles.

Viruses were first implicated as being a possible predisposing factor to bacterial pneumonia in bighorn sheep in the mid 1960's (Howe et al., 1966, Bull. Wildl. Dis. Assoc. 2: 34–37). The first respiratory virus isolated from bighorn sheep was PI-3 virus from a captive herd in Wyoming (Parks et al., 1972, J. Wildl. Dis. 6: 669–672). Later PI-3 virus was isolated from free-ranging bighorn lambs from Colorado (Spraker, 1979, Ph.D. Thesis, Colorado State University, Fort Collins, Colorado, 232 pp.). Respiratory syncytial virus has been isolated from domestic sheep (Evermann et al., 1985, Am. J. Vet. Res. 46: 947–952) and pneumonic lesions have been induced experimentally in sheep using challenges of both respiratory syncytial virus and *Pasteurella haemolytica* (Al-Barraji et al., 1982, Am. J. Vet. Res. 43: 236–240). Isolation of a respiratory syncytial virus from this 8-mo-old bighorn lamb and serological evidence of this virus within the herd documents the presence of another respiratory virus of bighorn sheep. The primary role of this bighorn sheep respiratory syncytial virus in the pathogenesis of bacterial bronchopneumonia observed in these two sheep and in producing the rhinitis and coughing in the herd was undetermined.

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Serologic Studies of Select Infectious Diseases of Moose (*Alces alces* L.) from Alaska

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Few serologic studies have been conducted on moose from Alaska. Serologic reactivity has, however, been demonstrated in moose from Alaska to select arboviruses (Zarnke et al., 1983, J. Wildl. Dis. 19: 175–179) and antibody to contagious ecthyma was detected in an experimentally exposed moose calf (Zarnke et al., 1983, J. Wildl. Dis. 19: 170–174). Sera of moose from Alaska were also positive for antibodies to bovine viral diarrhea virus and infectious bovine rhinotracheitis virus (Dieterich, 1981, *In* Alaskan Wildlife Diseases, Dieterich (ed.), Univ. of Alaska Press, Fairbanks, pp. 28–29). The present serologic survey was designed to determine the prevalence of certain infectious agents of free-ranging moose from Alaska.

Serum samples were obtained between 1974 and 1982 from 110 free-ranging moose from Alaska. Samples were obtained from one location on the Alaska Peninsula (12 samples), three locations

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Evaluating efficacy of fence markers in reducing greater sage-grouse collisions with fencing

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Evaluating efficacy of fence markers in reducing greater sage-grouse collisions with fencing

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ABSTRACT

Anthropogenic infrastructure routinely interferes with wildlife movement, habitat use, and survival. Grouse in the family *Phasianidae* may be particularly susceptible to collisions with fences due to their morphology and life history. Because many *Phasianid* species are of conservation concern, managers often deploy markers on fences to reduce collision-associated mortality. However, scarce information on the effectiveness of different marker styles or the effects of local and landscape features on collision risk exists. Our objectives were to (1) determine the effectiveness of different marker styles in reducing collisions, (2) estimate the effects of local and landscape features on collision risk, and (3) evaluate an existing greater sage-grouse (*Centrocercus urophasianus*) collision risk model. We conducted greater sage-grouse collision surveys within Sublette County, Wyoming, USA in March and April of 2014 and 2015. Data were analyzed in a multi-scale occupancy model accounting for incomplete detection of collisions. We found substantial evidence for the ability of all markers to reduce collisions (~57% reduction), with little difference between the tested marker types. We found strong evidence for lower collision probabilities at fences with wood posts and on fences farther from leks. Our results also indicated a negative relationship between collision probabilities and the difference between fence and vegetation heights. We observed little evidence for differences in collision risk between areas defined as "high" or "moderate" risk in a pre-existing collision risk map. We recommend integrating fence marking into conservation practices requiring fencing, and prioritizing fence marking near leks in areas with greater fence exposure.

1. Introduction

Anthropogenic infrastructure such as fences routinely interferes in the movements, habitat use, and survival of a wide variety of wildlife species (Bevanger 1994; Drewitt and Langston 2008; Linnell 2016). Unfortunately, the installation of human infrastructures, including fences, typically witnessed across landscapes of high-income nations is now occurring in low-income countries as well (Bevanger 1994; Drewitt and Langston 2008). The broad-scale erection of fencing has continued due to civil and political unrest throughout the world (Bevanger and Henriksen 1996; Hayward and Kerley 2009; Linnell 2016), the need for maintaining domesticated livestock within an enclosed area (Hayter 1939), the need to exclude undesired animals from certain parcels (Bevanger and Henriksen 1996; Hayter 1939), or to maintain biodiversity (Hayward and Kerley 2009; Linnell et al. 2016).

Wildlife collisions with fencing represent a direct impact on the survival of individuals. Mortality associated with fence collisions has been well documented for numerous avian species, including the *Phasianids* which are thought to be susceptible to collisions with infrastructure due to their high wing loading, lekking behavior, and afoveal retina (Bevanger 1994; Lisney et al. 2012; Sillman 1973). In North America, Wolfe et al. (2007) found that 39.8% of lesser prairie-chicken (*Tympanuchus pallidicinctus*) mortality was caused by collision with fences and, based on a subset of the same data set, Patten et al. (2005) observed elevated mortality rates for female lesser prairie-chickens where habitats were more fragmented by fences, power lines, and roads. Similarly, greater sage-grouse (*Centrocercus urophasianus*; hereafter, sage-grouse) collisions with fencing have been observed in two studies in western North America (Christiansen, 2009, Stevens et al. 2012a). In Europe, collisions with fences and power lines have been observed for the western capercaillie (*Tetrao urogallus*), black grouse (*Tetrao tetrix*), red grouse (*Lagopus lagopus scoticus*), and ptarmigan (*Lagopus spp.*) (Baines and Summers 1997; Bevanger 1995; Catt et al. 1994). Although the impact of this collision-associated mortality on populations is not particularly well understood, there is some evidence indicating infrastructure collisions may contribute substantially

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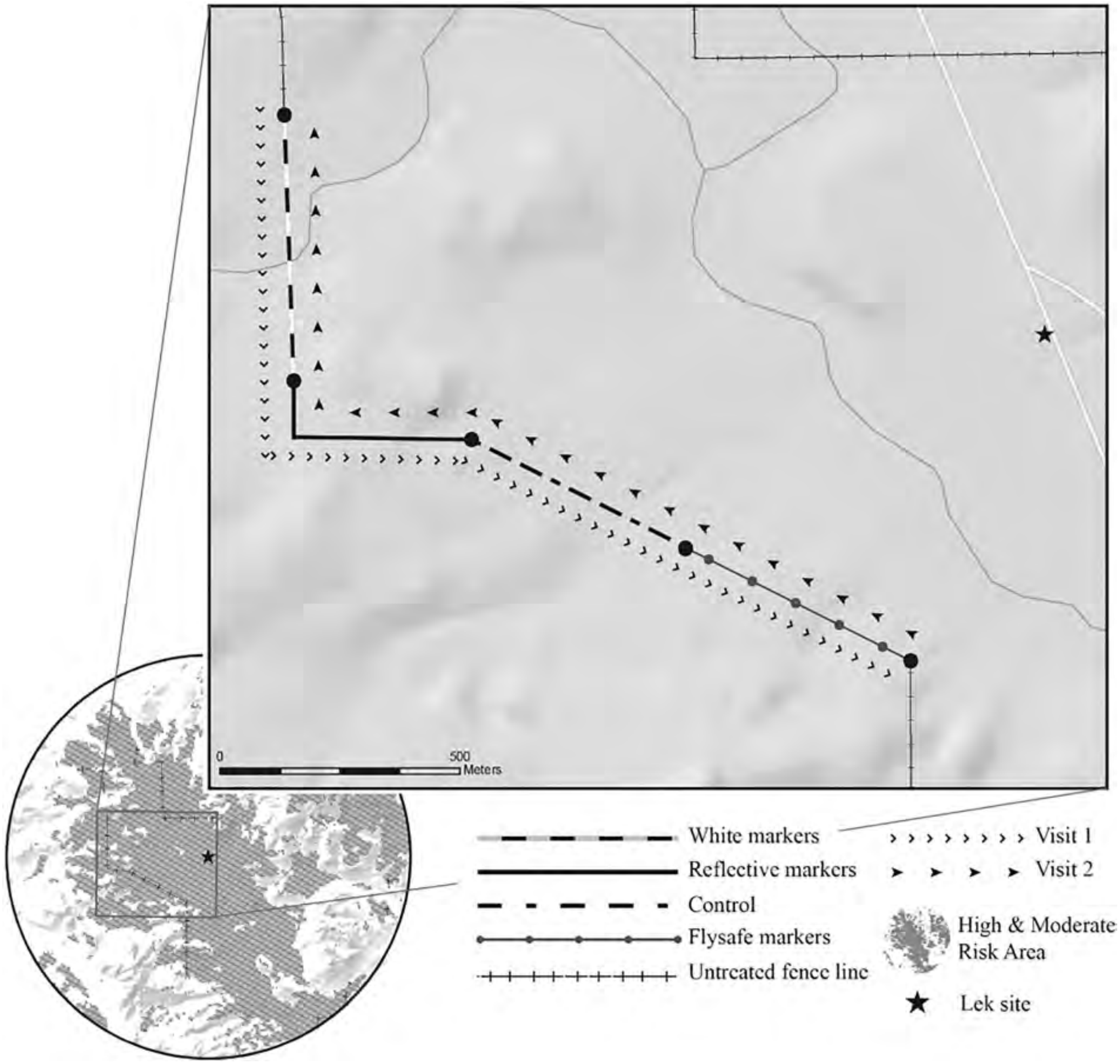

Fig. 1. Illustration of four treated segments of fence-line associated with a focal lek.

to population declines in some species (Baines and Andrew 2003; Bevanger 1995; Moss et al. 2000; Smith and Dwyer 2016).

The risk of wildlife collisions with fencing is likely impacted by a variety of site and landscape-scale factors (Stevens et al. 2012a). Site factors may include the density and height of local vegetation, fence height, type of fence, the type of fence posts, the distance between fence posts, the slope or ruggedness of the nearby landscape, and in the case of lekking species, the distance to surrounding leks and the number of individuals attending adjacent leks (Stevens et al. 2012a). Similarly, landscape-scale factors may include surrounding landcover types (Baines and Summers 1997), the density of individuals throughout the landscape (Baines and Andrew 2003), and movement corridors (including prominent ridges or other vegetative or topographic features that funnel animal movement) (Bevanger 1994; von Schweppenburg 1929).

Marking human infrastructure to increase its visibility is a common practice for reducing collisions for a variety of avian species (Luzenski et al. 2016), including *Phasianids* due to their predisposition for colliding with fences and the level of conservation concern regarding several species within this subfamily (Baines and Andrew 2003; Stevens et al. 2012b). The growing application of fence markers to reduce collisions has prompted government agencies and non-profit organizations to provide significant financial and personnel resources to install them at extensive scales (Natural Resources Conservation Service, 2015). This effort spurred one peer-reviewed study to evaluate the effectiveness of this practice. Stevens et al. (2012b) evaluated the effectiveness of fence markers in reducing greater sage-grouse collisions and found marked fences reduced collisions by 83%. Similarly, marking fences reduced black grouse (91%) and capercaillie (64%) collisions (Baines and Andrew 2003). Although these studies have shown that marking deer and stock fencing can reduce *Phasianid* collisions with fences, to date, no study has compared the efficacy of multiple marker types in reducing collisions, while accounting for imperfect detection, and considering site- and landscape-level factors that may influence collision rates. Durability concerns of marker types in Europe underscore the need for evaluating alternative marker styles (Baines and Andrew 2003). Additionally, few studies have empirically tested site- and landscape-scale factors that may influence the risk of grouse collisions with fencing.

