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BIGHORN SHEEP DEMOGRAPHICS FOLLOWING PNEUMONIA DIE-OFF EVENTS

by

Kylie Sinclair

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Wildlife Biology

Approved:

Kezia Manlove, Ph.D. Major Professor	Daniel MacNulty, Ph.D. Committee Member
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UTAH STATE UNIVERSITY
Logan, Utah

2021

ABSTRACT

Bighorn Sheep Demographics Following Pneumonia Die-off Events

by

Kylie Sinclair, Master of Science

Utah State University, 2021

Major Professor: Dr. Kezia Manlove
Department: Wildland Resources

Bighorn sheep (*Ovis canadensis*) populations across the Intermountain West are subject to disease pressure from the respiratory pathogen *Mycoplasma ovipneumoniae*. Although the effects of *M. ovipneumoniae*-associated disease die-offs on populations are well documented, less is known about the factors driving long-term variation in post-die-off demographic responses. While many herds experience years to decades in which recruitment is less than 20 lambs per 100 ewes, some herds' lamb survival rates are able to rebound rapidly following die-off events. Until lamb recruitment improves, population numbers will remain low. The reason why these herds recover quickly while others do not is currently unknown. Here, I assess the roles environmental, demographic, and pathogen-associated factors could play in shaping bighorn sheep herd recovery through improved lamb recruitment. My analysis relies on more than 30 years of data from over 58 bighorn sheep herds across the state of Nevada. My results suggest that herd demographic responses to *M. ovipneumoniae* vary across desert (*O. canadensis nelson*), California (*O. canadensis* *californiana*), and Rocky Mountain (*O. canadensis canadensis*) subspecies, and that environmental factors may be more important in shaping those demographic responses in desert bighorn than in Rocky Mountain or California bighorn herds. My results could have important implications on prioritization of bighorn sheep recovery efforts throughout the Intermountain West.

(96 pages)

PUBLIC ABSTRACT

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Bighorn sheep populations across the Intermountain West are subject to disease pressure from the respiratory bacteria *Mycoplasma ovipneumoniae*. Although the effects of *M. ovipneumoniae*-associated disease die-offs are well documented, less is known about the factors driving long-term differences in post-die-off population responses. While many herds experience years to decades in which recruitment is less than 20 lambs per 100 ewes, some herds' lamb survival rates are able to rebound rapidly following die-off events. The reason why these herds recover quickly while others do not is currently unknown. Here, we assess the roles environmental, demographic, and pathogen-associated factors could play in shaping bighorn sheep herd recovery. Our analysis relies on more than 30 years of data from over 58 bighorn sheep herds across the state of Nevada. Our results suggest that herd demographic responses to *M. ovipneumoniae* vary dramatically across subspecies, and that environmental factors may be more important in shaping those demographic responses in desert bighorn than in Rocky Mountain or California bighorn herds. Our results could have important implications on prioritization of bighorn sheep recovery efforts throughout the Intermountain West.

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Kylie Sinclair

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CHAPTER 1

INTRODUCTION

Emerging infectious diseases play a significant role in shaping wildlife population dynamics, both in the immediate aftermath of invasion, and also in the longer-term transition to endemism (Frick et al. 2010, Monello et al. 2014). While the etiology and immediate term effects of many diseases are reasonably well-documented, less is usually known about longer-term demographic consequences of pathogen establishment. This is true for a variety of wildlife disease systems, including white-nose syndrome in bats (Lilley et al. 2017), *Batrachochytrium dendrobatidis* in amphibians (Lips 2016), and chronic wasting disease in ungulates (DeVivo et al. 2017). We also find this to be true in regards to pneumonia in bighorn sheep (*Ovis canadensis).*

Like many species across the Intermountain West, bighorn sheep numbers have declined since the late 1800s due to overhunting and habitat encroachment (Spraker et al. 1984). These declines are exacerbated beyond what is typical of many big game species due to the additional burden of pneumonia caused by the bacteria *Mycoplasma ovipneumoniae* (*M/. ovipneumoniae*). This pathogen is carried asymptomatically by domestic sheep (*Ovis aries*) (Besser et al. 2013, Manlove et al. 2019) and transmitted to bighorn sheep through direct contact (Fox et al. 2011). Pneumonia outbreaks result in mortality rates ranging from 10% to 90% of the affected herd (Cassirer et al. 2018). After the initial outbreak, most surviving individuals acquire immunity to the infecting strain (Plowright et al. 2013) but some individuals continue to carry the pathogen asymptomatically

(Plowright et al. 2017) and can transmit it onward to lambs in subsequent years to decades, causing summer lamb mortality rates in excess of 80% (Cassirer and Sinclair 2007, Cassirer et al. 2018, Manlove 2016). Some herds, however, appear to clear the pathogen completely following the initial die-off, sparking a return to pre-introduction population dynamics (Manlove et al. 2016). The particular factors associated with this intermediate-term divergence in population responses following die-off events remain poorly understood.

Mycoplasma ovipneumoniae is a bacterial agent known to cause respiratory disease in Caprinae (Handeland et al. 2014, Nicholas et al. 2008, Plowright et al. 2017) and is frequently found in domestic sheep (Manlove et al. 2019). While infected domestic sheep are largely asymptomatic, infected bighorn sheep are subject to potentially severe disease (Besser et al. 2014). *M. ovipneuomoniae* impedes the ciliary escalator in an infected individual, allowing for downward progression of pathogens into the lower respiratory tract where pneumonia develops (Cassirer et al. 2018). Individuals who survive the initial disease event are able to clear the infection from the lower respiratory tract, but, for unknown reasons, are not always able to clear *M. ovipneumoniae* from the upper respiratory tract (Fig. 1.1).

Here I consider a suite of environmental, demographic, and pathogen-associated factors that could play a role in determining post die-off demographic dynamics. These include herd substructuring and density, host behavior and phenology, host physiology, host genetics, *M. ovipneumoniae* strain type, pathogen community, and environmental context (Fig. 1.2).

Herd substructuring and density

Bighorn sheep are social animals and frequently form subherds within a herd (Hass 1997). The stability of these subherds change between seasons and herd . I would expect herds with stable, discrete groups to have limited pathogen spread due to incomplete exposure, leading to local pathogen fade-out and promoting a quicker population recovery following a pathogen introduction event (Manlove et al. 2014). Well-mixed herds with individuals frequently changing between subherds would propagate pathogen spread, synchronizing infection and exposure statuses across the herd. Bighorn sheep also form sexually segregated groups (Ruckstuhl 1998), with rams and ewes rarely mixing outside of the mating season (rut) in the fall. This seasonal change in herd mixing dynamics introduces additional opportunities for pathogen introduction and spread (O'brien et al. 2014). Pathogen transmission rates often scale with population density (Begon et al. 2002; McCallum et al. 2001). Though some evidence suggests that this may not be the case at the population-level in bighorn sheep (Manlove et al. 2014), it certainly plays a role within groups. Additionally, predation and related apparent competition both place substantial constraints on bighorn sheep populations in some regions (Johnson et al. 2013; Rominger 2018).

Density could alter post-invasion disease dynamics in several ways. First, positive density-dependence could become relevant if populations decline to sizes where Allee effects (manifest through predation, mate finding, or inbreeding among others) become important drivers in the system.

On the other hand, negative density dependence might operate in two relevant ways. First, negative density dependence could shape pathogen transmission dynamics (especially for “low-contact” modes of transmission like respiratory droplets). Under density dependent transmission, the host population would be expected to eventually rebound once densities are reduced to a level where the pathogen can no longer reliably transmit. Second, negative density-dependence might become evident due to constraints imposed by environmental carrying capacities for populations that rebound rapidly.

Genetics

Genetic diversity can have important implications for population longevity. There is good evidence that genetic diversity has direct effects on population growth rates in some bighorn sheep herds (Hogg et al. 2006), though that signal may be confounded by the intensive translocation history of bighorns in Nevada (Jahner et al. 2019). Herds with reduced genetic diversity would be ideal candidates for augmentation efforts to increase that diversity.

