Bighorn movement and domestic sheep presence surrounding a case of acute fatal pneumonia in a bighorn sheep

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ABSTRACT: Mortality of bighorn sheep (*Ovis Canadensis*) after contact with domestic sheep (*Ovis aries*) has been documented in controlled pen studies, and through anecdotal observations in the wild. As a result, euthanasia of bighorns in contact with domestic sheep has become a routine management strategy for controlling introduction of pathogens to bighorn herds. Information regarding bighorn behavior before, during, and after contact with domestics may help further guide management practices. While studying the movements of a bighorn meta-population, we detected an acute pneumonia fatality of a GPS telemetered bighorn after contact with a recently introduced pen containing three hobby domestic sheep, immediately adjacent to a bighorn subherd's home range. GPS data on this and other telemetered bighorn in the vicinity revealed a variety of interesting movements temporally proximate to the contact event. Respiratory disease was not identified in any of the domestic sheep by routine veterinary exam. However, nasal and tonsil swabs confirmed the presence of respiratory disease-associated pathogens in all three domestic sheep. One domestic sheep was culled and necropsied revealing chronic upper respiratory disease. Coincidentally, in the years leading up to this event, this small bighorn subherd was already trending toward extirpation due to an aging herd and near-zero recruitment, likely facilitated by chronic respiratory disease in the herd. Although various factors prevented bighorn euthanasia as a management strategy, the GPS telemetry data from this herd provides insights as to how bighorn sheep movements may be affected by the presence of domestic sheep on the landscape, leading to interspecific contacts.

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INTRODUCTION

Respiratory disease is considered the most important limiting factor of maintaining and growing bighorn sheep (*Ovis canadensis*) populations (George *et al.* 2009, WAFWA 2017). Comingling between bighorn sheep and domestic sheep has been proven to result in fatal pneumonia in numerous experiments using captive animals (Wehausen *et al.* 2011, Subramaniam *et al.* 2011, Besser *et al.* 2014). These comingling experiments appear to be corroborated by various field accounts of wild bighorns dying after coming into contact with domestic sheep (Foreyt and Jessup 1982, Coggins 2002, George *et al.* 2008). In some cases, the wild-domestic interactions are circumstantially implicated for all-age die-off events of bighorn sheep (George *et al.* 2008, Cassaigne *et al.* 2010, Besser *et al.* 2021), causing sudden population declines. In at least two cases to date, the same bacterial genetic strain underlying a bighorn die-off event has even been found in nearby domestic caprinae flocks and is thus presumed to be the infection source (Kamath *et al.* 2019, Besser *et al.* 2021). Aside from a few published circumstantial accounts (George *et al.* 2008, Besser *et al.* 2021), detailed descriptions of natural comingling interactions between bighorn and domestic sheep leading to pathogen transfer are lacking. The ultimate source of these infection episodes are rarely, if ever, known given these transmission events are difficult to predict and presumably uncommon.

Despite a variety of controlled captive experiments that support observations from free-ranging animals, (Wehausen *et al.* 2011), it is not universally accepted by all stakeholders that wild-domestic comingling events can introduce respiratory pathogens to bighorn sheep populations. This is partially due to the inability to collect data during natural comingling events. In the rare instances when these events are detected, management practices to euthanize in-contact animals eliminate the opportunity for monitoring the interactions.

One potential source of wild-domestic comingling are small hobby flocks of domestic sheep housed in the proximity of established bighorn sheep herds. Current patterns of exurban (i.e., ranchette, high density rural) development in Western North America include the placement of homes adjacent to wildlife habitat (Riebsame *et al.* 1996), and this pattern is likely to continue with increased residential development. Exurban hobby domestic sheep are therefore a growing concern due to the risk of transferring disease from domestic to bighorn sheep (Sells *et al.* 2015, Heinse *et al.* 2016).

We provide a case study of a domestic sheep hobby flock introduced to a pen immediately adjacent to a free-ranging bighorn sheep herd, which coincided with the second year of a three-year study on bighorn sheep movements and distribution. One mature (~10 years of age) ram outfitted with a GPS satellite collar died of acute fatal bronchopneumonia ~500 m away from the occupied domestic sheep pen. The domestic sheep were removed by the owners shortly after this mortality was detected. The remaining GPS satellite collared bighorn were monitored for an additional fall season after removal of the domestic sheep.