Our research objectives were to 1) determine the effectiveness of different fence marker types, 2) estimate the effects of site and landscape features on collision risk and 3) evaluate an existing greater sage-grouse collision risk model. We evaluated the effectiveness of bright yellow FlySafe markers (FlySafe 2016), white markers with reflective

Fig. 2. Photographs of fence marker types deployed in our study. From left to right the above images represent the Flysafe, reflective, and white marker treatments.

tape and white markers without reflective tape compared to unmarked fence using a dataset collected in western Wyoming where sage-grouse densities are high and leks are abundant. Additionally, we investigated site and landscape features to identify areas with high collision risk and control for potentially confounding variables related to collision risk at multiple spatial scales. We evaluated an existing collision risk map (Stevens et al. 2013) to determine if observed sage-grouse collisions were correlated with areas predicted to have high or moderate collision risk.

2. Methods

2.1. Study area

Our study occurred on both private and public lands within Sublette County, Wyoming, USA. Sublette County contains some of the highest sage-grouse population indices within the occupied range (United States Fish and Wildlife Service, USFWS 2010). It lies within Management Zone II as identified by Stiver et al. (2006). The county covers approximately 3.2 million acres, of which, 80% is publicly owned. Elevations within Sublette County range from 6280 ft to 13,400 ft (Wyoming State Historical Society 2016). Lower elevations are largely characterized as sagebrush steppe habitat with riparian corridors along the Green River and its tributaries. Dominant vegetation within the lower elevation sagebrush steppe largely consists of Wyoming big sagebrush (*Artemesia tridentata* ssp. *wyomingensis*) and basin big sagebrush (*Artemesia tridentate* ssp. *tridentata*). Fencing within our study area largely consisted of three to four metal strands with barbs on all wires. A small amount of fencing within our study area consisted of metal woven wire fencing in which the bottom half of the fence consisted of both vertical and horizontal metal strands without barbs and forming rectangles 9 cm by 12 cm. Above the woven wires were typically one or two single horizontal metal wire strands with barbs.

2.2. Sampling design

We developed the sampling frame for Sublette County, Wyoming, using the 3 km-radius collision risk polygons (Stevens et al. 2013) for sage-grouse leks represented in the Wyoming Game and Fish Department lek database (Christiansen 2012). We reclassified the high and moderate risk zones into a single collision risk category and omitted the low risk zone for each of the 308 lek polygons in Sublette County (Fig. 1) using a Geographic Information System (GIS; ArcGIS Version 10.0, ESRI 2011). Next, we intersected the combined high and moderate risk zones for the lek polygons with the Bureau of Land Management (BLM) fence database (Bureau of Land Management - Pinedale Field Office, GIS Staff 2013). The sampling frame consisted of 77 lek polygons containing a minimum of 2 km of fence within the combined high and moderate risk zone of the lek polygons. We defined the sampling unit as the lek, which was represented by the 3 km-radius collision risk polygon (Stevens et al. 2013).

We selected a spatially balanced sample of 26 lek polygons (hereafter, we refer to randomly selected leks as “focal leks”) using Generalized Random Tessellation Stratification (GRTS; Stevens and Olsen 2004). We determined land ownership from the Sublette County Assessor's Office and requested permission to access the sampling units in the rank order of the GRTS sample selection. When landowners denied permission, we selected the next highest rank order of the GRTS sample selection. A useful feature of the GRTS design is the spatially balanced property of the sample was maintained when private landowners denied permission to access the sampling units (Stevens and Olsen 2004).

2.3. Treatments

Each of the four treatments was randomly applied to 500 m stretches of fencing within the selected sample units. Treatments were defined as control (no marker), white (approximately 7.5 × 5 cm piece of white undersill vinyl siding), reflective (white markers with a 7.5 × 1.8 cm strip of lime-yellow Identi-Tape V97 high intensity reflective tape applied to each side), and Fly Safe markers (approximately 12 × 9 cm yellow plastic markers) (FlySafe 2016) (Fig. 2). We selected the marker treatments because they are representative of the gamut of treatments being implemented within the western U.S. to reduce sage-grouse and lesser prairie-chicken collisions with fencing. For the 500 m stretches receiving the white, reflective, or Fly Safe treatments, markers were spaced approximately 1 m from fence-posts and other markers on the top wire of the fencing to be consistent with fence marking recommendations (United States Department of Agriculture, USDA 2016). The design with all three treatments and the control employed at each sampling unit corresponds to a repeated measures design with random order of the treatments levels (Morrison et al. 2008).

2.4. Sampling methods

A total of four observers trained in sage-grouse feather identification and possessing extensive biological survey experience conducted field work throughout the two year study. Observers were intensively trained to ensure they possessed a complete understanding of field protocols, a sufficient ability to identify collision events, and could positively identify sage-grouse remains.

Surveys were conducted approximately biweekly in March and April of 2014 and 2015. A survey of a site entailed either two or four visits. The first visit consisted of an observer walking along the site's fence while scanning for evidence of animal collisions. The observer then crossed the fence and conducted the second visit by doubling back and walking to the starting point of the first visit (Fig. 1). A survey consisted of four visits when a second observer, surveying separately from the first observer, visited the same site on the same day. Observers did not discuss findings during the course of the surveys in order to avoid influencing detection rates.

Observers maintained a distance of 1-2 m from the fence during each visit. While surveying, observers primarily searched the wires of the fence for signs of a collision. Additionally, observers scanned the

Table 1
Covariates included in analyses of fence collisions by Greater Sage-Grouse in Wyoming, 2014–2015, and their expected effect on the parameter of interest (positive effect, +; negative effect, −). Parameters include large-scale occupancy (ψ), small-scale occupancy (θ), and detection probability (*p*). Means and ranges are shown for continuous covariates and levels and frequencies for the categorical covariates.

Covariate	Description	Parameter	Means (ranges) and levels (frequencies)	Expected effect
Occ Lek	Number of occupied leks within 3 km of the focal lek	ψ	1.51 (0–3)	+
Lek Ct	Sum of lek counts for leks within 3 km of focal lek	ψ	72.88 (0–265)	+
Year	Year in which survey was conducted	ψ,θ	2014 (26), 2015 (25)	N/A
Trt	Fence marker type	θ	Control (50), FlySafe (51), White (51), Reflective (50)	Risk of control > white > reflective > FlySafe
Mark	Fence marked or not	θ	Control (50), Marked (152)	Lower for marked
Angle	Angle (°) created by the triangle between the lek and end of fence segment	θ	16.34° (1°–120°)	+
Distance	Distance (km) between the midpoint of the fence segment and the nearest lek	θ	1.85 km (0.15 km–4.60 km)	−
Near Ct	Mean max male lek count for the nearest lek from 2014 to 2015	θ	54.63 (1–265)	+
Fence Exp	Mean difference (cm) between the top strand of a fence and the top of the surrounding vegetation	θ	67.69 cm (26.67 cm–96.10 cm)	+
Risk	Percentage of the fence segment in high risk areas based on Stevens et al. (2013)	θ	45.8% (0.0%–100.0%)	+
Post	Type of posts used in a fence segment	θ	Wood (138), T-post (4), both (62)	Risk of t-post > both > wood
Surv	Biweekly survey (primary) period in which survey was conducted	θ, *p*	1 (200), 2 (202), 3 (189), 4 (189), 5 (188), 6 (190), 7(186)	None
Visit	Visit (secondary period) in which survey took place	*p*	1 (1019), 2 (1014), 3 (114), 4(112)	None
Obs	Observer conducting the survey	*p*	A (432), B (226), C (525), D (1076)	None
Trap	"Trap effects" for the 2nd and 4th visits to account for potential lack of independence between visits by the same observer	*p*	1st/3rd (1133), 2nd/4th (1126)	Higher for 2nd/4th visits
Trap2	"Trap effects" accounting for whether a collision was detected or not on the 1st visit	*p*	Non-detection (1135), detection (1080)	Higher if previously detected
Cloud	Cloud cover (%)	*p*	46.1% (0.0%–100.0%)	−
Snow	Snow cover (%)	*p*	33.8% (0.0%–100.0%)	+

bushes and ground approximately 10 m out from either side of the fence for feathers or carcasses. Observers recorded ocular estimates of average snow and cloud cover (0–100%) during the course of each survey.

We considered a collision to have occurred when sage-grouse feathers were observed in the wires or barbs of a fence. We believe this represents a more accurate count of collisions as other experts have determined carcass recovery can be low due to scavenging (Stevens et al. 2011) and we believe wounded grouse may travel significant distances after striking fences before they expire. Collisions were recorded on each visit during which they were observed. In the event that feathers were found on the fence at multiple locations between two fence posts (the fencing between two fence-posts hereafter is referred to as a "panel"), the evidence was considered a single collision unless the largest gap between feathers on the wire exceeded the average wingspan of a sage-grouse (Sibley 2000). Analyses did not include any evidence in a fence that may have resulted from perching, prey plucking, or preening events, which were generally characterized by a small amount of feathers loosely affixed to the barbs of the fence and primarily distributed near a wooden post.

Observers thoroughly documented all collisions found via photographs and written notes. Observers recorded collision locations with a hand-held Global Positioning System (GPS) unit. Additionally, observers recorded the following information pertaining to the collision evidence: the distance from the evidence on the fence to the nearest fence-post, the distance from the evidence on the fence to the nearest marker, the distance from the ground (or top of the snow layer, when applicable) to the highest evidence on the fence, and the strand of wire containing the collision evidence. Finally, the observers collected the following data to describe the collision site: the distance between the two fence-posts for the panel containing the evidence, the mean height of the vegetation along the fence panel containing the collision evidence, and the number of strands of wire on the panel of fencing containing the evidence. Photographs of feathers were sent to local experts if the field observers could not be sure of identification. Collision events were only included in analyses when species identification was possible (i.e., diagnostic feathers found).

2.5. Covariate data collection

We measured fence exposure by estimating the average height of woody vegetation and the height of the top strand of fencing in centimeters for each panel. We then subtracted the height of the woody vegetation from the height of the top wire of fencing to obtain a value of "fence exposure" in centimeters for the panel. If vegetation was taller than the fence, fence exposure had a negative value. We measured these values for six panels within each 500 m stretch. Values were calculated at the two panels representing the endpoints and systematically at four additional locations at 100 m intervals along each fence segment. The fence exposure values for each of the six panels per stretch were then averaged to derive a single mean fence exposure value for the 500 m stretch. With assistance from BLM personnel, we also noted whether posts within a fence segment were wood posts, metal t-posts, or a combination of the two.

Using ArcGIS 10.0 (ESRI) we calculated several covariates including: 1) the number of occupied sage-grouse leks within 3 km of the focal lek, 2) the sum of mean maximum male lek counts in 2014 and 2015 for all leks within 3 km of the fence segment midpoint, 3) the distance from the midpoint of each fence stretch to the nearest occupied sage-grouse lek and the mean maximum male count for that lek from 2014 to 2015, 4) the proportion of each fence stretch that fell within the high risk category of the collision risk map (Stevens et al. 2013), and 5) the angle of exposure for each stretch of fence (i.e., the angle created by the triangle between the ends of the fence segment and the associated lek).

Lastly, observers estimated cloud cover during each survey and percent of the ground covered by snow to the nearest 10%. In 2014 observers recorded a single value for the average snow cover values surrounding each of the four fence segments during a survey. In 2015 observers recorded a separate value for average percentage of snow cover along each fence segment. For analyses, we calculated the mean of the 2015 values for each survey to produce a single snow cover value consistent with the 2014 data. Table 1 summarizes all covariates included in our models.

2.6. Model justification and hypotheses

We used the method of working hypotheses (Chamberlin 1965) to evaluate alternate a priori hypotheses to understand how different marker types, site- and landscape-features and mapped collision zones affect sage-grouse fence collisions. We used the covariates in Table 1 to represent hypotheses for the objectives and translated the hypotheses into predictive models. We then used the predictive models to evaluate relative strength of evidence for the alternate hypotheses in a model selection framework (Burnham and Anderson 2002). We predicted detection of sage-grouse collisions at the fence segments would be incomplete, potentially biasing the measurement of effect sizes for the fence markers. Therefore, we evaluated several hypotheses for how observers and time occasions may influence the detectability of fence collisions. We predicted the detection of collisions would vary by observer (*Obs*), time of the biweekly surveys (*Surv*), and repeated visits (*Visits*, Table 1). We accounted for potential non-independence of detections when observers visited the fence segment twice on the same day using the *Trap2* covariate (Table 1). In addition, we hypothesized that snow cover (*Snow*) and cloud (*Cloud*) cover may interfere with the ability to detect the signs of collision (Table 1).