Behavior and phenology

Variation in behavior among bighorn sheep subspecies may have an impact on spread of disease. California (*Ovis canadensis californiana*) and Rocky Mountain (*O. canadensis canadensis*) bighorn sheep from subherds of 10-20 sheep within a population. They also have a compact birth pulse in the spring as a result of a compact rut in the fall (Hogg 1984). Desert bighorn sheep (*O. canadensis nelson*) tend to form smaller groups and have a broad birth pulse that can last for months (Hass 1997). Lambs tend to play within a cohort of other lambs around their age (Berger 2009), so this wide birth pulse could limit lamb-to-lamb contact and reduce the opportunity for pathogen spread.

Mycoplasma ovipneumoniae **exposure status**

Mycoplasma ovipneumoniae polymerase chain reaction (PCR) tests are used to identify actively infected animals. Competitive enzyme-linked immunosorbent assay (cELISA) tests are used to measure individual titers. While titers are only a proxy on the immune system response and not on current infection, elevated titer counts can be indicative of a carrier ewe in the population. This makes cELISAs an important metric for monitoring post-die-off herd health.

The broader pathogen community

Bighorn sheep are susceptible to additional pathogens of concern. Epizootic hemorrhagic disease and bluetongue viruses are transmitted by midges and capable of causing morbidity and mortality in bighorn sheep (Noon et al. 2002; Robinson et al. 1967; Singer et al. 1998). They typically do not have significant implications for populations but could have more severe impacts for bighorn sheep populations already struggling with pneumonia. Because these hemorrhagic diseases are closely related and because they are known to produce cross-reactive serological signals (Gorman 1990), I considered their effect as a single variable.

Another pathogen of concern are bacteria from the *Pasteurellaceae* family. These bacteria, like *M. ovipneumoniae*, are capable of causing fatal pneumonia in bighorn sheep (George et al. 2008; Shanthalingam et al. 2014), but not usually at the population scale of *M. ovipneumoniae* events, nor with the long-term effects. However, the presence of *Pasteurellaceae* bacteria during *M. ovipneumoniae* events could exacerbate the problem.

Environmental context

Environment can be a driver of host behavior, phenology, physiology, and substructuring. Here, I focused on the effects of drought across varying landscapes. I expected the effects of drought differ across geographic location and subspecies. In the north, drought restricts vegetation availability and can alter the timing of green-up (Festa-Bianchet 2018). Low snowpack and precipitation lead to poor quality forage, while too much snow may delay green-up and cause it to taper quickly, reducing the window of high quality forage (Pettorelli et al. 2007). In the south, drought reduces water availability, an already and valuable resource in the desert (Longshore et al. 2009).

Project objectives

The goal of this project was to consider a suite of environmental, demographic, and pathogen-associated factors that could play a role in determining post-die-off demographic dynamics in bighorn sheep populations. In Chapter 2, I examine *M. ovipneumoniae* dynamics in bighorn sheep herds across Nevada. I consider temporal trends of *M. ovipneumoniae* in the years following die-offs and prevalence and seroprevalence trends between bighorn sheep subspecies. I also take a closer look at overall serological trends and consider re-assessment for a lower cut-off value to call an individual seropositive for *M. ovipneumoniae*. In Chapter 3, I construct a state-space model to analyze the effects of proposed drivers on bighorn sheep populations on lamb recruitment in the wake of pneumonia-related die-offs using demographic bighorn sheep data from Nevada. Finally, in Chapter 4, I summarize my results and draw conclusions for management purposes.

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TABLES AND FIGURES

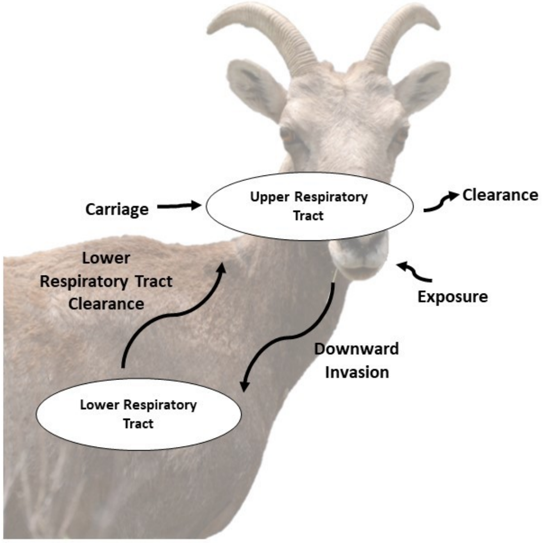

Figure 1.1. *Mycoplasma ovipneumoniae* in-host process. *Mycoplasma ovipneumoniae* is introduced through direct contact with an infected animal and infects the upper respiratory tract. *M. ovipneumoniae* impedes the function of the ciliary escalator, allowing for downward progression of pathogens into the lower respiratory tract. Bighorn sheep that survive the initial introduction event are able to clear pathogens from the lower respiratory tract. Most sheep seem to be able to also clear infection from the upper respiratory tract but for unknown reasons some sheep continue to carry *M. ovipneumoniae* asymptomatically, allowing for continued spread to susceptible lambs.

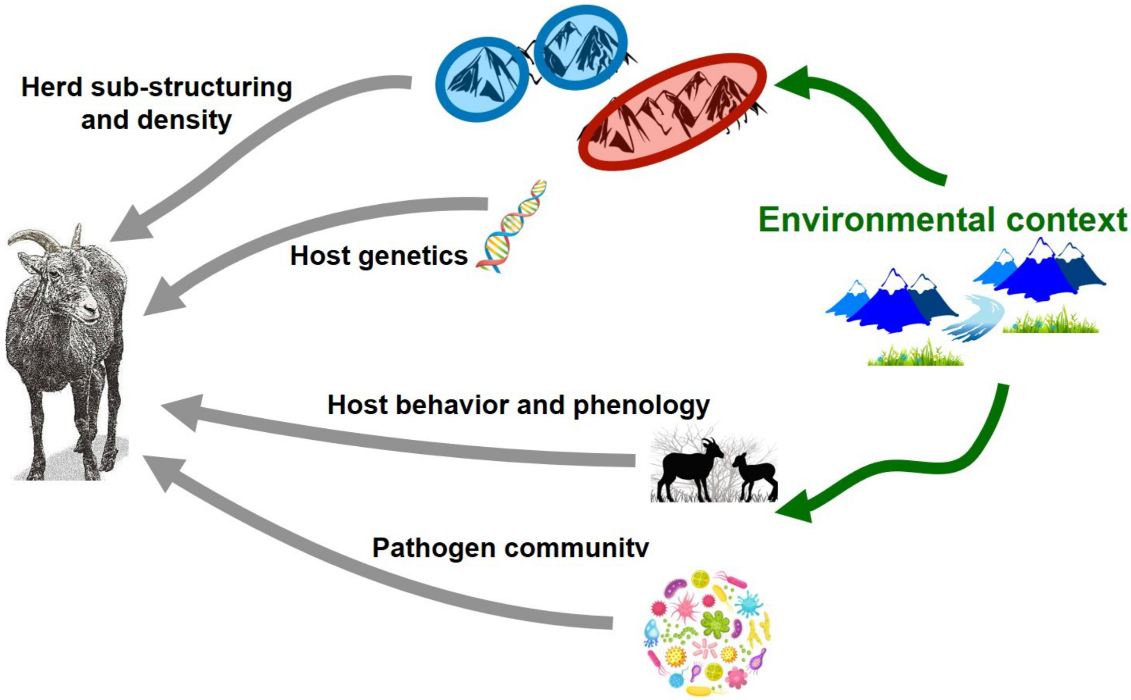

Figure 1.2. Drivers of host fate and population response. Proposed drivers of bighorn sheep population demography addressed in this thesis.

CHAPTER 2

MYCOPLASMA OVIPNEUMONIAE DYNAMICS IN NEVADA BIGHORN SHEEP HERDS

ABSTRACT

Many bighorn sheep (*Ovis canadensis*) populations are suffering from persistent *Mycoplasma ovipneumoniae*-related pneumonia events that can results in high rates of lamb mortalities. These events are the results of an initial pathogen introduction event that leads to an all age die-off. Recovering bighorn sheep have the potential to become asymptomatic carriers and continue to pass *M. ovipneumoniae* to susceptible individuals. Understanding pathogen persistence in bighorn sheep populations is a critical knowledge gap in the recovery of infected population. Here, we used 2,966 *M. ovipneumoniae* polymerase chain reaction and competitive enzyme-linked immunosorbent assay test results from 56 bighorn sheep populations across Nevada to analyze seroprevalence cut-off values, seroprevalence trends across herds and subherds, look at prevalence and seroprevalence trends across subspecies, and look at trends in immune response across age, sex, and subspecies. We found that current standards for a cELISA-positive animal may be set too high for the data we have from Nevada. Updating our cut-off value did not change sero-status for any herds but did change classifications for three subherds. Desert bighorn sheep herds exhibited lower prevalence and seroprevalence than was seen in California and Rocky Mountain subspecies and appeared to clear *M. ovipneumoniae* infections from their populations more quickly. There was no statistically significant trend in immune response by sex or age for any subspecies.