First, a summary of pathogens detected in the bighorn and domestic sheep is provided. Next, basic descriptive proximity measures are used to test for a shift in the bighorn's space use in relation to when domestic sheep occupied the pen (treatment period) versus when the pen was vacant (control period). Then, a step-selection movement model analysis is used to test the hypothesis that wild bighorn sheep exhibit attraction to domestic sheep (comparing control and treatment periods), by accounting for the spatio-temporally dynamic movements of bighorn ewes, spatio-temporally dynamic snow cover data, and various static landscape characteristics.

Study Area

This study focuses on a subherd (Colorado Bighorn Game Management Unit: S52) of the Central San Juan bighorn sheep population (Colorado Bighorn Data Analysis Unit: RBS-22). The RBS-22 population has ranged from approximately 50 bighorn sheep in 1965 to a peak of 450 in 1989. The S52 subherd once contained ~100 individuals during RBS-22's population peak in 1989. All-age die off events in 1989 and 1993 reduced the RBS-22 population size to 150, but have since slowly rebounded to approximately 325 in 2019. Males between S52 and a neighboring (≥4 miles away) subherd of RBS-22 frequently interact. Although records are sparse (Spicer 1999), contact between domestic and bighorn sheep in summer of 1989 occurred due to a one-time "trailing" permit issued to a nearby domestic sheep grazer of federal land, is suspected to have led to the winter 1989 die-off event in RBS-22. Potentially extirpated (Spicer 1999), the S52 subherd was augmented with approximately 33 bighorns in 2001. Evidence of disease, via low lamb recruitment and pneumonia related mortalities, continued to be exhibited throughout RBS-22 since the 1989 die-off event, and the 1993 die-off event (originating in the S36 subherd of RBS-22). This study's original intent was to monitor the GPS satellite collar movements of a sample of bighorns representing the RBS-22 population over a three-year period (January 16, 2016 - February 15, 2019). Ground surveys were implemented approximately monthly, where vehicle access permitted (GMUs: S52, S-36, and S-53), to monitor lamb survival (via lamb ratios), opportunistically record group composition, and estimate abundance via mark-resight.

METHODS

Bighorn Movement Study Design

Of 40 GPS satellite collars (Vectronic-aerospace, GmbH) deployed in the RBS-22 population, three were deployed in the S52 subherd via chemical immobilization with a dart projector. Collars were programmed to collect a location every four hours. All capture and handling followed that of the Colorado Parks and Wildlife (CPW) Bighorn Capture Guidelines (IACUC protocol # 04-2007). Within S52, GPS collared ewe BH0018 was monitored 2016/01/30 through 2019/01/28, collared ram BH0015 was monitored 2016/06/03 through 2018/11/22, and collared ram BH0020 was monitored 2016/12/09 through 2018/01/25. GPS collar monitoring periods of BH0015 and BH0020 concluded with their deaths, while BH0018 concluded with detonation of the collar's timed drop-off mechanism.

Over the three-year study period, field staff were present 54 days to collect bighorn group locations, composition observations, and resight surveys in S52, of which bighorns were observed on 42 of those days. A maximum of 17 adults (12 rams and 5 ewes) were observed to be present in S52 near the start of the study (June 2016). Simple mark-resight abundance indices (Chapman and Overton) estimated 12.2, 10.1, and 4.9 bighorn in the S52 subherd for 2016, 2017, and 2018 respectively. The S52 subherd was normally divided into ≤ 3 primary social groups. Ewes (and associated lambs) were observed in a single group all but twice and sometimes as solitary individuals during the lambing season. Rams were congregated in one or two groups of 4-7 animals. Ram group social dynamics changed drastically in response to the rut period (fall and early winter) and relatively fluid with inconsistent interchange with the neighboring subherd. Evidence of individual transient bighorns (young rams and 1 yearling ewe) immigrating or foraying into S52 did exist on three survey efforts. No evidence of lambs greater than 2-3 months of age existed the first two years (2016-2017). All resident bighorn groups in S52 were assumed to be known considering a relatively small amount of suitable bighorn habitat and consistent group composition observations across survey efforts.