When evaluating the effectiveness of fence markers (objective 1), we predicted that collision risk would be lower on fence segments with markers than fence segments without markers (*Mark*, Table 1) since fence marking has been shown to reduce collision risk for grouse species (Stevens et al. 2013). In addition, we hypothesized that collision risk would be lowest on fence segments with yellow Fly Safe markers, intermediate on segments with white markers with reflective tape, and greatest on segments with white markers without reflective tape (*Trt*, Table 1). Because *Phasianid* species are known to see carotenoid-based colors (Mougeot et al. 2007), we predicted the bright yellow Fly Safe markers would be more effective than white markers with reflective tape. We predicted white markers with reflective tape would be more effective than white markers without reflective tape because reflective tape is thought to provide greater visibility for low light and snow background conditions (Stevens et al. 2013). In addition, we hypothesized that fence segments with wood posts would be more effective in reducing collisions than fence segments with iron t-posts and fence segments with both types (*Post*, Table 1) because wooden posts may be more conspicuous than iron t-posts (Stevens et al. 2012a) and sage-grouse are known to avoid areas with vertical woody structure (Stiver et al. 2006).

We evaluated site- and landscape features to identify areas with greater collision risk (objective 2) at multiple scales and to control for potentially confounding variables when evaluating the effectiveness of different marker types (Morrison et al. 2008). At the local scale, we hypothesized that collision risk would be higher on fence segments near active leks (*Distance*) and near leks with greater lek attendance (*Near Ct*, Table 1) as has been shown in previous research (Stevens et al. 2012b). In addition, we predicted that collision risk would be greater on fence segments with greater fence exposure above vegetation and on fence segments (*Fence Exp*) with a larger "exposure angle" in relation to the focal lek (*Angle*, Table 1). Stevens et al. (2012a) considered a variable for the height difference between the fence and the nearest lateral shrub, but did not find strong evidence for this variable. Nevertheless, we felt sage-grouse were more likely to fly above the vegetation than between it and greater fence exposure would therefore lead to greater collision risk. Given the positive association of collisions with lek counts and small lek distances, we hypothesized that birds needing to cross fencing to attend or leave a lek would have a higher risk of collision and used the *Angle* covariate to test this hypothesis. At the landscape scale, we hypothesized that collision risk would be greater in lek polygons with high numbers of occupied leks (*Occ Lek*) and with high lek counts (*Lek Ct*, Table 1). Stevens et al. (2012a, 2012b) measured the distance between fence segments and leks to show that distribution and abundance of leks was related to collision risk at the site-scale. We measured lek density and sage-grouse abundance within the 3-km^2 radius lek buffers (28 km^2) to evaluate the extent that lek distribution and abundance influenced collision risk of lek polygons at the landscape scale. Because sage-grouse are known to move between leks on the landscape (Emmons and Braun 1984), we predicted that lek polygons containing a greater number of leks and greater numbers of birds would also have greater collision risk. If landscape measures of lek distribution and abundance prove important, these covariates can be used to account for the dependence of the treatments within 3-km^2 radius lek polygons using the repeated measures design.

To evaluate an existing collision risk map by Stevens et al. (2013) (objective 3), we predicted that collision risk would be greater along fence segments in areas characterized by high risk than on fence characterized by moderate risk (*Risk*, Table 1). Because the collision risk map was based on terrain ruggedness and distance to nearest lek (Stevens et al. 2013), this hypothesis evaluates collision risk in response to moving farther from a lek with increasing topographic relief.

2.7. Statistical analyses

We developed a multi-scale occupancy model (Nichols et al. 2008) to estimate occupancy probabilities of collision evidence, and the factors influencing them at site- and fence-segment levels. The model allowed estimation of three parameters that corresponded to each level in the nested sampling design. We used repeat visits nested within each survey to estimate detection, repeat surveys of fence segments nested within a site (i.e., lek) to estimate small-scale occupancy (the probability of a collision occurring within a 500 m fence segment), and replicate leks nested within the study area to estimate large-scale occupancy (the probability of a collision occurring within any of the four fence segments associated with the focal lek). All analyses were conducted using Program MARK (version 8.0; White and Burnham 1999) via RMARK (version 2.1.14; Laake 2013). We defined our three general parameters as: (1) the probability that evidence of ≥ 1 new sage-grouse collision was present on ≥ 1 fence segment at site i during any of the surveys, ψ_i, (2) the probability that evidence of ≥ 1 new collision was present at a fence segment during survey j, θ_{ij}, and (3) the probability that a new collision was detected on visit k, given the fence segment was occupied during survey j and visit k, p_{ijk}. The multi-scale occupancy model is well suited for the repeated measures design by allowing the investigation of covariates influencing occupancy at the large-scale (i.e., collisions at any fence segment associated with a focal lek) as well as treatments effects on conditional occupancy at the small-scale (i.e., collisions at individual fence) while accounting for non-independence of fence segments within a lek. This is analogous to how variance is estimated in a mixed model with a random effect on the focal lek (Pavlacky et al. 2012). We assumed fence segments were closed to changes in occupancy within each survey and that new collisions were accurately identified and recorded. The fence segments were allowed to be open between surveys. This model also assumes that detections are independent; however, observers conducted the second visit on the opposite side of the fence immediately after the first visit. We attempted to account for this potential lack of independence by estimating separate detection probabilities for the first and second visits by the same observer during a survey period along with whether a collision was detected during the first visit.

2.8. Model set

To investigate our hypotheses regarding the factors influencing large- and small-scale occupancy and detection, the models in our

Table 2
Model set for models explaining variation in detection probabilities (p) of Greater Sage-Grouse fence collisions in Wyoming, 2014–2015. We fit models using the most general small- (θ) and large-scale (ψ) occupancy probability model structures. Because two covariates on each occupancy probability were different measures of similar hypotheses, we included both model structures on each of those parameters. Covariates included to explain variation in detection probabilities included: fixed visit effects (Visit), fixed survey effects (Surv), fixed observer effects (Obs), "trap effects" for the 2nd and 4th visits (Trap), "trap effects" accounting for whether a collision was detected or not on the 1st visit (Trap.2), cloud cover (Cloud), and snow cover (Snow). Model structure on small-scale occupancy included: fence exposure (Fence Exp), proportion of fence segment in high risk areas (Risk), angle of fence in relation to lek (Angle), Year, biweekly (primary) period (Surv), an interaction between post type and marker type (Post × Trt), and an interaction between distance to nearest lek and the count at that lek (Distance × Near Ct). Model structures on large-scale occupancy included: Year and either the sum of lek counts at nearby leks (Lek Ct) or the number of nearby occupied leks (Occ Lek; indicated in ψ column). The number of parameters (npar), Akaike's Information Criterion adjusted for small sample size (AIC_c), difference between a model's AIC_c value and the minimum AIC_c value (ΔAIC_c), and AIC_c weights are also shown for models with $\Delta AIC_c \le 10$.

ψ	p	npar	AIC_c	ΔAIC_c	Weight
Occ Lek	Null	25	415.082	0.000	0.582
Lek Ct	Null	25	416.051	0.969	0.358
Occ Lek	Snow	26	423.116	8.034	0.010
Occ Lek	Surv	26	423.388	8.306	0.009
Occ Lek	Cloud	26	423.572	8.490	0.008
Occ Lek	Trap.2	26	423.582	8.500	0.008
Lek Ct	snow	26	424.084	9.002	0.006
Lek Ct	surv	26	424.358	9.275	0.006
Lek Ct	cloud	26	424.541	9.459	0.005
Lek Ct	trap.2	26	424.551	9.469	0.005

model set consisted of various combinations of covariates on each parameter. We included 3 covariates on large-scale occupancy (ψ), 10 on small-scale occupancy (θ), and 7 on detection (p; Table 1). We also included interactions between post type and marker, as well as minimum distance to the nearest lek and maximum male count for that lek on θ. Because the model set was very large when considering all possible combinations of covariates, we used a sequential approach to model selection (Lebreton et al. 1992). We fit models that included all possible additive combinations of covariates on detection, while including additive effects for all covariates for large- (ψ) and small-scale (θ) occupancy. There were two covariates on large-scale occupancy that were different measures of the same hypothesis: (1) the number of occupied leks within 3 km of the focal lek (Occ Lek, Table 1) and (2) the sum of the lek counts for leks within 3 km of the focal lek (Lek Ct). We did not include both covariates in the same model. Therefore, we fit a global model containing all other additive combinations of covariates with Occ Lek and Lek Ct. separately, resulting in two global models. Then, using the most parsimonious detection structure(s), we evaluated hypotheses related to large-scale occupancy. Retaining the best large-scale occupancy model structure(s), we fit models that included all possible combinations of covariates thought to influence small-scale occupancy, including the two interaction terms.

We used an information-theoretic approach for model selection and used Akaike's Information Criterion (AIC) adjusted for sample size (AIC_c) for model comparison (Burnham and Anderson 2002). We used Akaike weights, w_i, as a measure of the relative amount of evidence for each model. Our model set for small-scale occupancy was not balanced because of the interaction terms and mutually exclusive covariates (i.e., Mark and Trt), so we used a modified version of cumulative weights based on the frequency of the covariate in the model set [$w_+(j)$] (Doherty et al. 2012) to determine the relative importance of our covariates,

$$w_+(j) = \left[\frac{w}{1-w}\right] \Big/ \left[\frac{f}{1-f}\right],$$

where w is the cumulative Akaike weight (sum of Akaike weights for models containing the covariate) and f is the frequency of models containing the covariate in the model set. Weights ≫ 1 indicate support for the importance of that variable, weights near 1 are inconclusive, and weights ≪1 indicate little support for importance. We used the odds ratio to express the effect sizes (β) in terms of the percentage increase in the odds of collision.

3. Results

We found evidence of 64 confirmed fence collisions by sage-grouse during the study, with 15 detected in 2014 and 49 detected in 2015. Additionally, we observed 96 instances of possible or likely collisions which were not included in analyses. Over 60% of sites (16 of 26) and 26% of fence segments (27 of 104) contained evidence of ≥ 1 confirmed collision. Only two fence segments were constructed using t-posts exclusively, and no collisions were detected at those segments; therefore, we fixed small-scale occupancy (θ) of those segments to zero to assist with numerical convergence.

Our global models used in the sequential model selection, included year and either the number of nearby occupied leks or the sum of the lek counts at those leks effects on large-scale occupancy, ψ (Year + Occ Lek) or ψ (Year + Lek Ct); year, survey, treatment × post type, distance to nearest lek × count for nearest lek, fence angle to lek, proportion in high risk areas, and fence exposure effects on small-scale occupancy, θ (Year + Surv + Distance + Angle + Risk + Fence Exp + Post × Trt + Distance × Near Ct); and observer, cloud cover, snow cover, and visit effects on detection, p (Obs + Cloud + Snow + Visit).

3.1. Detection probabilities

Using these two global models, we explored 40 other detection structures, representing simplifications of our general detection structure (Tables 2 and A1). The most parsimonious model included a constant detection probability (w = 0.59), as did the 2nd best model, cumulatively accounting for 95.4% of the weight; thus, we retained this detection structure, p (.), in our subsequent models. We estimated the probability of detecting ≥ 1 collision at 0.935 (SE = 0.026).