Mycoplasma ovipneumoniae has been extensively studied in bighorn sheep, however these studies have largely occurred in a few well known populations or systems, and focus almost exclusively on disease in the Rocky Mountain bighorn subspecies (Cassirer and Sinclair 2007; Cassirer et al. 2013; Plowright et al. 2013, 2017). There are reasons to think the in-host physiological dynamics, as well as patterns in the resulting diagnostic tests, might differ among bighorn subspecies, especially since responses among other Caprinae hosts vary widely (Ziegler et al. 2014).

Differences in pathogen exposure could also occur between bighorn sheep based on age, sex, or co-infections with other pathogens. Ewes and rams rarely mix outside of mating season (rut) in the fall, which could lead to exposure levels that differ between sexes. Younger rams are seen with ewe groups occasionally, so they might better match ewe group exposure patterns. Even within ewe groups, groups with lambs could experience increased levels of pathogen exposure due to extensive shedding from acutely infected lambs. In addition to exposure through contact with other ewes, lambs interact with other lambs, and then come back in contact with their dams, allowing for additional pathogen exposure opportunities. For co-infections, individuals which are already allocating immune resources to combating another pathogen could be in more compromised states then animals who have otherwise low pathogen burdens.

Another driver of differing pathogen response could be subherd mixing dynamics, which vary among herds. Some groupings of ewes may rarely, or never, come into contact with other ewes, but may still be connected through shared rams that move between ewe-groups during rut. Other herds are well-mixed with the preponderance of ewes coming in contact with the rest of the herd throughout the year. This can have important implications on disease dynamics if a pathogen becomes “trapped” in a particular subherd (Sah et al. 2017). This social trapping can lead to incomplete exposure, where only one subherd encounters the pathogen. Incomplete exposure can in turn dilute the overall seroprevalence (proportion of animals testing seropositive) within the herd, so accounting for herd mixing is important when assessing patterns of herd serology. This may have important implications on the spread of the pathogen and on its long-term persistence (Sah et al. 2017; Manlove et al. 2014; Hass 1997).

Additionally, management actions such as translocation and augmentation hinge on understanding a herd's infection history. If disease tests have fundamentally different performances for some sexes, ages, or subspecies of animals, then a single testing standard could prove problematic. Understanding the factors that drive host immune responses to *M. ovipneumoniae*, how those responses relate to long-term demographics, and how consistent responses are across host subspecies sex and ages is important for effective management of bighorn sheep populations.

Preliminary data exploration pertinent to a broader analysis of post-pathogen-introduction demographic patterns (Chapter 3) indicated some disagreement between field data and laboratory testing cut-off values. These cut-off values define what test result level an individual is classified as positive or negative for an antibody response. That observation sparked the following questions, which I address in this chapter: 1) what serological cut-off values are best supported by our NDOW field data? 2) do serological signals vary systematically among *M. ovipneumoniae* strains? and 3) do serological signals differ systematically among animals of different subspecies, sexes, or ages.

STUDY AREA

We used two different data sources to describe *M. ovipneumoniae* infection status in each of 58 bighorn sheep herds from across the state of Nevada (Table 1). The first consisted of results from real-time polymerase chain reaction (PCR) tests, which provide information about current pathogen infection status. Samples were obtained from nasal swabs acquired during live capture or post-mortem necropsy and sent to the Washington Animal Disease Diagnostic Lab (WADDL) for testing. There, DNA was isolated from the swab sample and amplified using a commercially available real-time PCR process. Results were reported as both a binary (present/not present) result and a continuous cycle threshold that ranged from 0 to 40, with 40 indicating that no gene copies of *M. ovipneumoniae* could be amplified from the sample.

The second infection-related dataset consisted of results from a competitive enzyme-linked immunosorbent assay (cELISA), a serological test that identifies the presence of antibodies, and thus past exposure, to *M. ovipneumoniae*. Blood samples were obtained from live animals or (rarely) post-mortem, and serum was separated and shipped to WADDL. Results were again reported as both a categorical read-out describing individual status as positive, indeterminate, or negative; and a quantitative metric describing the specific percent inhibition the individual's serum exhibited. The standard for assessing cELISA serology results comes from WADDL. Inhibition below 40% is considered negative, between 40% and 50% is indeterminate, and over 50% is positive.

METHODS

Re-assessment of cut-offs for categorizing herds as "seropositive"

We used density plots generated by the R package sm (Bowman and Azzalini 2018) to analyze the distribution of cELISA percent inhibition by individuals classified as either positive or negative using the WADDL 50% cut-off value. We found an unexpected bimodal response in the negative density plot, likely reflecting groups of individuals who had not encountered the pathogen, corresponding to "true" negative results, and some individuals what had encountered that pathogen but were exhibiting a lower inhibition value than was captured using WADDL's standard (Fig. 2.1).

We used the R package mclust (Scrucca et al. 2016) to determine the means and variances associated with two modes, or categories, of results corresponding with a 95% likelihood of being a positive or a negative result. mclust creates Gaussian mixture models with hierarchical clustering, and then uses Bayesian information criterion to select the optimal model. Following fitting with mclust, we used the results to identify alternative cut-off values that better matched the field data *M. ovipneumoniae* positive and negative animals, as well as determine pathogen prevalence within a herd.

Home-range mixing patterns and assessment of incomplete exposure

We used the R package adehabitatHR (Calenge 2006) in combination with information gathered from radiocollared individuals to estimate quantities describing within-herd mixing dynamics adehabitatHR. We identified seasonally-specific individual home ranges using the 95% isopleth determined by a Brownian bridge kernel estimate (Horne et al. 2007), relying on a smoothing parameter estimated through the liker function in the same adehabitatHR package. We split the year into two seasons: rut and non-rut (rut was defined as September to December in Rocky Mountain and California bighorns, and July to December in desert bighorns). Next, we calculated the percent overlap between each pair of individuals within each herd to build seasonally-specific home range overlap networks. We calculated modularity of each resulting network using the walktrap community detection algorithm as implemented in R's igraph package, and stored modularity estimates for each herd (Csardi and Nepusz 2006). Modularity is a metric that describes the extent to which subherds are disjoint from one another within a population. Scaled from 0 to 1, modularity increases with increasing subherd stability, meaning connectivity between individuals of different subherds is low. We then used these modularity values to consider differences in herd seroprevalence between the WADDL positive cut-off value and our updated positive cut-off value.

Patterns in seroprevalence according to subspecies, sex, and age

We examined seroprevalence (according to WADDL's 50% cut-off value, and under our updated seroprevalence cut-off estimated from the Gaussian mixture model) and prevalence of *M. ovipneumoniae* in the years following an introduction event. We considered bighorn sheep subspecies separately, combining the Rocky Mountain subspecies with California subspecies due to small sample size.

We fit separate logistic regression models for California/Rocky Mountain and desert subspecies that related prevalence (number of PCR-positive animals out of all animals sampled), WADDL seroprevalence (number of animals with cELISA percent inhibition > 40 out of all animals sampled), and the seroprevalence determined by analysis using mclust to time since initial die-off.

We examined individual cELISA percent inhibition by sex and age. Specifically, for each subspecies, we examined differences percent inhibition changed sex or age separately using linear regression. California and Rocky Mountain subspecies were considered as one subspecies. Here, we focused on results that were considered positive from our updated seroprevalence cut-off from our first goal.