Using a quasi-experimental design (Butsic *et al.* 2017), we defined the treatment period as the study's second year (2017) fall and early winter, when a group of three domestic sheep (adult female, yearling female, adult male) were introduced to a small pen and remained for approximately four months (~2017/09/27 – 2018/02/07), within 300 m line of site of an area commonly used by the GPS collared bighorns. The pen's structure was physically present on site during the control period, but only occupied by the domestic sheep during the treatment period, allowing us to mask out the influence of other static landscape features on bighorn movement patterns. One GPS collared ram suffered acute fatal pneumonia after presumed infrequent interaction with the domestic sheep pen. No records exist of direct contact between bighorns and the domestic sheep, but one bighorn was documented (via photograph) at the pen's fence. Control periods were assigned as the fall and early winter of the study's first year (2016) and third year (2018) matching the Julian calendar dates of the treatment period. Exact dates of the treatment and control periods varied by individual given that one bighorn capture event occurred in mid-fall of the first year, and two of the bighorn died during the treatment period or during the control period (see exact dates of bighorn GPS collar monitoring above).

Pathogen and Disease Diagnostics

Serum samples were collected at the time of wild bighorn capture to test for presence of *Mycoplasma ovipneumoniae* antibodies (Washington Animal Disease Diagnostic Laboratory). The bighorn ram that died on January 24, 2018 remained in the field under cold conditions and was necropsied five days after death. Lung tissue was fixed in 10% neutral buffered formalin and prepared for histologic examination by paraffin embedding, sectioning at approximately 5 µm, and staining with hematoxylin and eosin. Samples were not suitable for culture, but PCR diagnostics included testing for *Pasteurellaceae* leukotoxin A gene (Fox *et al.* 2015), and *M. ovipneumoniae* (McAuliffe *et al.* 2003) of the lung and upper respiratory sinus tissues. Additional PCR and strain-typing methods were developed for this project, and results of those assays were pending at the time of submission of this paper. The domestic sheep owners agreed to relinquish one adult ram to a local veterinarian for euthanasia (Gunnison Valley Veterinary Clinic), which was then transferred immediately to the authors for necropsy and ancillary diagnostics on 2018/02/07. Lung and upper respiratory sinus tissues were submitted to the Wyoming Game and Fish Health Laboratory for aerobic culture and PCR for *Mannheimia* species, *Bibersteinia* species, *Pasteurellaceae* leukotoxin gene, and *M. ovipneumoniae*. The samples were also tested in-house at the CPW wildlife health lab for *Pasteurellaceae* leukotoxin A and *M. ovipneumoniae*. Prior to euthanasia of the domestic ram, the domestic ram and both domestic ewes were sampled by swabbing of the nasal passages and tonsil crypts. Swabs were preserved in transport media (ESwab: *COPAN Diagnostics Inc.* and BD Culture Swab*, Becton Dickinson and Company*) for bacterial culture and PCR as described above for the domestic sheep necropsy tissues (Wyoming Game and Fish Health Laboratory). Lung and upper respiratory sinus tissues from both the bighorn ram mortality and the euthanized domestic ram were submitted to the Texas A&M Veterinary Medical Diagnostic laboratory for a PCR-based respiratory pathogen screen including bovine respiratory syncytial virus, bovine viral diarrhea virus, bovine parainfluenza-3 virus, bovine herpesvirus-1, and bovine coronavirus.

Basic Proximity Analysis

For each bighorn GPS collar location, a proximity measure (Euclidean distance) was calculated with respect to the domestic sheep pen's location and the other bighorns' temporally dynamic location. Proximity was summarized by mean and median values. We used a generalized additive model (R package: 'MGCV') with a first order autoregressive correlation structure, accounting for serial dependencies in the GPS collar location data, to assess whether statistically significant differences in the proximity measure existed between the control and treatment periods. With the proximity as the response variable, a model with a factor indicating domestic sheep occupancy of the pen (experimental group), was compared using delta AIC scores to a null model without the experimental group factor.