3.2. Large-scale occupancy

Large-scale occupancy of collisions increased as the sum of nearby lek counts increased and was higher in 2015. However, the 95% confidence intervals for both of these effects included zero. Because of this uncertainty, the most parsimonious model for ψ was the constant model, which accounted for a majority of the AIC_c weight (w = 0.85) (Table 3). On average, large-scale occupancy was estimated to be 0.717

Table 3
Model set for models explaining variation in large-scale occupancy probabilities (ψ) of Greater Sage-Grouse fence collisions in Wyoming, 2014–2015. We fit models using the most parsimonious model on detection probabilities (i.e., null) and the global model structure on small-scale occupancy probabilities (θ). Model structures on large-scale occupancy included: Year and either the sum of counts at leks with 3 km (Lek Ct) or the number of occupied leks within 3 km (Occ Lek; indicated in ψ column). Model structure on small-scale occupancy included: fence exposure (Fence Exp), proportion of fence segment in high risk areas (Risk), angle of fence in relation to lek (Angle), Year, biweekly (primary) period (Surv), an interaction between post type and marker type (Post × Trt), and an interaction between distance to nearest lek and the count at that lek (Distance × Near Ct). We also include the number of parameters (npar), Akaike's Information Criterion adjusted for small sample size (AIC_c), difference between a model's AICc value and the minimum AICc value (ΔAIC_c), and AIC_c weights.

ψ	npar	AIC_c	ΔAIC_c	Weight
Null	23	402.913	0.000	0.852
Lek Ct	24	408.447	5.534	0.054
Year	24	408.498	5.585	0.052
Occ Lek	24	409.084	6.171	0.039
Year + Occ Lek	25	415.082	12.170	0.002
Year + Lek Ct	25	416.051	13.139	0.001

Table 4

Cumulative AIC_c model weights for variables thought to influence small-scale occupancy (θ) of greater sage-grouse fence collisions in Wyoming, 2014–2015. Cumulative weights were adjusted based on the frequency of the covariate in the model set (Doherty et al. 2012). Variables included in the model set are: fence exposure (Fence Exp), proportion of fence segment in high risk areas (Risk), angle of fence in relation to lek (Angle), Year, biweekly (primary) period (Surv), wood post or wood and t-post (Post), marker type (Trt), whether a fence was marked or unmarked (regardless of marker type; Mark), the distance to the nearest occupied lek (Distance), the count at the nearest lek (Near Ct), an interaction between post type and marker type (Post × Trt), an interaction between post type and whether a fence was marked (Post × Mark), and an interaction between distance to nearest lek and the count at that lek (Distance × Near Ct). Modified cumulative model weights ≫ 1 suggest strong support for that variable, weights near 1 are ambiguous, and weights ≪ 1 suggest little support for that variable.

Variable	Cumulative weight
Post	12.797
Mark	4.188
Distance	3.349
Fence Exp	1.699
Year	1.261
Risk	1.246
Near Ct	1.078
Post × Mark	0.908
Surv	0.790
Distance × Near Ct	0.658
Angle	0.476
Trt	0.065
Post × Trt	0.001

(SE = 0.127).

3.3. Small-scale occupancy

We found strong evidence for effects of post type [w_+(Post) = 12.80], whether a fence was marked or not [irrespective or marker type, w_+(Mark) = 4.19], and distance to the nearest lek [w_+(Distance) = 3.35] on small-scale occupancy (Tables 4, 5, and A2). There was some support for the effects of fence exposure [w_+(Fence Exp) = 1.70], year [w_+(Year) = 1.26], the amount of fence segment within the high risk areas based on Stevens et al. (2013) [w_+(Risk) = 1.25], and the count at the nearest lek [w_+(Near Ct) = 1.08; Tables 4 and A2]. Consistent with our hypotheses, wood posts, fence marking, and increasing distance to nearest lek resulted in lower collision occupancy probabilities (Tables 6, A3, and A4 and Fig. 3). The amount of fence exposure and the proportion of fence in high risk areas increased the probability of a collision, as we predicted. Occupancy probabilities were higher in 2015 and as the count at the nearest lek increased, though these coefficients were not significant (Table 6). All marker types performed similarly [β = − 0.843,(95% CI = − 1.545, − 0.141); odds ratio: 0.430, (0.128, 0.732)], with reflective [β = − 1.018,(95% CI = − 1.967, − 0.068); odds ratio: 0.361, (0.018, 0.705)] and white markers [β = − 0.808, (− 1.703, 0.087); odds ratio: 0.446, (0.047, 0.857)] reducing occupancy probabilities slightly more than Fly Safe markers [β = − 0.725, (− 1.634, 0.184); odds ratio: 0.484, (0.044, 0.924)] based on the model including treatment and all other covariates with cumulative weights > 1.

4. Discussion

We adapted the multi-scale occupancy framework to investigate landscape- and local-scale features influencing the probability of fence collision, and our results support the anecdotal and limited empirical evidence for the threat of fences to sage-grouse (Christiansen 2009; Flake et al. 2010; Scott 1942; Stevens et al. 2012a, 2012b). Our study also provided insight into the factors influencing fence collisions at two spatial scales by using a multi-scale occupancy model. In addition to accounting for imperfect detection of collisions, this approach allowed us to account for the lack of independence between fence segments associated with a particular lek (Nichols et al. 2008; Pavlacky et al. 2012).

Table 5

Model set for models explaining variation in small-scale occupancy probabilities (θ) of Greater Sage-Grouse fence collisions in Wyoming, 2014–2015. We fit models using the most parsimonious model on detection probabilities (i.e., null) and large-scale occupancy probabilities (i.e., null). Model structures on small-scale occupancy included: distance to nearest lek (Distance), the count at the nearest lek (Near Ct), fence exposure (Fence Exp), wood post or t-post (Post), proportion of fence segment in high risk areas (Risk), angle of fence in relation to lek (Angle), marker type (Trt), marked or unmarked fence (regardless of marker type; Mark), Year, biweekly (primary) period (Surv), an interaction between Distance and Near Ct, and an interaction between Post and Mark or Trt. The number of parameters (npar), Akaike's Information Criterion adjusted for small sample size (AIC_c), difference between a model's AICc value and the minimum AICc value (ΔAIC_c), and AIC_c weights are also shown for the top 10 models.

θ	npar	AIC_c	ΔAIC_c	Weight
Fence Exp + Mark + Distance + Post + Risk + Near Ct	9	364.644	0.000	0.030
Fence Exp + Mark + Distance + Post + Risk + Year	9	364.756	0.111	0.028
Fence Exp + Mark + Distance + Post + Risk + Year + Near Ct	10	364.903	0.259	0.026
Fence Exp + Mark + Post + Risk + Distance × Near Ct	10	365.270	0.626	0.022
Surv + Fence Exp + Mark + Distance + Post + Risk + Year	15	365.647	1.003	0.018
Fence Exp + Mark + Distance + Post + Near Ct	8	365.762	1.118	0.017
Fence Exp + Mark + Post + Risk + Year + Distance × Near Ct	11	365.794	1.150	0.017
Surv + Fence Exp + Mark + Distance + Post + Year	14	365.810	1.166	0.017
Fence Exp + Mark + Distance + Post + Year + Near Ct	9	365.998	1.354	0.015
Fence Exp + Mark + Distance + Post + Risk	8	366.015	1.371	0.015

Table 6

Coefficient estimates, standard errors (SE), and 95% confidence intervals (CI) for all variables explaining variation in small-scale occupancy (θ) probabilities of Greater Sage-Grouse fence collisioins in Wyoming, 2014–2015. Variables include fence exposure, whether a fence was marked (regardless of marker type; Mark), the distance to nearest lek (Distance), fences with wood and t-posts (wood and t-post), proportion of fence segment in high risk areas (Risk), year (2015), and the count at the nearest lek (Near Ct). The intercept represents an unmarked fence with wood posts in 2014 with all continuous variable values set to 0. Variables included had modified cumulative AIC_c weights > 1. Estimates from the third best model are reported because it is the best model including all variables with cumulative weights > 1. All significant coefficients (i.e., 95% CIs do no overlap 0) are indicated by an asterisk.

Parameter	Mean	SE	95% CI
Intercept*	− 5.544	1.123	(− 7.745, − 3.342)
Fence Exp*	0.031	0.013	(0.005, 0.058)
Mark*	− 0.843	0.358	(− 1.545, − 0.141)
Distance*	− 0.586	0.192	(− 0.962, − 0.210)
Wood and T-post*	1.774	0.382	(1.025, 2.523)
Risk*	1.150	0.565	(0.042, 2.258)
2015	0.821	0.473	(− 0.105, 1.747)
Near Ct	0.004	0.002	(− 0.001, 0.009)

Studies regarding potential risk of collision with human-associated infrastructure have noted that risks to lekking species may be higher in close proximity to lek locations (Baines and Summers 1997; Bevanger 1994; Stevens et al. 2012a, 2012b). Therefore, we tested four hypotheses relating to the risk of collision in association to the number of leks, the number of individuals observed at nearby leks, the position of fencing (angle) in relation to a nearby lek, and the distance to the nearest lek. Unlike Stevens et al. (2012a), we found little evidence for an effect of the number of birds using nearby leks on collision probabilities and therefore failed to confirm our hypothesis. Similarly, there

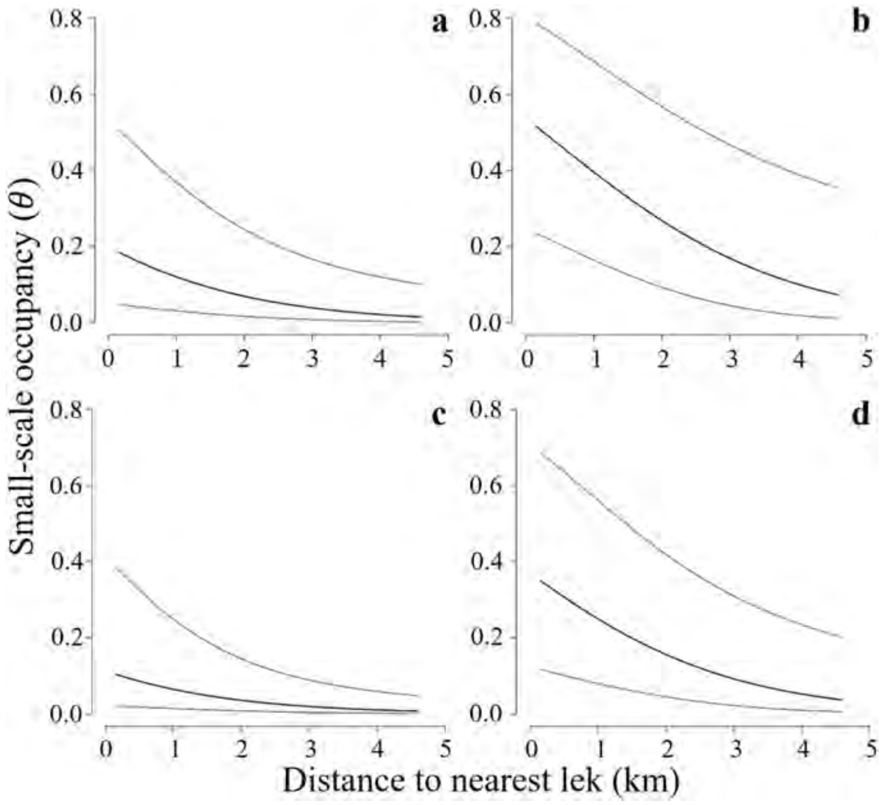

Fig. 3. Small-scale occupancy probability (θ, heavy lines) and associated 95% confidence intervals (light lines) as a function of distance to nearest lek for a) unmarked, wood post, b) unmarked, wood and t-post, c) marked, wood post, and d) marked, wood and t-post fence segments.

was no evidence to support an increased risk of collision near fencelines that are near multiple leks. Baines and Andrew (2003) similarly found no effect of lek indices on collision risk indicating that other factors may be more predicitive. Our findings may be partially due to using presence-absence data to detect differences among leks of various sizes, such that the probability of ≥ 1 collision is high for a fence near even a single smaller lek. Addtionally, lek counts have been criticized for their inability to accurately reflect abundance of sage-grouse (Beck and Braun 1980; Johnson and Rowland 2007; Walsh et al. 2004) but have been shown to be a reasonable index of the population of breeding males when standard survey protocols are followed (Jenni and Hartzler 1978; Emmons and Braun 1984; Walsh et al. 2004; Johnson and Rowland 2007). However, lek counts may not accurately represent the number of birds in the area surrounding a lek, and therefore, may be a poor indicator of the likelihood of a collision. We therefore recommend that future efforts to estimate or account for collision risk use estimated densities when possible.