RESULTS

Re-assessment of cut-offs for categorizing herds as "seropositive"

When density plots of results over and under 50% (based on WADDL's cut-off standards) were generated with R package sm (Bowman and Azzalini 2018) (Fig. 2.1), the negative plot still showed a bimodal response. Analysis with mclust (Fraley and Raftery 2006) indicated that positive values may begin as low as 25.2% inhibition (Fig. 2.2). Statewide serological data from Nevada were clearly bimodal, with one mode reflecting exposed individuals (mean = 57.21, sd = 9.16) and a second mode likely reflecting unexposed animals (mean = -2.025, sd = 20.30; Table 2.2). These modes were estimated to account for 50.3% and 49.7% of the total results, respectively. Analysis of cELISA percent inhibition cut-off values indicated that positive animals may have a lower-than-expected percent inhibition when considering WADDL's 50% cut-off value.

Home-range mixing patterns and assessment of incomplete exposure

For herd and subherd mixing dynamics, we limited analysis to populations with serology testing on at least five animals. We plotted proportion of population that was seronegative against the proportion of the population that was seropositive by WADDL standards (50% cut-off) and proportion of the population that was originally considered negative but would be positive by our update cut-off value of 25.2% (Fig. 2.3a). We found that all herds previously classified as seronegative from WADDL's cut-off remained seronegative with our updated cut-off. While seroprevalence remained the same in many herds, several showed an increase in seroprevalence (Candelaria Hills, NNSS, Stonewall Mountain, Snowstorm Mountains, Spring Mountain, Garfield Hills, Ruby Mountain) with some herds switching to a seroprevalence of greater than 50% (Excelsior Mountains, Toiyabe Range, Mt. Jefferson). Next, we took the subherds as determined by the Brownian bridge kernel estimation and analyzed seroprevalence trends in the same way. We only considered subherds with serology test results on at least five animals. Here we found three subherds previously classified as seronegative were 100% seropositive with the updated cut-off. We found trends similar to what was seen at the herd level, with seroprevalence in many herds remaining the same and several switching to greater than 50% seroprevalence.

Patterns in seroprevalence according to subspecies, sex, and age

We analyzed differences in *M. ovipneumoniae* persistence and severity between bighorn sheep subspecies (Fig. 2.4; Table 2.3). We considered Rocky Mountain bighorn sheep herds with the California subspecies in this analysis due to the limited number of

Rocky Mountain samples. We began by looking at prevalence. We determined the California/Rocky Mountain subspecies responded to *M. ovipneumoniae* in a manner similar to pat- terns documented in the Rocky Mountain subspecies in studies conducted in other states (Cassirer and Sinclair 2007; Manlove et al. 2016), with a noticeable decrease in prevalence in the years following an *M. ovipneumoniae* introduction event ($\hat{\beta}_{year} = -0.134$; $p < 0.001$). This corresponds to a scenario where for each additional year post-die-off, the odds that an animal is seropositive change by a multiplicative factor of 0.875. We next looked at seroprevalence with the WADDL determined 50% cut-off for positives. While there was a visual drop in seroprevalence following die-offs, statistically, the difference was insignificant ($p = 0.132$). Desert bighorn sheep, however, initially exhibited a lower seroprevalence following introduction, and, while no obvious trend is evident (coefficient estimate = 0.029, $p = 0.0457$), we can see that desert bighorn sheep exhibit lower seroprevalence values relative to California and Rocky Mountain bighorn sheep in the years following die-offs. Desert bighorns also displayed a more rapid decline in prevalence levels in subsequent years ($\hat{\beta}_{year} = 0.798$ corresponding to a multiplicative change in odds of 0.45 each year; $p < 0.001$). When we considered our revised seroprevalence value at 25.2%, we found the California subspecies trend was more obvious and exhibited elevated seroprevalence in all years (0.049, $p = 0.0176$). Once again, the desert subspecies exhibited no obvious trend but seroprevalence was still evidently lower than seen in the California subspecies (0.0194, $p = 0.135$).

We examined trends of cELISA inhibition by age and sex separately for each subspecies, considering California and Rocky Mountain bighorn sheep together, using linear regression (Fig. 2.5). We considered only animals with cELISA inhibition levels over 25.2%. We found no evidence of trends in cELISA percent inhibition by age or sex for either subspecies (Table 2.4).

DISCUSSION

The distribution of cELISA values in Nevada bighorn sheep indicates that current WADDL positive cut-off standards may be set too high, particularly for desert bighorn sheep. This could mean we are underestimating extent of exposure and infection in Nevada bighorn sheep populations. There could be a few reasons for this. Desert bighorn sheep may be better-equipped to tolerate *M. ovipneumoniae*, leading to a reduced immune response relative to that of Rocky Mountain bighorns. Evolving *M. ovipneumoniae* strains could also be playing a role. There are numerous strains of the pathogen, and some strains may simply elicit lower immune responses in bighorn sheep. Moving forward, reconsidering an appropriate cut-off value for classifying positive animals could have important implications for populations. Some herds may be misclassified as sero-negative which hinders pathogen tracking and might alter their potential desirability as source populations for translocation. Re-assessing herd serological status with our updated cut-off value changed the estimated seroprevalence in some populations significantly. When diving further into subherds, we found some subherds went from negative seroprevalence to 100% seroprevalence. In- creased substructuring could lead to limited pathogen exposure across the population, resulting in lower than expected immune response in most individuals, so it stands to reason that a lowered cut-off value would more accurately capture pathogen presence in such population. As seroprevalence could be a metric on asymptomatic carrier ewes in a population, accurate detection of positive seroprevalence and an understanding of mixing dynamics between subherds in a population are critical for managing bighorn sheep herds in the years following a die-off.

We found prevalence and seroprevalence response varies by subspecies. Using our updated seroprevalence value, we found a strengthened signal in the California response with little change over time. The desert response is quite varied regardless of cut-off. Further investigation will be needed to better understand what is driving the large variance in desert seroprevalence over time. While we did not find any significant trend in age or sex amongst individuals, this is not entirely surprising, particularly for age. Bighorn sheep ewes are difficult to accurately age after about age 4, making it difficult to parse accurate trends from the data.

MANAGEMENT IMPLICATIONS

In order to effectively manage bighorn sheep populations, we need to be correctly classifying populations as seropositive or seronegative. A major point of concern with correct classification has to do with translocations. Translocating individuals into a population that may still have the pathogen will result in high mortality rates of introduced individuals. Translocating animals out of that population may result in pathogen spread to naive populations. At this time, we see no evidence to support management of group composition to manage for pathogen persistence.

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TABLES AND FIGURES

Table 2.1. Number of *Mycoplasma ovipneumoniae* tests by subspecies used in analysis.

Pathogen Tests			
Subspecies	# of herds sampled	# of ELISA tests	# of PCR tests
California	11	372	481
Desert	40	787	1182
Rocky Mountain	5	51	93

Table 2.2. Gaussian mixture model estimates.

	Mean	SD
Seropositive mode	57.208	9.161
Seronegative mode	-2.025	20.30

Table 2.3. Prevalence and seroprevalence trends in years following a die-off. Results from a logistic regression on subspecies prevalence and seroprevalence as a function of years since a *M. ovipneumoniae* die-off.

Subspecies	Test	Untransformed coefficien estimate (CI)	Multiplicative change in odds (CI)	P-value
California/ Rocky Mountain	Prevalence	-0.134 (-0.197, -0.077)	-0.87 (-1.22, -1.08)	<0.001
Desert	Prevalence	-0.798 (-1.26, -0.469)	-0.45 (-3.53, -1.60)	<0.001
California/ Rocky Mountain	Seroprevalence @50%	0.034 (-0.011, 0.077)	1.03 (-1.01, 1.08)	0.132
Desert	Seroprevalence @50%	0.029 (0.001, 0.056)	1.03 (1.00, 1.06)	0.0457
California/ Rocky Mountain	Seroprevalence @25.2%	0.049 (0.008, 0.090)	1.05 (1.01, 1.09)	0.0176
Desert	Seroprevalence @25.2%	0.0194 (-0.004, 0.041)	1.02 (1.00, 1.04)	0.135

Table 2.4. Estimates of linear regression analysis on cELISA inhibition by age or sex in bighorn sheep subspecies. All results were not significant.

cELISA Inhibition Trends by Age and Sex				
Subspecies	Demographic factor	Estimated effect	t value	P-value
California/ Rocky Mountain	Age	-0.969	-1.298	0.196
Desert	Age	0.3486	1.046	0.296
California/ Rocky Mountain	Sex	1.235	0.39	0.697
Desert	Sex	2.08	1.412	0.159