Movement Analysis

A step-selection function based movement model (Thurfjell *et al.* 2014) was used to measure attraction of bighorns to various landscape features including the domestic sheep pen. Each bighorn sheep GPS collar location were input as "use" locations. For each ending node of a movement step, a set of 20 matched available locations were generated by projecting a set of potential step distances and turning angles from the initial node of the step. These available locations were ones that a bighorn could have chosen at the end of a step but did not for whatever reason (Thurfjell *et al.* 2014). Each matched available location was generated by drawing a random step length and turning angle from a negative binomial and wrapped normal distribution (respectively). Mu and theta parameters of the negative binomial distribution, and mu and rho parameters of the wrapped normal distribution, were derived from the observed step length and turning angle for each location. Generated step distances were restricted to be less than 20 km. Spatial environmental covariates of used (response variable = 1) and available locations (response variable = 0) were compared in a case-control design using a conditional logistic regression model (Therneau 2012). Each pair of the used corresponding available locations were assigned as stratum cases to partition variance appropriately.

A candidate set of models, based on all combinations of 28 landscape covariates found in prior studies to influence bighorn sheep habitat selection, was built to inform basic landscape use decisions, outside those potentially determined by the locations of other bighorn or domestic sheep on the landscape. This basic model included topographic variables (slope, roughness, terrain ruggedness, aspect, topographic position index, and distance to perennial stream), vegetation landcover based (grass patch, canopy cover percentage, and distance to forest edge), daily winter precipitation (snow depth and snow water equivalent), and anthropogenic covariates (Euclidean distance to nearest roofed structure, distance to nearest road). The roofed structure covariate included a shed (barn) in the middle of the domestic sheep pen, but also all other houses and other man-made buildings within the vicinity of S52. Various spatial scales and forms of the topographic covariates regarding aspect, terrain ruggedness, and topographic position, were tested. Winter precipitation metrics were available at a daily temporal resolution, where snow depth and snow water equivalent for each step's use and available location was updated accordingly. Collinear predictor variables were screened with a Pearson correlation coefficient threshold of 0.6. Model selection was then used to choose the most parsimonious candidate models (Burnam and Anderson 2002) to create a baseline bighorn movement selection model that disregards bighorn selection with respect to the domestic sheep pen's location or other bighorns. Data for all three bighorns were consolidated in this model.

In a second iteration of model construction, the proximity (Euclidean distance) to the domestic sheep pen was included as a covariate. This model was run separately for each of the three collared bighorns and the two experimental groupings to allow comparison of avoidance/attraction coefficients across individuals and to contrast the treatment period to the control period within each individual. The conditional logistic regression beta coefficient estimates (+/- 95% confidence intervals) for domestic pen proximity were extracted for the control and treatment periods. Inverse of coefficient estimates were displayed to ensure that positive coefficients indicate attraction to the domestic sheep pen, while negative coefficients indicate avoidance of the domestic sheep pen. Coefficient estimates near zero (approximately -0.2 to 0.2) with confidence intervals overlapping zero indicated ambivalence to the domestic sheep pen.

In a third iteration of the model, the proximity of the collared bighorn rams to each other, on a movement step-by-step basis, were added as covariates to the baseline model. Beta coefficient estimates for these paired bighorn proximity metrics were extracted and examined similarly to the domestic sheep pen coefficients for each bighorn and experimental grouping.

RESULTS

Pathogen and Disease Diagnostics

Results of the respiratory diagnostics conducted on eight of the estimated 12 resident bighorns known to exist in S52 during the study, and all three domestic sheep inhabiting the pen, are shown in Table 1. Four of the eight bighorns showed evidence of exposure to *M. ovipneumoniae* based on serology, or active infections with *Mycoplasma ovipneumoniae* based on PCR diagnostics. Three of the eight bighorns were PCR positive for *Pasteurellaceae* leukotoxin A in lung and sinus lining tissues. The collared bighorn ram (BH0020) that died near the domestic sheep pen was determined to have acute bronchopneumonia via gross pathology and histopathology, and confirmed to have *Pasteurellaceae* leukotoxin A and *M. ovipneumoniae* present in sinus lining and lung tissues via PCR. BH0020 serology was also positive for *M. ovipneumoniae* antibodies when captured approximately one year earlier.