Although there is an abundance of peer-reviewed work indicating that flight paths may greatly increase the risk of bird collisions with human infrastructure (Bevanger 1994; Bevanger 1998; Everaert and Stienen 2007; Henderson et al. 1996; Scott et al. 1972), we found no evidence for increased collision risk with an increased angle of fence exposure in relation to the lek which failed to confirm our hypothesis. It is possible this covariate was confounded with the distance to the nearest lek (closer distances having a larger angle) which we tested and describe in the following text. Nevertheless, we maintain that flight paths may be important in determining collision risk for some systems and species and encourage researchers to consider other potential vegetative, topographical, biological, and environmental factors that may influence or create flight paths in future studies.

We found the proximity of a fence segment to a lek influenced the probability of a collision (Distance); the average occupancy probability decreased by approximately 39% between distances of 153 m (i.e., smallest distance observed) and 1 km. This is consistent with the findings of Stevens et al. (2012a, 2012b) and confirmed our hypothesis. This relationship is likely due to increased encounters between birds and fences when a fence is closer to an area where birds congregate. We therefore recommend that marking efforts preferentially mark fence close to leks in the future. Additionally, we encourage future studies investigating risks of collisions with human-related infrastructure to consider accounting for water and/or food sources, geophagy sites, or other features that may lure large numbers of individuals into a localized area.

As in Stevens et al. (2012a), our results suggest that fence post type has the largest effect on the occupancy probability of sage-grouse collisions, with the lowest occupancy probabilities for fence segments with wooden posts, which confirmed our hypothesis. Only two fence segments in our study had t-posts exclusively and neither of those segments had evidence of a collision on them; therefore, we were unable to estimate occupancy probabilities for segments with only t-posts. Unmarked fence segments with wooden posts had lower occupancy probabilities than segments with both wooden and t-posts and any of the fence markers; yet, collision rates for fence segments with wooden posts were reduced further by the use of fence markers. These results are consistent with those found by Summers and Dugan (2001), in which, they found full length paling (which resemble wooden posts) to be the most visible fence marker. As such, we recommend future marking efforts consider testing the effectiveness of wooden stays woven into the fencing. Additionally, preferentially marking fencing with t-posts or a mixture of wood and t-posts could maximize the reduction in potential *Phasianid* collisions with fencing as our results indicated fences without wooden posts may have high rates of collisions.

We found a small effect of the amount of exposed fencing on collision risk. As vegetation height near a fence decreased, the probability of a collision increased which supported our hypothesis. *Phasianids* are generally classified as "poor flyers" (Bevanger 1994; Rayner 1988) which characteristically engage in short flights (Viscor and Fuster 1987). These morphological constraints likely result in *Phasianids* engaging in proportionately more of their flight at low altitudes, often near the top of exposed vegetation, than many birds with lower wing loading. As the top of vegetation approaches or exceeds the top of human infrastructure there is thought to be less risk of collisions (Bevanger 1994). Although we observed a weak relationship between the amount of exposed fence and collision risk, we maintain areas with short vegetation may benefit more from the use of markers by making the fence more visible. Similarly, we suggest that taller "elk fences" in the western U.S. and "deer fences" in Europe may increase collision risk beyond that of stock fencing due to the potential for additional fence projection above the vegetation as well as a general increase in total fence area. This idea was not explicitly tested in our study and represents an area for future research.

Our study design was largely based on the collision risk map developed by Stevens et al. (2013) which predicted high risk of collisions in areas close to leks and with little topography. The authors acknowledged their range-wide model was created using data collected within a relatively small geographic area in Idaho. As such, they recommended additional validation efforts be conducted. Our findings suggested a slightly increased collision probability in high risk areas, but this effect was weak. Because we attempted to select fence-line segments within the high and moderate risk areas of this map, much of the fence-line included in our study fell within these areas. Therefore, low risk areas were not well represented in our study, precluding an evaluation of the low risk portions of the risk map. We recommend further investigation of the efficacy of the collision risk map in predicting collision risk, particularly to determine if greater slopes associated with topography do impact collision risk range-wide and to determine if low risk areas on the collision risk map have a lower number of associated fence collisions. Until the collision risk map can be evaluated further, we recommend that managers seeking to reduce sage-grouse collisions focus their fence-marking efforts on fence-lines in both the high and moderate risk zones which are both close to leks and possess local site characteristics which have been shown to increase collision risk in our study and/or in previous studies.

We estimated a detection rate of 0.94, suggesting a false absence rate of 6% in the raw collision data. Our detection rate was similar to the collision detection rate calculated by Baines and Andrew (2003) when they simulated collision events with grouse carcasses. This indicates that detection of collision events is likely quite high when conducting walking surveys, provided that evidence of the collision still persists on the landscape. Stevens et al. (2011) calculated much lower detection rates when conducting walking surveys within 15 m of bird carcasses which were placed in the field; however, their estimates accounted for both detectability and scavenging bias. We suspect the scavenging bias was the driving factor in the reduced detection rates; however, they also placed carcasses beyond the search window of both our study and that of Baines and Andrews (both, of which had an effective search strip width of approximately 5 m). Furthermore, Stevens et al. placed piles of feathers and the carcasses within the habitat whereas in the Baines and Andrews study the carcasses were "vigorously thrown at the fence to simulate flight collisions". Given that we regularly witnessed feathers widely strewn across areas of 30 m or more in our study, we feel the methods used by Stevens et al. (2011) may not have accurately created conditions similar to that of an actual collision event, ultimately underestimating detection probabilities of *Phasianid* collision evidence.

Our results suggest that all three types of fence markers employed in our research were effective at reducing collision probabilities and confirmed our hypothesis, with stretches of marked fence having a 57% (27% - 87%) lower probability of containing ≥ 1 collision. These results align with previous studies by Stevens et al. (2012b) and Baines and Andrew (2003) which found marking fences reduced *Phasianid* collisions with fencing. Our results provided weak evidence that reflective markers were the most effective marker type in our study, with a 64% (30%–98%) reduction in collision probability. Stevens et al. (2012b) saw an 83% reduction in sage-grouse collisions using reflective markers. The smaller effect observed in our study may be due in part to less resolution to detect covariate effects when using occupancy models compared to abundance measures because counts are summarized to presence or absence. In addition, the smaller effect observed in our study may be partially related to accounting for incomplete detection of sage-grouse collisions, despite detection being quite high. The collision reduction estimated in our study aligns well with the estimated 64% reduction for capercaillie, 91% reduction for black grouse, and 49% reduction for red grouse estimated by Baines and Andrew (2003).

Overall, we found little difference in the effectiveness of the three marker types, as models with a marker effect (for any marker type) had substantially more cumulative AIC_c weight than models with effects for all marker types individually. However, contrary to our hypothesis, Fly Safe markers were slightly less effective than both white and reflective markers. We estimated average per marker costs for white markers at $0.14, reflective markers at $0.71, and Fly Safe markers at $0.40 (USD). Therefore, using the plain white markers without reflective tape, may represent the most cost-effective sage-grouse marking strategy of those we tested. In Europe, the only study to our knowledge, which investigated marker utility in preventing *Phasianid* collisions employed two strips of orange plastic netting on the fence (Baines and Andrew 2003). The authors acknowledged that, although effective in reducing collisions within woodlands, this marker style was not suitable for deployment in areas exposed to weather (i.e., open moorland), where red grouse densities may be high. We witnessed very little damage to the three types of markers we deployed and therefore recommend trials using these marker types in open habitats of Europe.

The effectiveness of the fence markers in reducing *Phasianid* collisions highlights the importance of integrating fence marking into ongoing conservation efforts. Prescribed grazing is often recommended to improve nesting and wintering habitat conditions for lekking-species of conservation concern such as the greater-sage-grouse (Monroe et al. in review) and lesser prairie-chicken (Hagen et al. 2016). Because the implementation of rotational grazing systems involves additional fencing to subdivide an area into several pastures (United States Fish and Wildlife Service, USFWS 2010), we recommend marking exposed fence near leks even in areas thought to have only moderate collision risk due to topography. We suggest fence marking may reduce the potential for ecological traps (Battin 2004) associated with conservation practices that require the creation of additional fencing.

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Appendix A

Table A1
Model set for models explaining variation in detection probabilities (p) of Greater Sage-Grouse fence collisions in Wyoming, 2014–2015. We fit models using the most general small- (θ) and large-scale (ψ) occupancy probability model structures. Because two covariates on each occupancy probability were different measures of similar hypotheses, we included both model structures on each of those parameters. Covariates included to explain variation in detection probabilities included: fixed visit effects (Visit), fixed survey effects (Surv), fixed observer effects (Obs), "trap effects" for the 2nd and 4th visits (Trap), "trap effects" accounting for whether a collision was detected or not on the 1st visit (Trap.2), cloud cover (Cloud), and snow cover (Snow). Model structure on small-scale occupancy included: fence exposure (Fence Exp), proportion of fence segment in high risk areas (Risk), angle of fence in relation to lek (Angle), Year, biweekly (primary) period (Surv), an interaction between post type and marker type (Post × Trt), and an interaction between distance to nearest lek and the count at that lek (Distance × Near Ct). Model structures on large-scale occupancy included: Year and either the sum of lek counts at nearby leks (Lek Ct) or the number of nearby occupied leks (Occ Lek; indicated in ψ column). The number of parameters (npar), Akaike's Information Criterion adjusted for small sample size (AIC_c), difference between a model's AIC_c value and the minimum AIC_c value (ΔAIC_c), and AIC_c weights are included.

ψ	p	npar	AIC_c	ΔAIC_c	Weight
Occ Lek	Null	25	415.082	0.000	0.582
Lek Ct	Null	25	416.051	0.969	0.358
Occ Lek	Snow	26	423.116	8.034	0.010
Occ Lek	Surv	26	423.388	8.306	0.009
Occ Lek	Cloud	26	423.572	8.490	0.008
Occ Lek	Trap2	26	423.582	8.500	0.008
Lek Ct	Snow	26	424.084	9.002	0.006
Lek Ct	Surv	26	424.358	9.275	0.006
Lek Ct	Cloud	26	424.541	9.459	0.005
Lek Ct	Trap2	26	424.551	9.469	0.005
Occ Lek	Surv + Snow	27	432.197	17.115	< 0.001
Occ Lek	Cloud + Snow	27	432.347	17.265	< 0.001
Occ Lek	Snow + Trap2	27	432.355	17.273	< 0.001
Occ Lek	Surv + Cloud	27	432.568	17.486	< 0.001
Occ Lek	Surv + Trap2	27	432.627	17.545	< 0.001
Occ Lek	Trap	27	432.720	17.637	< 0.001
Occ Lek	Cloud + Trap2	27	432.811	17.729	< 0.001
Lek Ct	Surv + Snow	27	433.166	18.084	< 0.001
Lek Ct	Cloud + Snow	27	433.315	18.233	< 0.001
Lek Ct	Snow + Trap2	27	433.323	18.241	< 0.001
Lek Ct	Surv + Cloud	27	433.537	18.455	< 0.001
Lek Ct	Surv + Trap2	27	433.597	18.514	< 0.001
Lek Ct	Trap	27	433.688	18.606	< 0.001
Lek Ct	Cloud + Trap2	27	433.780	18.698	< 0.001
Occ Lek	Obs	28	439.208	24.126	< 0.001
Lek Ct	Obs	28	440.177	25.095	< 0.001
Occ Lek	Visit	28	440.748	25.665	< 0.001
Lek Ct	Visit	28	441.716	26.633	< 0.001
Occ Lek	Surv + Cloud + Snow	28	442.205	27.123	< 0.001
Occ Lek	Surv + Snow + Trap2	28	442.276	27.194	< 0.001
Occ Lek	Snow + Trap	28	442.373	27.290	< 0.001
Occ Lek	Cloud + Snow + Trap2	28	442.426	27.344	< 0.001
Occ Lek	Surv + Trap	28	442.621	27.538	< 0.001
Occ Lek	Surv + Cloud + Trap2	28	442.647	27.565	< 0.001
Occ Lek	Cloud + Trap	28	442.789	27.707	< 0.001
Lek Ct	Surv + Cloud + Snow	28	443.173	28.091	< 0.001
Lek Ct	Surv + Snow + Trap2	28	443.245	28.163	< 0.001
Lek Ct	Snow + Trap	28	443.342	28.260	< 0.001
Lek Ct	Cloud + Snow + Trap2	28	443.394	28.312	< 0.001
Lek Ct	Surv + Trap	28	443.589	28.507	< 0.001
Lek Ct	Surv + Cloud + Trap2	28	443.616	28.534	< 0.001
Lek Ct	Cloud + Trap	28	443.758	28.676	< 0.001
Occ Lek	Snow + Obs	29	449.910	34.828	< 0.001
Occ Lek	Cloud + Obs	29	450.240	35.158	< 0.001
Occ Lek	Surv + Obs	29	450.246	35.164	< 0.001
Lek Ct	Snow + Obs	29	450.877	35.795	< 0.001
Lek Ct	Cloud + Obs	29	451.208	36.126	< 0.001
Lek Ct	Surv + Obs	29	451.215	36.133	< 0.001
Occ Lek	Visit + Snow	29	451.315	36.233	< 0.001
Occ Lek	Visit + Surv	29	451.656	36.573	< 0.001
Occ Lek	Visit + Cloud	29	451.786	36.704	< 0.001
Occ Lek	Visit + Trap2	29	451.786	36.704	< 0.001
Lek Ct	Visit + Snow	29	452.283	37.200	< 0.001