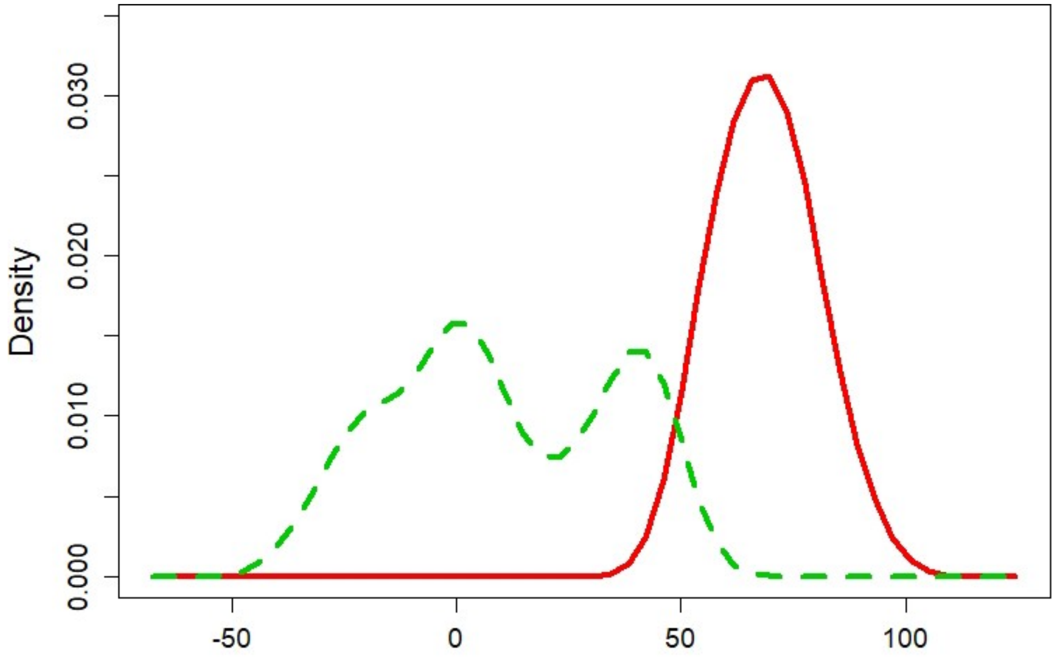

Figure 2.1. Density plot of cELISA values from all samples in the state of Nevada. Density plot of bighorn sheep samples (n = 207) with the WADDL standard of 50% inhibition. The WADDL defined negative density line in green shows a bimodal response, indicating the presence of two classes, not just negative. The positive line in red is unimodal, indicating positives are being correctly classified as positive.

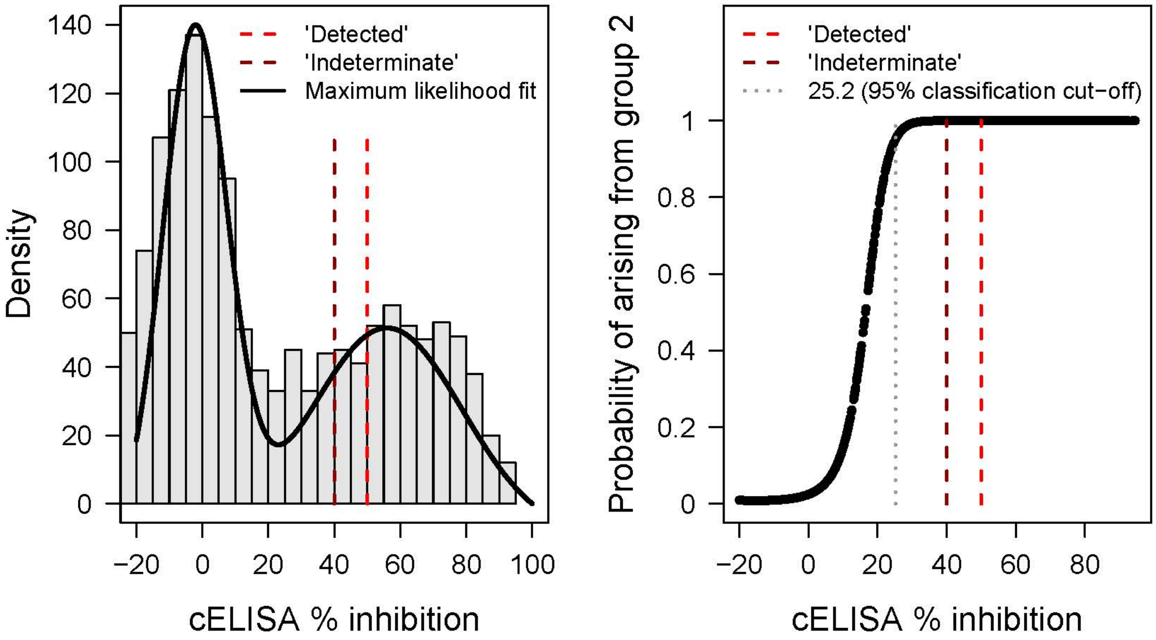

Figure 2.2. Distribution of cELISA values aggregated from all samples in the state of Nevada.
A) Empirical cELISA measurements from all bighorn sampled (n > 800) in the state of Nevada from 2008 to 2019. The dark line indicates the maximum likelihood fit of a two-Gaussian mixture model to the raw cELISA data. The dark red and red dashed lines indicate current cut-offs for classification as indeterminate and seropositive, respectively.
B) Probability a datapoint arises from the seropositive mode using the maximum likelihood model shown in A. Dark red and red lines indicate current cut-off values. The grey dotted line shows the cut-off value above which points have a 95% probability of having arisen from the seropositive mode under the fit in A.

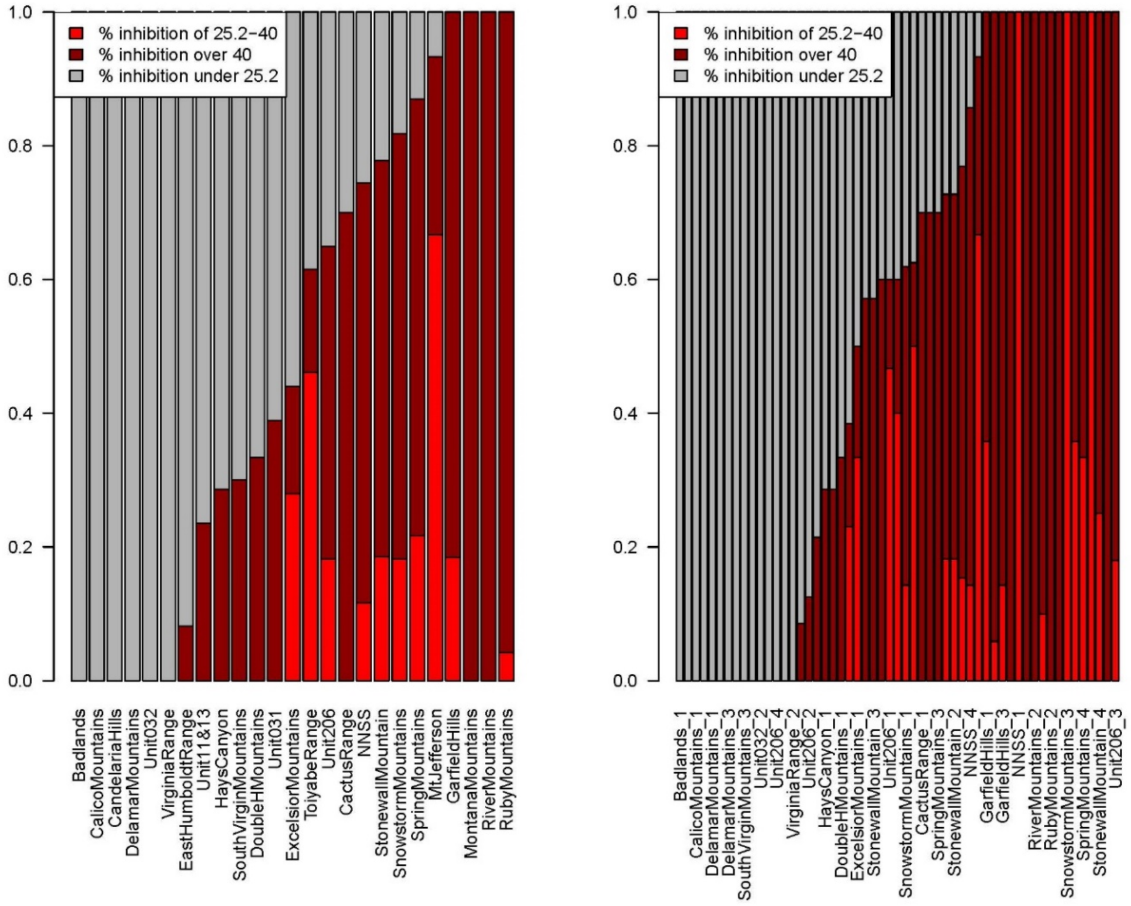

Figure 2.3. Seroprevalence by herd and subherd. Composition of cELISA status (clearly positive, clearly negative, or in the potentially misclassified range) within all communities of bighorn sheep identified using the NDOW GPS dataset. Limited to communities with at least five members. Left panel is grouped by herd; right panel is grouped by community within herds.