Necropsy examination of the adult domestic ram showed abundant fat stores, an overall body condition within normal limits, and evidence of chronic respiratory disease including chronic sinusitis of the nasal and ethmoid turbinates, and mild chronic tracheitis. PCR of cultured swabs (nasal and tonsil swabs) from the domestic sheep verified hemolytic *Bibersteinia trehalosi, Mannheimia hemolytica, Pasteurella multocida, Pasteuellaceae* leukotoxin A, and *Mycoplasma ovipneumoniae.* Overall, different sample sources within and among the three domestic sheep revealed slightly differing bacteriology results.

Table 1. Respiratory pathogens detected in S52 during the study for wild bighorn sheep and domestic sheep.

Species	Animal ID	Date	Source	Method	Respiratory Pathogens/Diagnostic
Wild Bighorn	BH0018 (collared ewe)	2016/01/30	Serum	Antibody	Not detected
	BH16_225 (unmarked ram)	2016/02/11	Whole carcass	Gross- and histo-pathology	Not detected
			Serum	Antibody	*M. ovipneumoniae*
	BH17_089 (unmarked ewe)	2017/01/16	Sinus lining	PCR	Not detected
	BH18_235 (unmarked ram)	2017/05/27	Lung	PCR	Not detected
			Sinus lining	PCR	Not detected
	BH0020 (collared ram – disease related mortality)	2016/12/09	Serum	Antibody	*M. ovipneumoniae* positive
		2018/01/29	Whole carcass	Gross- and histo-pathology	Acute bacterial bronchopneumonia
		2018/01/29	Lung	PCR	*Pasteurellaceae* leukotoxin A, *M. ovipneumoniae*
		2018/01/29	Sinus lining	PCR	*Pasteurellaceae* leukotoxin A, *M. ovipneumoniae*
Domestic Sheep	DS18_219 (adult ram)	2018/02/07	Whole carcass	Gross- and histo-pathology	Chronic sinusitis
			Lung	PCR	Hemolytic *B. Trehalosi, Mannheimia hemolytica, P. multocida*
			Sinus lining	PCR	*Pasteurellaceae* leukotoxin A*, Mannheimia hemolytica, P. multocida, M. ovipneumoniae*
			Tonsil swab	PCR & culture	Hemolytic *B. Trehalosi, Mannhaeimia hemolytica, P. multocida*
			Nasal swab	PCR & culture	*Mannhaimia hemolytica, Bibersteinia trehalose, P. multocida, M. ovipneumoniae*
	DS18_220 (yearling ewe)	2018/02/07	Nasal swab	PCR & culture	*M. ovipneumoniae*
			Tonsil swab	PCR & culture	*Pasteurellaceae* leukotoxin A, *Bibersteinia trehalosi*
	DS18_221 (adult ewe)	2018/02/07	Nasal swab	PCR & culture	Not detected
			Tonsil swab	PCR & culture	*Pasteurellaceae* leukotoxin A, *Mannheimia hemolytica, Mannheimia glucosida*
Wild Bighorn	BH18_635 (unmarked ram)	2018/04/14	Whole carcass	Gross- and histo-pathology	Not detected
			Lung	PCR	Not detected
			Sinus lining	PCR	Not detected
	BH18_663 (unmarked ewe – disease related euthanasia)	2018/05/14	Whole carcass	Gross- and histo-pathology	Chronic bronchopneumonia
			Lung	PCR	*Pasteurellaceae* leukotoxin A, *M. ovipneumoniae*
			Sinus lining	PCR	*Pasteurellaceae* leukotoxin A, *M. ovipneumoniae, Mannheimia* w/leukotoxin
			Nasal swab	PCR	*M. ovipneumoniae*
	BH0015 (collared ram)	2018/11/22	Whole carcass	Gross- and histo-pathology	Not detected
			Lung	PCR	*Pasteurellaceae* leukotoxin A
			Sinus lining	PCR	*Pasteurellaceae* leukotoxin A

However, we detected *P. multocida* and *M. hemolytica* in all samples from this ram. Results from a PCR-based respiratory virology panel did not indicate presence of bovine respiratory syncytial virus, bovine viral diarrhea virus, bovine parainfluenza-3 virus, bovine herpesvirus-1, or bovine coronavirus.