Lek Ct	Visit + Surv	29	452.624	37.542	< 0.001
Lek Ct	Visit + Cloud	29	452.754	37.672	< 0.001
Lek Ct	Visit + Trap2	29	452.755	37.672	< 0.001
Occ Lek	Surv + Cloud + Snow + Trap2	29	453.244	38.162	< 0.001
Occ Lek	Surv + Snow + Trap	29	453.256	38.173	< 0.001
Occ Lek	Cloud + Snow + Trap	29	453.403	38.320	< 0.001
Occ Lek	Surv + Cloud + Trap	29	453.607	38.525	< 0.001
Lek Ct	Surv + Cloud + Snow + Trap2	29	454.212	39.130	< 0.001
Lek Ct	Surv + Snow + Trap	29	454.225	39.143	< 0.001
Lek Ct	Cloud + Snow + Trap	29	454.372	39.290	< 0.001
Lek Ct	Surv + Cloud + Trap	29	454.576	39.494	< 0.001
Occ Lek	Surv + Snow + Obs	30	462.022	46.940	< 0.001
Occ Lek	Cloud + Snow + Obs	30	462.034	46.951	< 0.001
Occ Lek	Surv + Cloud + Obs	30	462.383	47.300	< 0.001
Lek Ct	Surv + Snow + Obs	30	462.989	47.907	< 0.001
Lek Ct	Cloud + Snow + Obs	30	463.000	47.917	< 0.001
Lek Ct	Surv + Cloud + Obs	30	463.351	48.269	< 0.001
Occ Lek	Visit + Surv + Snow	30	463.354	48.271	< 0.001
Occ Lek	Visit + Cloud + Snow	30	463.458	48.376	< 0.001
Occ Lek	Visit + Snow + Trap2	30	463.458	48.376	< 0.001
Occ Lek	Visit + Trap	30	463.600	48.517	< 0.001
Occ Lek	Visit + Surv + Cloud	30	463.780	48.698	< 0.001
Occ Lek	Visit + Surv + Trap2	30	463.799	48.716	< 0.001
Occ Lek	Visit + Cloud + Trap2	30	463.929	48.847	< 0.001
Lek Ct	Visit + Surv + Snow	30	464.321	49.239	< 0.001
Lek Ct	Visit + Cloud + Snow	30	464.425	49.343	< 0.001
Lek Ct	Visit + Snow + Trap2	30	464.425	49.343	< 0.001
Lek Ct	Visit + Trap	30	464.567	49.485	< 0.001
Lek Ct	Visit + Surv + Cloud	30	464.748	49.666	< 0.001
Lek Ct	Visit + Surv + Trap2	30	464.767	49.685	< 0.001
Lek Ct	Visit + Cloud + Trap2	30	464.897	49.815	< 0.001
Occ Lek	Surv + Cloud + Snow + Trap	30	465.335	50.252	< 0.001
Lek Ct	Surv + Cloud + Snow + Trap	30	466.304	51.222	< 0.001
Occ Lek	Visit + Obs	31	474.083	59.000	< 0.001
Lek Ct	Visit + Obs	31	475.051	59.969	< 0.001
Occ Lek	Surv + Cloud + Snow + Obs	31	475.438	60.355	< 0.001
Lek Ct	Surv + Cloud + Snow + Obs	31	476.404	61.322	< 0.001
Occ Lek	Visit + Snow + Trap	31	476.629	61.547	< 0.001
Occ Lek	Visit + Surv + Cloud + Snow	31	476.755	61.673	< 0.001
Occ Lek	Visit + Surv + Snow + Trap2	31	476.775	61.692	< 0.001
Occ Lek	Visit + Cloud + Snow + Trap2	31	476.879	61.797	< 0.001
Occ Lek	Visit + Surv + Trap	31	476.984	61.902	< 0.001
Occ Lek	Visit + Cloud + Trap	31	477.020	61.938	< 0.001
Occ Lek	Visit + Surv + Cloud + Trap2	31	477.201	62.119	< 0.001
Lek Ct	Visit + Snow + Trap	31	477.597	62.515	< 0.001
Lek Ct	Visit + Surv + Cloud + Snow	31	477.723	62.641	< 0.001
Lek Ct	Visit + Surv + Snow + Trap2	31	477.742	62.660	< 0.001
Lek Ct	Visit + Cloud + Snow + Trap2	31	477.846	62.764	< 0.001
Lek Ct	Visit + Surv + Trap	31	477.952	62.870	< 0.001
Lek Ct	Visit + Cloud + Trap	31	477.988	62.906	< 0.001
Lek Ct	Visit + Surv + Cloud + Trap2	31	478.169	63.087	< 0.001
Occ Lek	Visit + Snow + Obs	32	488.636	73.554	< 0.001
Occ Lek	Visit + Cloud + Obs	32	488.987	73.905	< 0.001
Occ Lek	Visit + Surv + Obs	32	488.994	73.912	< 0.001
Lek Ct	Visit + Snow + Obs	32	489.603	74.521	< 0.001
Lek Ct	Visit + Cloud + Obs	32	489.955	74.873	< 0.001
Lek Ct	Visit + Surv + Obs	32	489.962	74.880	< 0.001
Occ Lek	Visit + Surv + Snow + Trap	32	491.496	76.413	< 0.001
Occ Lek	Visit + Cloud + Snow + Trap	32	491.542	76.459	< 0.001
Occ Lek	Visit + Surv + Cloud + Trap	32	491.893	76.811	< 0.001
Lek Ct	Visit + Surv + Snow + Trap	32	492.464	77.382	< 0.001
Lek Ct	Visit + Cloud + Snow + Trap	32	492.510	77.427	< 0.001
Lek Ct	Visit + Surv + Cloud + Trap	32	492.861	77.778	< 0.001
Occ Lek	Visit + Surv + Snow + Obs	33	505.266	90.184	< 0.001
Occ Lek	Visit + Cloud + Snow + Obs	33	505.279	90.197	< 0.001
Occ Lek	Visit + Surv + Cloud + Obs	33	505.653	90.571	< 0.001

Lek Ct	Visit + Surv + Snow + Obs	33	506.233	91.150	< 0.001
Lek Ct	Visit + Cloud + Snow + Obs	33	506.245	91.163	< 0.001
Lek Ct	Visit + Surv + Cloud + Obs	33	506.621	91.539	< 0.001

Table A2
Model set for models explaining variation in small-scale occupancy probabilities (θ) of Greater Sage-Grouse fence collisions in Wyoming, 2014–2015. We fit models using the most parsimonious model on detection probabilities (i.e., null) and large-scale occupancy probabilities (i.e., null). Model structures on small-scale occupancy included: distance to nearest lek (Distance), the count at the nearest lek (Near Ct), fence exposure (Fence Exp), wood post or t-post (Post), proportion of fence segment in high risk areas (Risk), angle of fence in relation to lek (Angle), marker type (Trt), marked or unmarked fence (regardless of marker type; Mark), Year, biweekly (primary) period (Surv), an interaction between Distance and Near Ct, and an interaction between Post and Mark or Trt. The number of parameters (npar), Akaike's Information Criterion adjusted for small sample size (AIC_c), difference between a model's AICc value and the minimum AICc value (ΔAIC_c), and AIC_c weights are included for models with $\Delta AIC_c < 4$.

θ	npar	AIC_c	ΔAIC_c	weight
Fence Exp + Mark + Distance + Post + Risk + Near Ct	9	364.644	0.000	0.030
Fence Exp + Mark + Distance + Post + Risk + Year	9	364.756	0.111	0.028
Fence Exp + Mark + Distance + Post + Risk + Year + Near Ct	10	364.903	0.259	0.026
Fence Exp + Mark + Post + Risk + Distance × Near Ct	10	365.270	0.626	0.022
Surv + Fence Exp + Mark + Distance + Post + Risk + Year	15	365.647	1.003	0.018
Fence Exp + Mark + Distance + Post + Near Ct	8	365.762	1.118	0.017
Fence Exp + Mark + Post + Risk + Year + Distance × Near Ct	11	365.794	1.150	0.017
Surv + Fence Exp + Mark + Distance + Post + Year	14	365.810	1.166	0.017
Fence Exp + Mark + Distance + Post + Year + Near Ct	9	365.998	1.354	0.015
Fence Exp + Mark + Distance + Post + Risk	8	366.015	1.371	0.015
Fence Exp + Mark + Distance + Post + Year	8	366.230	1.586	0.014
Surv + Mark + Distance + Post + Year	13	366.584	1.940	0.011
Surv + Fence Exp + Distance + Post + Risk + Year	14	366.689	2.045	0.011
Surv + Fence Exp + Mark + Distance + Post + Risk	14	366.791	2.147	0.010
Surv + Fence Exp + Mark + Distance + Post + Near Ct	14	366.803	2.159	0.010
Surv + Fence Exp + Mark + Distance + Post + Risk + Near Ct	15	366.871	2.227	0.010
Surv + Fence Exp + Distance + Post + Year	13	366.883	2.239	0.010
Surv + Mark + Distance + Post + Risk + Year	14	366.897	2.253	0.010
Fence Exp + Distance + Post + Risk + Year	8	366.926	2.282	0.010
Surv + Distance + Post + Risk + Year	13	366.997	2.353	0.009
Surv + Distance + Post + Year	12	367.005	2.361	0.009
Angle + Surv + Post + Year	12	367.072	2.428	0.009
Surv + Fence Exp + Mark + Distance + Post	13	367.177	2.533	0.008
Fence Exp + Distance + Post + Risk + Year + Near Ct	9	367.183	2.538	0.008
Angle + Surv + Distance + Post + Year	13	367.336	2.692	0.008
Surv + Fence Exp + Mark + Distance + Post + Year + Near Ct	15	367.365	2.721	0.008
Angle + Surv + Mark + Post + Year	13	367.420	2.776	0.007
Fence Exp + Distance + Risk + Near Ct + Post × Mark	10	367.457	2.813	0.007
Mark + Distance + Post + Risk + Year + Near Ct	9	367.459	2.815	0.007
Fence Exp + Distance + Risk + Year + Post × Mark	10	367.587	2.942	0.007
Fence Exp + Mark + Distance + Post	7	367.590	2.946	0.007
Surv + Fence Exp + Mark + Distance + Post + Risk + Year + Near Ct	16	367.591	2.946	0.007
Fence Exp + Distance + Risk + Year + Near Ct + Post × Mark	11	367.717	3.073	0.006
Angle + Fence Exp + Mark + Distance + Post + Risk + Near Ct	10	367.748	3.104	0.006
Angle + Fence Exp + Mark + Distance + Post + Risk + Year	10	367.821	3.177	0.006
Mark + Distance + Post + Risk + Year	8	367.882	3.238	0.006
Angle + Surv + Mark + Post	12	367.902	3.258	0.006
Mark + Distance + Post + Risk + Near Ct	8	367.992	3.348	0.006
Angle + Surv + Mark + Post + Near Ct	13	368.029	3.385	0.006
Fence Exp + Distance + Post + Risk + Near Ct	8	368.075	3.431	0.005
Surv + Mark + Distance + Post + Year + Near Ct	14	368.076	3.432	0.005
Angle + Surv + Post	11	368.076	3.432	0.005
Fence Exp + Distance + Post + Year + Near Ct	8	368.107	3.463	0.005
Angle + Fence Exp + Mark + Distance + Post + Risk + Year + Near Ct	11	368.160	3.516	0.005
Fence Exp + Distance + Post + Year	7	368.210	3.566	0.005
Mark + Distance + Post + Year + Near Ct	8	368.239	3.595	0.005
Angle + Surv + Mark + Distance + Post + Year	14	368.255	3.611	0.005
Surv + Distance + Post + Year + Near Ct	13	368.264	3.620	0.005
Fence Exp + Mark + Post + Distance × Near Ct	9	368.276	3.632	0.005
Fence Exp + Risk + Post × Mark + Distance × Near Ct	11	368.284	3.640	0.005
Surv + Mark + Distance + Post + Near Ct	13	368.308	3.664	0.005
Surv + Distance + Post + Risk + Year + Near Ct	14	368.328	3.684	0.005
Angle + Fence Exp + Mark + Post + Risk + Distance × Near Ct	11	368.379	3.735	0.005
Angle + Surv + Post + Near Ct	12	368.397	3.753	0.005