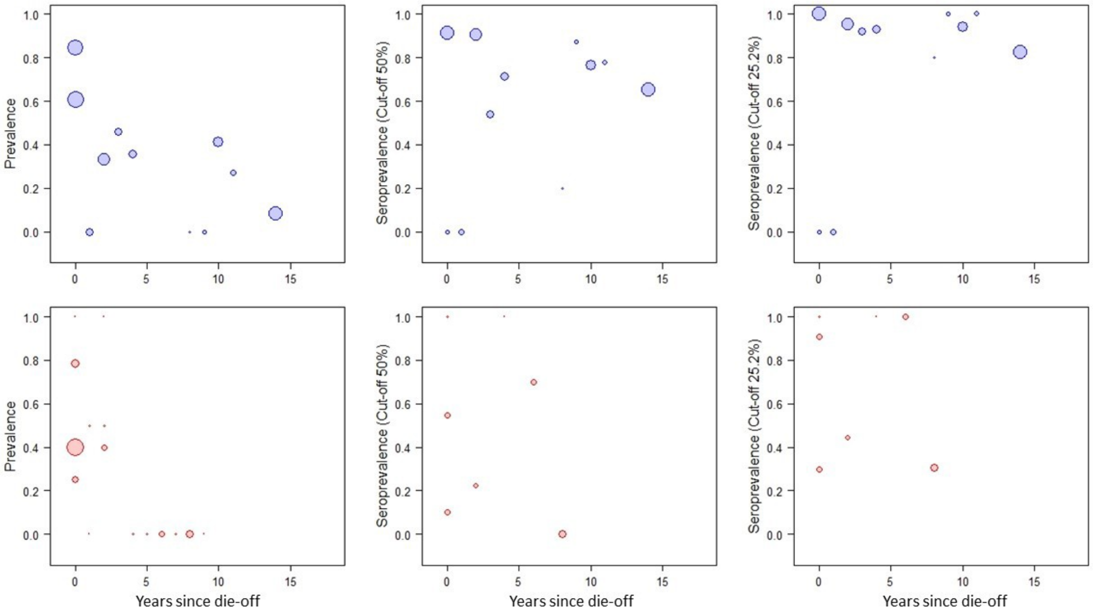

Figure 2.4. *M. ovipneumoniae* seroprevalence and prevalence patterns in two bighorn sheep subspecies. Data showing trends in waning *M. ovipneumoniae* sero- prevalence and prevalence in years following disease-related die-offs. Seroprevalence trends reflect WADDL standard cut-off values for positives (50%) and our updated classification for cut-off values (25.2%). Point size is determined by number of animals tested.

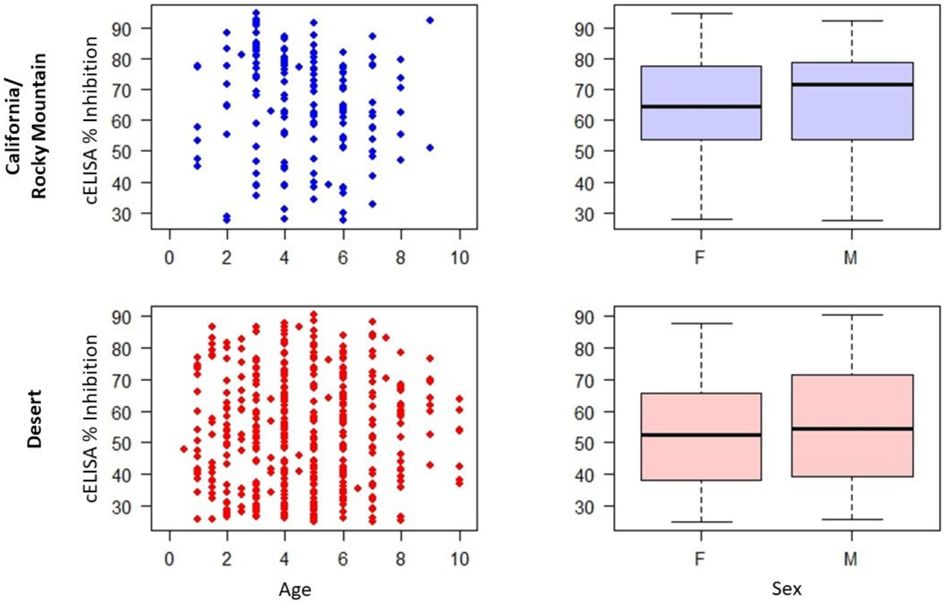

Figure 2.5. cELISA inhibition trends by age and sex. We considered cELISA inhibition trends by age and sex. California and Rocky Mountain subspecies were considered together in the blue panels and the desert subspecies was considered in the red panels. No trend was detected.

CHAPTER 3

EVALUATING HOW INDIVIDUAL VITAL RATES RELATE TO FACTORS DRIVING HERD RECOVERY

ABSTRACT

Pneumonia caused by the pathogen *Mycoplasma ovipneumoniae* is known to reduce populations of bighorn sheep (*Ovis canadensis*) and limit their recovery in the years following pathogen introduction. Understanding what drives continued poor population recovery is critical for effective management of bighorn sheep populations. Here, we considered a suite of hypothesized drivers to analyze their effect on lamb recruitment using 2500 herds-years of data from 58 bighorn sheep herds across Nevada. We used a Poisson regression model and included covariates addressing herd substructuring and density, host genetics, host behavior and phenology, pathogen community, and environmental context. Our results indicate that continued *M. ovipneumoniae* persistence in the years following a die-off is possibly the biggest concern in regards to lamb recruitment. We also found positive density dependence, indicating that populations may be experiencing an Allee effect, making them ideal candidates for translocation effects. We found desert bighorn sheep herds had better lamb recruitment than California and Rocky Mountain subspecies following pathogen introduction. Both genetics and substructuring showed some effect on lamb recruitment but these metrics were lacking in long-term data and will require further investigation.

Emerging infectious diseases play a significant role in many wildlife populations (DeVivo et al. 2017; Frick et al. 2010; Lilley et al. 2017; Lips 2016). Even if the immediate effects of pathogen introduction can be anticipated, long-term impacts are more difficult to assess and mitigate (Monello et al. 2014). This holds true for bighorn sheep populations across the Intermountain West (Spraker et al. 1984). Introduction of the respiratory pathogen *Mycoplasma ovipneumoniae* typically leads to mortality rates of 10-90% of a population (Cassirer et al. 2018), but how a herd responds after this initial introduction event is currently not predictable. Frequently herds experience persistent infections that lead to high lamb mortality rates which can persist for years to decades after the pathogen is introduced (Cassirer and Sinclair 2007; Manlove et al. 2016). Other herds appear to clear the pathogen completely, allowing populations to rapidly return to pre-die-off population dynamics (Coggins 1988; Manlove et al. 2016). Understanding what is driving these differences among herds is critical for effective management of bighorn sheep populations.

There are many proposed drivers of bighorn sheep demographic dynamics in the wake of pneumonia-related die-offs. Herd substructuring and density have the potential to limit or increase pathogen spread (Begon et al. 2002; Manlove et al. 2014; McCallum 2001). Genetic diversity within a population may limit the ability to recover following die-offs. Bighorn sheep behavior and phenology has the potential to propagate or limit pathogen spread. Pathogen community and burden can also contribute to reduced individual health and increased opportunity of disease consequence. The environment also plays a role in shaping other factors, including herd substructuring and bighorn sheep behavior. More detail on these factors can be found in Chapter 1.