Basic Proximity Analysis

Mean and median proximity measures for each bighorn and experimental group with respect to the domestic sheep pen are shown in Table 2. Proximity to the pen significantly differed between the control and treatment periods for ram BH0015, ram BH0020, and ewe BH0018 based on delta AIC scores comparing a model with the experimental group variable to a null model without (Table 2). During the treatment period, the two collared rams were usually closer to the pen, while BH0018 appeared to be usually further from the pen. Including the experimental grouping variable into the statistical model explaining proximity to the pen greatly improved model parsimony (ΔAIC) for BH0018 and BH0020: BH0018 was significantly further from the pen and BH0020 was statistically significantly closer to the pen when domestic sheep were occupying it during the treatment period. However, the change in proximity was much less explainable by the experimental grouping for BH0015 due to only minor improvement (ΔAIC = 3.4) of AIC change over the null model.

Mean and median proximity measures among the unique bighorn sheep pairings by experimental group are shown in Table 3. Proximity between bighorns significantly differed between the control and treatment periods for all three pairings based on delta AIC scores comparing a model with the experimental group variable to a null model without (Table 3). During the treatment period, bighorn rams were a median 2.52 (BH0015) and 1.89 (BH0020) times further away from bighorn ewe BH0018, than during the control period (Table 3)."

Movement Analysis

The most parsimonious bighorn movement step-selection model was informed by a combination of four topographic covariates (southeastern aspects, terrain ruggedness at the 250 m scale, terrain ruggedness at the 2000 m scale, and topographic position at the 500 m scale), four vegetation covariates (grass habitat landcover type, canopy cover percentage, distance to nearest forest edge, and the interaction between canopy cover and forest edge), and one anthropogenic covariate (quadratic form of distance to nearest road). Spatio-

Table 2. Mean, median, and minimum distance (m) proximity of three collared bighorn sheep to pen during control (domestic sheep absent) and treatment (domestic sheep present) experimental grouping periods. Statistical significance described by comparing AIC of a null model with the experimental grouping. Increasing AIC, when > 2, indicate greater significance and effect size.

Bighorn ID	Control			Treatment			Significance (ΔAIC improvement over null model)
	Mean	Median	Minimum	Mean	Median	Minimum	
BH0015	3704.1	3202.6	298.0	2685.0	1286.8	316.8	3.4
BH0018	2017.2	1790.9	270.8	2408.7	1887.1	339.6	93.1
BH0020	1285.6	1053.4	489.3	1033.8	733.6	338.5	252.6

Table 3. Mean and median distance (m) proximity of three collared bighorn sheep to each other during control (domestic sheep absent) and treatment (domestic sheep present) experimental grouping periods. Statistical significance described by comparing AIC of a null model with the experimental grouping. Increasing AIC, when > 2, indicate greater significance and effect size.

Bighorn Pairing	Control		Treatment		Significance (ΔAIC improvement over null model)
	Mean	Median	Mean	Median	
BH0018 & BH0015	3375.0	1976.3	6242.1	4944.8	77.9
BH0018 & BH0020	562.9	532.4	3911.5	1011.5	56.4
BH0015 & BH0020	452.4	38.7	634.1	26.0	12.8

temporally dynamic snow covariates (snow depth and snow water equivalent) and distance to nearest roofed structure were not explanatory variables in the most parsimonious model. Coefficients and model selection outputs for the most parsimonious baseline model are available upon request.