Surv + Mark + Distance + Post + Risk + Year + Near Ct	15	368.414	3.770	0.005
Surv + Fence Exp + Distance + Post + Year + Near Ct	14	368.431	3.787	0.005
Distance + Post + Risk + Year + Near Ct	8	368.445	3.801	0.004
Angle + Fence Exp + Mark + Distance + Post + Near Ct	9	368.449	3.805	0.004
Angle + Fence Exp + Mark + Distance + Post + Year	9	368.468	3.824	0.004
Fence Exp + Post + Risk + Year + Distance × Near Ct	10	368.499	3.855	0.004
Fence Exp + Distance + Near Ct + Post × Mark	9	368.531	3.886	0.004
Mark + Distance + Post + Year	7	368.550	3.906	0.004
Surv + Fence Exp + Distance + Post + Risk + Year + Near Ct	15	368.591	3.947	0.004
Mark + Distance + Post + Near Ct	7	368.623	3.979	0.004

Table A3
Coefficient estimates, standard errors (SE), and 95% confidence intervals (CI) for all variables from the best model explaining variation in small-scale occupancy (θ) probabilities of Greater Sage-Grouse fence collisions in Wyoming, 2014–2015. Variables include fence exposure (Fence Exp), whether a fence was marked (regardless of marker type; Mark), the distance to nearest lek (Distance), fences with wood and t-posts (wood and t-post), proportion of fence segment in high risk areas (Risk), and the count at the nearest lek (Near Ct). The intercept represents an unmarked fence with wood posts with all continuous variable values set to 0. All significant coefficients (i.e., 95% CIs do no overlap 0) are indicated by an asterisk.

Parameter	Mean	SE	95% CI
Intercept*	− 5.104	1.068	(− 7.197, − 3.012)
Fence Exp*	0.033	0.013	(0.007, 0.059)
Mark*	− 0.922	0.359	(− 1.623, − 0.217)
Distance*	− 0.500	0.197	(− 0.886, − 0.113)
Wood and T-post*	1.783	0.387	(1.025, 2.541)
Risk*	1.128	0.565	(0.020, 2.235)
Near Ct	0.005	0.002	(0.000, 0.010)

Table A4
Coefficient estimates, standard errors (SE), and 95% confidence intervals (CI) for all variables from the second best model explaining variation in small-scale occupancy (θ) probabilities of Greater Sage-Grouse fence collisioins in Wyoming, 2014–2015. Variables include fence exposure (Fence Exp), whether a fence was marked (regardless of marker type; Mark), the distance to nearest lek (Distance), fences with wood and t-posts (wood and t-post), proportion of fence segment in high risk areas (Risk), and the count at the nearest lek (Near Ct). The intercept represents an unmarked fence with wood posts with all continuous variable values set to 0. All significant coefficients (i.e., 95% CIs do no overlap 0) are indicated by an asterisk.

Parameter	Mean	SE	95% CI
Intercept*	− 5.181	1.090	(− 7.317, − 3.046)
Fence Exp*	0.032	0.013	(0.006, 0.058)
Mark*	− 0.818	0.356	(− 1.515, − 0.121)
Distance*	− 0.650	0.186	(− 1.015, − 0.285)
Wood and T-post*	1.685	0.374	(0.952, 2.418)
Risk*	1.161	0.557	(0.069, 2.253)
2015*	0.875	0.431	(0.030, 1.720)

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A Bighorn Sheep Die-off in Southern Colorado Involving a *Pasteurellaceae* Strain that May Have Originated from Syntopic Cattle

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A Bighorn Sheep Die-off in Southern Colorado Involving a *Pasteurellaceae* Strain that May Have Originated from Syntopic Cattle

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ABSTRACT: We investigated a pasteurellosis epizootic in free-ranging bighorn sheep (*Ovis canadensis*) wherein a *Pasteurellaceae* strain carried by syntopic cattle (*Bos taurus*) under severe winter conditions appeared to contribute to pneumonia in affected bighorns. Twenty-one moribund or dead bighorn sheep were found on the "Fossil Ridge" herd's winter range, Colorado, USA, between 13 December 2007 and 29 February 2008. Eight carcasses examined showed gross or microscopic evidence of acute to subacute fibrinous bronchopneumonia. All eight carcasses yielded at least one β-hemolytic *Mannheimia haemolytica* biogroup $1^{(\pm G)}$ strain, and seven also yielded a β-hemolytic *Bibersteinia trehalosi* biogroup 4^{CDS} strain; evidence of *Pasteurella multocida*, *Mycoplasma ovipneumoniae*, and parainfluenza 3 and bovine respiratory syncytial viruses was also detected. Isolates of β-hemolytic *Manneimia haemolytica* biogroup 1^{G} from a bighorn carcass and a syntopic cow showed 99.5% similarity in genetic fingerprints; *B. trehalosi* biogroup 4^{CDS} isolates were ≥94.9% similar to an isolate from a nearby bighorn herd. Field and laboratory observations suggested that pneumonia in affected bighorns may have been caused by a combination of pathogens including two pathogenic *Pasteurellaceae* strains—one likely of cattle origin and one likely of bighorn origin—with infections in some cases perhaps exacerbated by other respiratory pathogens and severe weather conditions. Our and others' findings suggest that intimate interactions between wild sheep and cattle should be discouraged as part of a comprehensive approach to health management and conservation of North American wild sheep species.

Key words: *Bibersteinia trehalosi*, bighorn sheep, cattle, pneumonia, *Mannheimia haemolytica*, *Mycoplasma*, *Ovis canadensis*, *Pasteurella multocida*.

The decline of bighorn sheep (*Ovis canadensis*) abundance throughout much of western North America appears attributable to historical overharvest, habitat loss or degradation and, in large part, to epizootics caused by introduced pathogens, some of which have now become enzootic. The earliest reports of epizootics in bighorn sheep (e.g., accounts in Warren, 1910; Grinnell, 1928; Shillinger, 1937; Honess and Frost, 1942) closely followed the advent of domestic livestock grazing in bighorn habitat, suggesting that bighorn populations in some areas first may have been exposed to novel pathogens in the 1800s. More than a century later, recurring respiratory disease epizootics remain obstacles to recovering bighorn sheep populations to historic levels (Miller, 2001). Understanding and, where feasible, controlling specific risk factors that may cause or precipitate pneumonia epizootics in bighorn sheep has become an imperative of this species' conservation. Unfortunately, post hoc investigations of epizootics under field conditions rarely yield clear answers regarding source(s) of the responsible pathogen(s) and the role of potential contributing stressors like weather. Here, we describe a case wherein exposure to a pathogen carried by syntopic cattle (*Bos taurus*) under severe winter conditions may have contributed to the onset of epizootic pasteurellosis in a free-ranging bighorn herd. Our objectives are to report the findings of our field and laboratory investigations of this epizootic and to broaden conventional thinking about risk factors that may affect the health and perpetuation of North American wild sheep species.

The "Fossil Ridge" bighorn herd in

southern Colorado, USA (38°30–41′N, 106°34–48′W) was started with a translocation of 20 individuals and had grown to >60 animals by 2006 (George et al., 2009). Available range was restricted during most winters and recreation activity may have further reduced the area occupied by bighorns. As a likely consequence of limited winter range, a local Hereford breed cattle rancher reported that for about 15 yr some bighorns had come into his cattle feed lines on private land at times during fall and winter. On the basis of the belief that such interactions were not particularly risky to bighorn sheep, this behavior was not discouraged by local wildlife managers.

The winter of 2007–08 was one of the most severe in recorded history for the Gunnison Basin (Colorado Division of Wildlife, 2009), an area that included the Fossil Ridge herd's range. On the basis of data compiled at the Gunnison County Electric Association weather station for the United States National Oceanic and Atmospheric Administration, about 51 cm of heavy, wet snow fell during 6–7 December 2007, burying mountain shrub communities across the basin; below-average temperatures ranging from −7 C to −20 C precluded any appreciable snowmelt thereafter. Apparently healthy bighorn sheep were seen on traditional winter range during the week of 16 December, although no lambs from the previous summer were observed.

The epizootic at Fossil Ridge was first reported on 23 December 2007 by the local rancher, who noticed fewer bighorns in the area and subsequently found three carcasses and two sick animals nearby. Clinical signs included depression, thick nasal discharge, and dyspnea, but little coughing. One sick animal was shot; the other was found dead the next day. Subsequent field investigation on 23 and 24 December revealed additional carcasses and sick animals. In the course of discussing the situation, the rancher mentioned finding an adult female bighorn carcass 10 days earlier. He also noted that this had been a particularly bad year for respiratory disease problems in his cattle herd, perhaps because recently purchased replacement animals had "brought something in" (e.g., Frank et al., 2003); however, no previous diagnostic work had been done on the cattle herd.

Twenty-one moribund or dead bighorn sheep were found on the Fossil Ridge herd's winter range between 13 December 2007 and 29 February 2008; three additional carcass remains were found in October 2008. Eight relatively intact carcasses were necropsied; other carcasses were too scavenged, decomposed, or inaccessible to examine. Lung, tonsil, and other select tissues were submitted to the Caine Veterinary Teaching Center (CVTC; University of Idaho, Caldwell, Idaho, USA) for bacterial culture with emphasis on *Pasteurellaceae* (modified from Jaworski et al., 1998), to Microbial Research, Inc. (MRI; Fort Collins, Colorado, USA) and the Washington Animal Disease Diagnostic Laboratory (Pullman, Washington, USA) for *Mycoplasma* spp. culture, and to the CVTC, MRI, and the Colorado State University Veterinary Diagnostic Laboratory (CSUVDL; Fort Collins, Colorado, USA) for PCR assays to detect *Mycoplasma* spp. DNA (Baird et al., 1999; Besser et al., 2008; D. Bade, unpubl. data; G. Weiser, pers. comm.). Antibody titers to parainfluenza 3 (PI3; recent or active infection titer ≥1:256) virus and bovine respiratory syncytial virus (BRSV; recent or active infection titer ≥1:64) were measured by virus neutralization tests at the CSUVDL. Select representative *Pasteurellaceae* isolates from carcasses and live animals (sampling detailed below) were further compared by repetitive DNA sequence genotyping by Newport Laboratories (Worthington, Minnesota, USA) using PCR and boxA1R primer (Goldberg et al., 2006). Select *Mycoplasma* spp. isolates were identified by DNA sequencing at the University of Minnesota Veterinary Diagnostic Labora-

TABLE 1. Respiratory disease agents detected from dead and surviving bighorn sheep (*Ovis canadensis*) and syntopic cattle (*Bos taurus*) during and after the December 2007 epizootic at "Fossil Ridge" in southwestern Colorado, USA. Evidence of infection or exposure came from culture data for *Pasteurellaceae*, from culture and PCR data for mycoplasmas, and from serology data for the two respiratory viruses. See text for methods and interpretation.