To better understand what processes account for demographic variation in this post- die-off time-frame, we constructed a state-space model that compared the effect of drivers on lamb recruitment before and after *M. ovipneumoniae*-related die-offs., including factors related to herd substructuring and density, genetic diversity, environment, demography, and broader pathogen community structure (Fig. 3.1).

STUDY AREA

Our analysis relies on data from 58 bighorn sheep herds (consisting of 2,900 herd- years) across Nevada collected by the Nevada Department of Wildlife (NDOW) (Fig. 3.2). NDOW aggregates their survey data at the hunt unit level (with a hunt unit consisting of one or several spatially proximal populations). Wherever possible we disaggregated hunt unit counts into herd-level counts (with a "herd" typically defined as the individuals living within one mountain range) to improve spatial resolution (See Appendix A). We refer to these disaggregated units as "herd-years" throughout, and they form the basis of our analysis going forward. Populations ranged from 42.000N degrees latitude in the north to 35.139N degrees latitude in the south. Herd environments were typically either Mojave Desert or Northern Basin and Range (Omernik and Griffith 2014). We used count data to capture population dynamics. Aerial surveys were conducted approximately every two years in most herds, with the earliest counts beginning in 1970. We incorporated 600 herd-years of demography data on California bighorn sheep, 2050 herd-years of data on desert bighorn sheep, and 250 herd-years of Rocky Mountain bighorn sheep data for 2900 herd-years of data total.

Animal health data were collected during capture events by Nevada Department of Wildlife personnel using established NDOW protocols. Capture methods varied, but helicopter capture was the predominant method employed. Age and sex composition of sampled animals varied by herd but was typically intended to be a random sampling of ewes or ewes and rams. Animal health sampling usually included collection of a genetic sample, nasal swabs for culture, blood for serology, and a fecal sample, along with basic animal health evaluations. Animals were usually also fitted with GPS collars to gather location information at fixed time intervals.

METHODS

We examined annual counts of lambs present in a herd through a Poisson model to estimate lamb recruitment, a key vital rate to characterize herd demographic response following *M. ovipneumoniae* introduction. The model included ewe count in the same herd- year as an offset, a term reflecting density in the previous year, and covariates corresponding to each of our hypothesized recruitment drivers. We allowed most covariates to interact with an indicator variable for whether the year was pre- or post-die off to allow effects to change following pathogen introduction events.

Herd density and substructuring

We accounted for herd size and density in the models using NDOW's aerial survey estimates of herd size and composition. These counts were categorized into ram, ewe, and lamb counts. Surveys were conducted approximately every two years in most herds with the earliest counts occurring in 1970. Counts were adjusted according to NDOW records of the number and classification of sheep removed from the population due to harvest or translocation each year and the number and classification of sheep added to the population through augmentation efforts. We included the estimated total population size in the previous year to account for density-dependent effects.

We measured the extent of substructuring within each herd by first building Brownian Bridge home ranges for all radiocollared animals within each herd in each of two seasons (a six-month season including rut which ran from September to February for California bighorn sheep and from July to January for desert bighorns, and another season spread across the rest of the year to cover lambing) (Fig. 3.3) (Horne et al. 2007). Home ranges were built using function kernelbb in R's adehabitatHR package (Calenge 2006). We extracted the 95% isopleth for each individual's home-range, and then calculated (potentially asymmetric) percent overlap between each pair of individuals. Those overlap weights were then used to build a season-herd-specific homerange overlap network upon which we calculated modularity (a measure that increases as the graph separates into increasing distinct subherds) (Csardi and Nepusz 2006). We included two modularity values – one from rut and one from non-rut – for each population, based on networks built using both sexes (whenever possible) or ewes only (when no radiocollar data existed on rams) as covariates in the lamb count model.

Phenology, behavior, and subspecies

The three subspecies of bighorn sheep found in Nevada for which we had data – Desert (*Ovis canadensis nelsoni*), California (*O. canadensis californiana*), and Rocky Mountain (*O. canadensis canadensis*) (Fig. 3.2) – differ fundamentally in their phenology and behavioral ecology, and we used subspecies classification as a proxy for phenology and behavioral differences here. California and Rocky Mountain bighorn sheep phenology and behavioral ecology are similar. They form subherds of ten to twenty individuals and have a short mating season in the fall (Berger 1978; Hogg 1984; Manlove et al. 2014). This results in a temporally compact pulse of lamb births in the spring, with most lambs in a herd being born in a span of two to four weeks (Hass 1997). Desert bighorn sheep tend to form smaller groups and exhibit a more prolonged mating and lambing season, perhaps due to environmental heterogeneity (Hass 1997). This difference in ecology could alter expected disease trends for several reasons. Smaller groups reduce the number of individuals that infected animals contact, potentially limiting pathogen spread through social trapping (Sah et al. 2017). Additionally, lambs tend to participate in contact play with other lambs very near to them in age (Berger 2009). The prolonged birth pulse in the desert subspecies may lead to enough age-variation in lambs to reduce the frequency of playful contacts. If lambs limit contact play only to those within their age group, this will also limit pathogen spread and decrease disease-related lamb mortalities. Subspecies data were provided by NDOW at the hunt-unit level and used in our model at the herd level.

Genetic diversity

We used metrics of genetic diversity to examine how local inbreeding and genetic diversity might affect lamb recruitment. Genetic samples were collected both during capture and through a hunter harvest return program (N = 1,644), and were sequenced using genotyping-by-sequencing approach (Parchman et al. 2012) following the same specifications employed by Jahner et. al. (2019). Based on variations found in 1,138 individuals with high quality genetic data, two measures of genetic diversity - nucleotide diversity (Tajima 1983) and Watterson's estimator (Watterson 1975) - were quantified at the hunt unit level with angsd (Korneliussen et al. 2013, 2014; Jahner et al. unpubl. data). We also estimated Tajima's D (Tajima 1989), which is based on the difference between nucleotide diversity and Watterson's estimator and can give insight into the recent demographic history of a population. A positive Tajima's D value indicates more common alleles than rare and can be an indication of a population bottleneck. In contrast, a negative Tajima's D represents more rare alleles than expected and can be consistent with a recent population expansion. We would expect lamb recruitment to increase or remain stable with a decreasing Tajima's D.

Herd *M. ovipneumoniae* status and history

We used an infection-related dataset consisting of results from the competitive enzyme- linked immunosorbent assay (cELISA) that was the focus of inquiry in Chapter 2. The cELISA test is a serological test that identifies the presence of antibodies, and thus past exposure, to *M. ovipneumoniae*. Blood samples were obtained from live animals or (rarely) post-mortem, and serum was separated and shipped to WADDL. Results were again reported as both a categorical metric describing individual status as positive, indeterminate, or negative; and a quantitative metric describing specific percent inhibition that the individual's serum exhibited. Based on our results from Chapter 2, we considered samples to be "positive" on the cELISA results if they exhibited percent inhibitions over 25.2%. We calculated herd seroprevalence from the number of individuals meeting this criterion divided by the number of individuals tested within a herd and year.

Beyond these direct physiological data, we also used several variables reflecting timing of pathogen introduction events. First, we built a categorical variable identifying die-off years, in which each herd-year was categorized as either a known die-off, a known no die-off, or unknown. We additionally determined the number of years since a die-off occurred in the focal herd by counting years since a die-off event, with the die-off year classified as year 0. We also marked years prior to year 0 of known die-offs as pre-die-off years and those at and following year 0 as post-die-off. If die-off timing was unknown, the variables were left as NAs until the first sampling-based evidence of *M.*

ovipneumoniae (either PCR or serology) was recorded, at which time the post-die-off indicator was set to one and pre-die-off years were set to known no die-off.