Expanding this baseline model with the additional pen proximity covariate and running the model individually, bighorn's attraction to the pen varied according to the experimental grouping (Figure 1). During the control period, all three bighorns were ambivalent to the domestic sheep pen's location; 95% confidence intervals of the beta coefficient greatly overlapped zero (Figure 1). During the treatment period, the degree of attraction to the pen (when occupied by the domestic sheep) increased for all three bighorn (Figure 1). Ewe BH0018 showed the least attraction and was slightly insignificant given the lower confidence interval extending to just -0.14. Compared to the other two bighorn, ram BH0020 showed a significantly high level of attraction to the pen during the treatment period (Figure 1).

Applying the baseline model to each ram separately and adding the proximity of ewe BH0018 as a covariate showed mixed results.

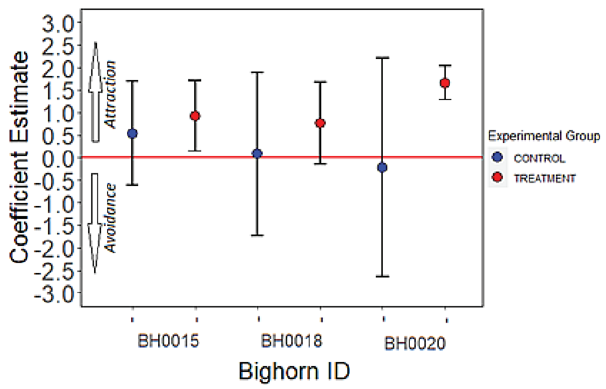

Figure 1. Step-selection movement behavior analysis by individual bighorn with respect to the domestic sheep pen's location during the control and treatment periods. Positive and negative coefficients represent attraction and avoidance respectively. Error bars (95% confidence intervals of the model selection coefficient) overlapping zero represent ambivalence behavior.

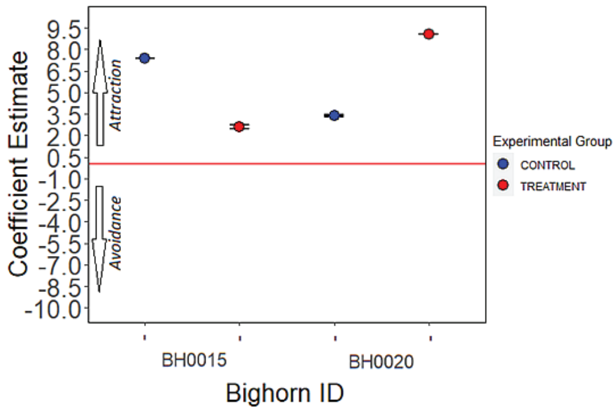

Figure 2: Spatio-temporally dynamic step-selection movement analysis by the individual bighorn rams with respect to the GPS collared bighorn ewe's (BH0018) locations during the control and treatment periods. Positive and negative coefficients represent attraction and avoidance respectively. Error bars (95% confidence intervals of the model selection coefficient) overlapping zero represent ambivalence behavior.

Although both bighorn rams were always attracted to the bighorn ewe, BH0015 showed less attraction to the ewe during the treatment year (relative to the control period), while BH0020 showed more attraction to the ewe during the treatment year (relative to the control period) (Figure 2).

DISCUSSION

In this case study, we provide a rigorous demonstration of free-ranging bighorn sheep movements in the presence of three penned domestic sheep. One wild bighorn sheep demonstrated behaviors suggesting attraction to the penned sheep, and subsequently died due to acute fatal pneumonia in the same season.

Step-selection movement models of the bighorn showed ambivalence toward the pen during the control periods (the year before and year after pen was occupied by the domestic sheep), and attraction during the treatment period, when the domestic sheep were present. The attraction was statistically significant in both rams (BH0015 and BH0020), but not for the ewe (BH0018). Of the three, ram BH0020 demonstrated the highest degree of attraction, and BH0020 was also anecdotally observed at the pen's fence line during the treatment period, while no anecdotal evidence exists of BH0015 and BH0018 at the pen. Ram BH0020 is also the only bighorn known to have died of pneumonia during the domestic sheep's occupation of the pen.

The attraction of BH0015 and BH0020 to the domestic sheep was not explainable by bighorn ewes. The two resident bighorn ewes in S52, with movements represented by GPS collared BH0018, were nearly always observed together during the study. Although both ram BH0020 and BH0015 were very attracted to ewe BH0018, the ewe was statistically further away from the domestic sheep pen during the treatment period, and showed little attraction to the pen, if any.