	Bighorn sheep			Cattle
Agent	Dead (December 2007), $n=8$	Alive (February–March 2008), $n=10$	Alive (February 2009), $n=11$	Alive (February 2008), $n=27$
Mannheimia haemolytica				
Biogroup 1 (β)[a]	5[b]	0	0	0
Biogroup 1^{G} (β)	7	0	0	1
Biogroup 1^{AG} (β)	1	0	0	0
Biogroup 3 (β)	0	0	2	0
Biogroup 3^{A}	0	0	1	0
Biogroup $16^{AG(\pm E)}$	0	0	0	20
Bibersteinia trehalosi				
Biogroup 4^{CDS} (β)	7	1	0	0
Biogroup $4^{(\pm \text{various})}$	0	5	2	0
Biogroup $2^{(\pm \text{various})}$	0	3	1	0
Pasteurella multocida	5	4	1	0
Mycoplasma spp.	8	3	1	15
Mycoplasma ovipneumoniae	8	sd[c]	sd	9
Mycoplasma bovirhinis	0	sd	sd	9
Bovine respiratory syncytial virus (titer ≥1:64)	2 (of 2)	2	nr[d]	8
Parainfluenza 3 virus (titer ≥1:256)	2 (of 2)	2	nr	19

[a] Isolates showed β-hemolysis on blood agar.

[b] Number of individuals positive; total sample size is shown in the column heading except where noted.

[c] Samples discarded by reference laboratory before species-specific PCR being performed.

[d] Not reported because prior vaccination confounded interpretation of titers.

tory (UMVDL; Saint Paul, Minnesota, United States). Select liver trace mineral concentrations were measured at the CSUVDL using established methods (Rosen et al., 2009) to rule out feed-associated intoxication.

All carcasses examined showed gross and microscopic evidence of acute to subacute fibrinous bronchopneumonia. The predominant microscopic lesion was severe, subacute bacterial bronchopneumonia associated with oat-shaped macrophages, edema, fibrin, and a few neutrophils filling and expanding alveolar spaces. Three dominant β-hemolytic *Pasteurellaceae* strains were recovered (Table 1). All eight carcasses yielded at least one *Mannheimia haemolytica* strain, including biogroup 1^{G} isolates from seven and biogroup 1 isolates (<83% genetic fingerprint similarity to the biogroup 1^{G} strain) from five. A *Bibersteinia trehalosi* biogroup 4^{CDS} strain also was isolated from seven carcasses. In addition, *Pasteurella multocida* (subsp. b or biotype U) was isolated from five carcasses. Lung or tonsil tissue samples from all eight bighorn carcasses tested PCR positive for *Mycoplasma ovipneumoniae* (Table 1). Serology in two cases where blood was available also suggested exposure to PI3 and BRSV. Liver tissue mineral concentrations (mean±95% confidence interval; range) for copper (146.1±78.6 parts per million [ppm] dry weight; 12.1–316 ppm), manganese (6.7±1.4 ppm; 3.8–9.7 ppm), molybdenum (3.7±1.2 ppm; 1.9–5.8 ppm), and zinc (122.7±37.3 ppm; 60.3–208 ppm) were all within acceptable limits (Rosen et al., 2009; CSUVDL, unpubl. data; L. L. Wolfe, unpubl. data), but selenium concentrations (0.7±0.2 ppm; 0.5–1.5 ppm) were lower than reported for healthy bighorns (Rosen et al., 2009).

We captured 10 of the 11 known surviving bighorns (nine adult females and one adult male) via darting about 5 or 9 wk after the die-off was first reported. Two adult females were equipped with very-high-frequency radiocollars and the other eight animals were marked with unique plastic ear tags. We collected blood and oropharyngeal swabs and treated each animal with tulathromycin (DRAXXIN®, Pfizer Animal Health, New York, New York, USA), doramectin (DECTOMAX®, Pfizer Animal Health), and a commercial vaccine containing killed PI3, BRSV, infectious bovine rhinotracheitis virus, and bovine viral diarrhea virus (Triangle 4, Fort Dodge; Fort Dodge, Iowa, USA). In addition, we collected blood and triplicate nasopharyngeal swabs from a subset of the rancher's cattle ($n=27$) and treated all of the syntopic cattle with tulathromycin ($n=70$). We placed swabs in Port-A-Cul™ tubes (Becton, Dickinson and Company, Sparks, Maryland, USA) and hand-delivered one set to MRI and shipped the other overnight to the CVTC for culture and *Mycoplasma* spp. PCR. A third swab in brain–heart infusion broth was submitted to CSUVDL for *Mycoplasma* spp. PCR. Serum antibody titers to PI3 and BRSV were measured by serum neutralization (CSUVDL).

Nonhemolytic strains of *B. trehalosi* biogroups 2 and 4^{CDS} and *P. multocida* were the primary *Pasteurellaceae* isolated from the surviving bighorns (Table 1); *Mannheimia haemolytica* was not isolated. Nonhemolytic *M. haemolytica* biogroup $16^{\mathrm{AG}(\pm\mathrm{E})}$ were the most abundant *Pasteurellaceae* isolated from cattle, although a β-hemolytic *M. haemolytica* biogroup 1^{G} also was isolated from one of the cattle (Table 1); *B. trehalosi* were not isolated from cattle. Three of the surviving bighorns and 15 of the sampled cattle were PCR positive for *Mycoplasma* spp. (Table 1); both *Mycoplama ovipneumoniae* and *Mycoplasma bovirhinis* were detected in the cattle by PCR or culture. A proportion of both the surviving bighorns and the sampled cattle had antibody titers suggesting exposure to PI3 and BRSV (Table 1).

In February 2009, surviving Fossil Ridge bighorn sheep were again baited and recaptured via drop netting. Of 11 animals captured, three (one lamb, two adult females) were unmarked and thus had not been handled in 2008. We sampled and tested bighorns as above and treated each with tulathromycin, doramectin, and two commercial vaccines, Triangle 4 and a *Mannheimia haemolytica* type A1 bacterin-toxoid (One Shot®, Pfizer Animal Health). Cultures yielded β-hemolytic *M. haemolytica* biogroup 3 (Table 1), along with nonhemolytic *B. trehalosi*, *M. haemolytica*, and *P. multocida* isolates. On the basis of numbers of non-*Pasteurellaceae* recovered, shipping and processing delays likely biased culture results. Only the lamb was PCR positive for *Mycoplasma* spp. Cattle were not resampled.

Laboratory findings linked a combination of pathogens to this epizootic. Despite sampling lags and some heterogeneity among the *Pasteurellaceae* isolated from pneumonic bighorns, a β-hemolytic, *M. haemolytica* biogroup 1^{G} isolate from a bighorn carcass showed 99.5% similarity in its genetic fingerprint to the *M. haemolytica* biogroup 1^{G} isolate from one of the syntopic cattle; moreover, these two isolates' fingerprints were ≥95.5% similar to fingerprints of other *M. haemolytica* biogroup 1^{G} isolates from temporally and geographically separate cases of domestic sheep (*Ovis aires*)-associated acute pasteurellosis in bighorns (Foreyt, 1989; George et al., 2008). These findings support the notion that domestic ruminants can harbor *Pasteurellaceae* strains that are pathogenic in bighorn sheep. The β-hemolytic *B. trehalosi* biogroup 4^{CDS} also isolated from most pneumonic Fossil Ridge bighorns (but none of the syntopic cattle) has been recovered from several Colorado bighorn herds (Green et al., 1999; L. L. Wolfe and M. W. Miller, unpubl. data); *B. trehalosi* biogroup 4^{CDS} isolates from both dead and surviving Fossil Ridge bighorns were ≥94.9% similar by genetic fingerprinting to isolates from the nearby Taylor River bighorn herd where this strain (called "ribotype E_{CO}" elsewhere; Green et al., 1999) has been enzootic since at least the early 1990s (M. W. Miller and L. L. Wolfe, unpubl. data). These findings support the notion that enzootic *Pasteurellaceae* also can contribute to pneumonia during epizootics in bighorn sheep. In addition to *Pasteurellaceae*, both bighorns and syntopic cattle showed evidence of exposure to *Mycoplasma ovipneumoniae* (most likely of bighorn origin), PI3, and BRSV.

On the basis of findings from necropsy and live animal sampling, we believe that this pneumonia epizootic was caused by a combination of pathogens including two or more pathogenic strains of *Pasteurellaceae*—a *Mannheimia haemolytica* strain most likely of cattle origin and a *B. trehalosi* strain most likely of bighorn origin—with some cases perhaps exacerbated by exposure to *Mycoplasma* spp. and viruses of cattle or bighorn origin. Despite what we believe to be compelling support for this explanation, however, we recognize that identifying the true cause(s) of this and other pasteurellosis epizootics in bighorn sheep retrospectively under field conditions cannot be done with certainty. For example, interpretation of culture data is complicated by the heterogeneity and dynamics of *Pasteurellaceae* in bighorns and in domestic sheep and cattle (Miller et al., 1997; Jaworski et al., 1998; Miller, 2001; Safaee et al., 2006; Kelley et al., 2007; George et al., 2008; Tomassini et al., 2009), and is further confounded by influences of sample handling and laboratory methods (Safaee et al., 2006; George et al., 2008; Dassanayake et al., 2009a; L. L. Wolfe, unpubl. data) and the potential for pathogenicity to change within strains via horizontal transfer of the gene encoding leukotoxin (Kelley et al., 2007). In addition to the pathogens we detected, weather conditions may have contributed at Fossil Ridge either as a stressor on bighorns or cattle, or simply by increasing interactions between bighorns and cattle (Fig. 1). Notably, however, we did not observe epizootic pasteurellosis in a bighorn herd wintering in the nearby Taylor River drainage despite equally severe winter conditions and the presence of several pathogens also present in the Fossil Ridge herd (β-hemolytic *B. trehalosi* biogroup 4^{CDS}, PI3, BRSV, *Mycoplasma ovipneumoniae*; L. L. Wolfe, unpubl. data), suggesting that the presence of *Mannheimia haemolytica* or *Mycoplasma bovirhinis* in syntopic cattle may have helped trigger the Fossil Ridge epizootic.

FIGURE 1. The intensity and duration of interactions between bighorn sheep and cattle on feed lines during December 2007 may have contributed to the apparent exchange of respiratory pathogens associated with a pasteurellosis epizootic in the "Fossil Ridge" bighorn herd that resided in southern Colorado, USA.

Segregating wild sheep from domestic sheep has long been recognized as important to preventing epizootics in bighorn sheep (Warren, 1910; Shillinger, 1937; Foreyt and Jessup, 1982). Thus far, similar emphasis has not been placed on preventing interactions between cattle and bighorn sheep, most likely because species differences and a tendency toward interspecies avoidance are thought to help minimize opportunities for pathogen exchange (Foreyt and Lagerquist, 1996). However, the similarities between *Pasteurellaceae* and other respiratory pathogens of cattle and domestic sheep suggest similar adverse consequences to bighorn sheep if pathogen transmission were to occur between cattle and bighorns (Onderka et al., 1988; Singer et al., 2000). Such consequences have been demonstrated experimentally: five of eight bighorns died within 4 days of receiving intradermal injections of a cattle vaccine containing attenuated, live *Mannheimia haemolytica* (Onderka et al., 1988), four bighorns died within 2 days after intratracheal inoculation with *M. haemolytica* isolated from cattle (Dassanayake et al., 2009b), and one of five captive bighorns died 6 days after being copastured with Holstein calves (Foreyt and Lagerquist, 1996). We conclude from our findings, combined with other published observations, that intimate interactions between wild sheep and cattle (e.g., shared feed lines or troughs) also should be discouraged as part of a comprehensive approach to health management and conservation of North American wild sheep species.

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