Other environmental variables do not explain the bighorn movements and proximity to the pen. In a nearby (~65 km) case study, extreme snow depths were implicated as a factor for increasing interactions (and subsequent all-age die off event) between a wild bighorn sheep herd and a winter-feeding operation of domestic cattle (Wolfe *et al.* 2010). However, snow depth was not a driver in our baseline movement model selection results, and the treatment year's snow depth was lower than the control year's (National Operational Hydrological Remote Sensing Center data). Other geographic covariates, which would be associated with inherent annual differences in vegetation quality and quantity did not provide a confounding explanation for the attraction observed. Bighorn's forage quantity and quality appeared excellent for the bighorn sheep during the treatment period and snow was not limiting bighorn access to forage as seen elsewhere (Wolfe *et al.* 2010); there is no reason to believe that bighorn were seeking the domestic sheep's food.

Respiratory pathogens were detected in all three of the domestic sheep, and chronic respiratory disease was observed in the domestic ram at necropsy. However, clinical disease was not observed in the domestic sheep by the owners or at a veterinary examination performed at the time that the ram was euthanized. Asymptomatic bighorn sheep and domestic sheep individuals are well documented in the wild and in confinement. Other wild bighorn and domestic sheep, marked or unmarked, may have been an undetected pathogen source for BH0020. Ewe BH18_663, was euthanized five months after ram BH0020's death and was revealed to have a case of chronic pneumonia that likely pre-dated the arrival of the domestic sheep. This uncollared ewe was known to interact with the ram (BH0020) that died. During the treatment period, BH0020 made one long distance foray (~35 km straight-line) into an area unoccupied by bighorn but which had recently been occupied by a large band of domestic sheep from a nearby federal allotment. Stray domestic sheep from multiple federal grazing allotments near (7 – 35 km) S52, each with a history of stray domestic sheep, may have also made contact with BH0020.

Poor herd performance characterized S52 long before this study, likely due to chronic pneumonia in lambs, which can impact bighorn population performance for over a decade (Grigg *et al.* 2017, Manlove *et al.* 2016). Other respiratory pathogens were detected in S52 bighorns prior to the arrival of the hobby domestic sheep (Table 1). Based on ground observations, none of the five lambs detected at 1-3 months of age in S52 during the first two years of the study reached adulthood. Facing extirpation, the S52 subherd was reduced to one resident ewe (BH0018) expected to be the sole matrilineal founder moving forward. In the three years (2018 – 2020) subsequent to the deaths of the other S52 bighorn ewes, all of BH0018's offspring survived to at least one year of age.

Efforts are ongoing at the time of this publication's submission date to strain type the *Pasteurellaceae* and *M. ovipneumoniae* bacteria detected in the wild and domestic sheep. This pairing of movement observations and strain typing diagnostics may be useful in future efforts to document wild/domestic sheep interactions and pathogen transfer.

Studies in free-ranging animals have unavoidable limitations and challenges. Regardless of strain-typing outcomes, this study cannot explicitly prove that directional disease transfer from domestics to wild sheep took place, as a full examination of pathogens present in the wild and domestic sheep was not conducted before the arrival of the domestic sheep. Proving directional disease transfer from domestics to wild sheep can be done in experimental pen studies where the interactions between wild and domestic sheep are controlled. However, experimental pen studies of confined animals can be accused of not representing realistic field conditions. In the wild, quasi-experimental analysis (Butsic *et al.* 2017) have potential to shed light on bighorn movement (as done in our retrospective movement analysis), but pre-comingling pathogen data of both wild and domestic sheep are impractical to collect, as comingling events are difficult to predict. Also, if a wild-domestic comingling event was known to be imminent, wildlife managers will often err on the side of caution and take action through wild bighorn euthanasia or removal of domestics to prevent comingling. We implore wildlife management agencies and land managers to engage in research efforts that capitalize on inevitable wild and domestic sheep interactions with a carefully planned prospective analysis of the pathogens in wild and domestic parties before and after interactions occur.

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