Other pathogens

We incorporated data on the status of other pathogens postulated to drive demography in bighorn sheep herds through two variables that we generated *de novo*. The first accounted for recent exposure to epizootic hemorrhagic disease virus (EHD) or bluetongue virus (BTV). EHD and BTV are both caused by double-stranded RNA viruses of the genus *Orbivirus*. Both are transmitted primarily through bites from midges of the *Culicoides* family and are important conversation concerns in wildlife and livestock. EHD and BTV are closely related to one another, and can produce cross-reactive serological signals (Gorman 1990), so we combined serological data from both together into one covariate, which reflected the proportion of animals with antibodies to EHD or BTV above 80% out of the total number of animals tested. Since EHD and BTV effects tend to be isolated to particular years, we allow this covariate to vary within a herd over time.

We constructed a second variable to capture the presence of potentially problematic bacteria from the family *Pasteurellaceae*. Pasteurellas have been researched extensively in the context of bighorn sheep pneumonia (George et al. 2008; Miller et al. 1991; Shanthalingam et al. 2014) and are known to cause fatal pneumonia in isolation in some cases. There is on-going debate about whether particular species (e.g., *Mannheimia haemolytica*) or functional attributes (e.g., leukotoxin-positivity status (Shanthalingam et al. 2014)) of Pasteurella are particularly salient. Here, we did not pursue that nuance explicitly, and instead built a variable that calculated an aggregate herd-level “Pasteurella score”. Pasteurella testing relies on culturing swabs, and then categorizing bacterial colonies in the culture by both size and species. We gave animals non-zero scores if their samples produced colonies of *Pasteurella multocida*, *Mannheimia haemolytica*, or *Bibersteinia trehalosi*. The magnitude of the score was determined by the number of colonies the animal produced. The animal received a score of 1 if there were “few” colonies, 2 if there was a “moderate” number of colonies, 3 if there were “many” colonies, and 4 if there were “very many” colonies. We then averaged scores across all animals sampled in a given year to generate a single herd-level bacterial burden value, which we held constant over time.

Drought

Water is a limiting resource in the desert and a potential driver of movement in the desert bighorn sheep subspecies (Longshore et al. 2013). In times of drought, scarcity of water will be exacerbated forcing sheep to congregate at the limited sources. Larger numbers of congregating animals would increase the chance for pathogen spread (Cross et al. 2005; Paull et al. 2012). We used county level drought data available through drought.gov to create our drought metric. Drought severity was classified as D0 (abnormally dry), D1 (moderate drought), D2 (severe drought), D3 (extreme drought), or D4 (exceptional drought) and applied to percent area experiencing that level of drought within a county each week. A drought severity and coverage index (DSCI) score by taking each category's percentage, multiplying it by a multiplier corresponding to that category level (i.e. D0 = 1, D1 = 2, etc.) and adding each product together. We considered weeks with DSCI scores over 250 to be in significant drought and included number of days in a year above this score as our drought metric. The county score was applied to all herds within the county for each year.

Post-*M. ovipneumoniae*-introduction interaction terms

To determine whether various covariates affected recruitment differently before and after *M. ovipneumoniae* introduction events, we examined a set of interaction terms between target covariates and an indicator variable taking on the value 1 after an introduction event and 0 otherwise. We examined the following covariates to assess whether their effects on recruitment changed following introduction events: aggregate bacterial load, modularity (both during rut and out of rut), Tajima's D, subspecies, ELISA prevalence, density, and EHD/BTV prevalence.

Other terms

Based on differences in behavioral ecology and life history, we also considered an interaction term between the offset (logged number of ewes) and the subspecies, allowing background lamb-to-ewe ratios to vary between desert and California/Rocky Mountain bighorn sheep. This decision was based on detailed work showing that bighorn sheep are capital breeders (Jonsson 1997). Since desert ewes may be subject to more severe resource constraints than California and Rocky Mountain bighorn ewes, we might expect aggregate per capita reproduction rates to be lower in the desert subspecies.

Statistical modeling

We initially explored the response data through time series plots annotated with disease introduction events and pathogen testing data for each hunt unit to visually assess data gaps and potential undocumented die-off events. We additionally examined univariate distributions of each covariate to identify necessary transformations, which led us to center and scale our cELISA prevalence, drought, and modularity predictors.

We used a hierarchical state-space model with state and observation processes as described by Kêry and Schaub (2012) to analyze recruitment in each herd-year, with the state variable (namely, the true number of lambs present during year t at herd h, zh,t) treated as Poisson ($z_{h,t}$ ~ Poisson ($\lambda_{h,t}$)), and the observed number of lambs, $y_{h,t}$ varying from that value according to a Gaussian distribution, $y_{h,t}$ ~ Gaussian ($z_{h,t}, \sigma_{obs}$) (Kêry and Schaub 2012).

We allowed $\lambda_{h,t}$, the rate parameter of the Poisson, to vary according to a linear combination of covariates including herd subspecies, herd-level *M. ovipneumoniae* PCR and cELISA prevalence, *M. ovipneumoniae* strain, yearly drought values, herd modularity, herd genetic diversity, EHD/Bluetongue exposure status, herd Pasteurella burden, and several interactions.

We additionally included an offset term corresponding to the log of the total number of ewes counted in the herd in the focal year, and a lagged population size term accounting for density dependence. In full, the model can be written as:

$$y_{h,t} \sim Normal(z_{h,t}, \sigma_{obs})$$

$$z_{h,t} \sim Poisson(\lambda_{h,t})$$

$$\log(\lambda_{h,t}) = \alpha_{subsp}[\mathrm{subsp}_h] + \alpha_{dieoff}[\mathrm{dieoff}_{h,t}] + \overrightarrow{\beta}\overrightarrow{X}_{h,t},$$

where subsp_h and $\mathrm{dieoff}_{h,t}$ contain category levels for the subspecies and die-off status of herd h in year i, and $\overrightarrow{\beta}$ and $\rightarrow - \overrightarrow{X}_{h,t}$ are vectors of continuous coefficients and corresponding covariate measurements, respectively.

We used uninformative or minimally informative priors wherever possible. For our categorical predictors (subspecies, α_{subsp}; and die-offs, α_{dieoff}) we used Normal priors with means of 0 and a standard deviations of 50. We used a Normal distribution with means of 0 and standard deviations of 100 for the offset and density terms (β_{nEwes} and β_{nTot}). For all other predictors, we used a Normal distribution with means of 0 and standard deviations between 0.5 and 2. The standard deviation for the observational model was created with a Uniform distribution running from 0 to 25.

Model implementation

Missing population counts, seroprevalence, modularity, EHD, bacteria, and Tajima’s D values were generated at random from covariate-specific Gaussian distributions within the model (Kêry and Royle 2015). We ran our models using Monte Carlo Markov Chain methods available in Nimble (de Valpine et al. 2017, 2020a,b) and accessed through R (R Core Team 2020). MCMC sampling was conducted as follows:

- We ran three chains, each consisting of 50,000 steps. 25,000 of these were treated as burn-in and excluded.
- We assessed sampler convergence using standard Gelman-Rubin diagnostics, as well as effective sample size. We sampled until a minimum of 500 effective samples were obtained for each inference-associated parameter in the model.
- We refit the model multiple times to verify consistency of coefficient estimates across different prior distributions.

We based all inferences on examination of 95% posterior credible intervals for each model coefficient.

Follow-up analysis: Isolated effects of select covariates

To confirm broad trends in our model, and in order to understand broad patterns in the data, we conducted several follow-up analyses. First, we examined the effects of *M. ovipneumoniae* seroprevalence and drought on lamb recruitment in isolation. To do this, we first created two subsets of the data, one consisting of only those years in which *M. ovipneumoniae* seroprevalence was equal to 0, and another in which drought values were equal to zero. We then modeled the lamb-to-ewe ratio as a function of drought in the entirely seronegative dataset, and the lamb-to-ewe ratio as a function of seroprevalence in the drought-free dataset. In both cases, we used linear regression with seroprevalence and drought treated as continuous variables.

RESULTS

This analysis included a total of 2,584 pre-die-off herd-years and 316 post-die-off herd- years. Of the 2,584 pre-die-off herd-years, 778 herd-years were of California and Rocky Mountain bighorn sheep herds and 1,806 herd-years were of desert bighorn sheep herds. Of the post-die-off herd-years, 72 herd-years were of California and Rocky Mountain bighorn sheep herds and 244 herd-years were desert bighorn sheep. We found a median lamb to ewe ratio for California and Rocky Mountain bighorn sheep of 0.468 (interquartile range (IQR) from 0.349 to 0.616) prior to die-offs and dropping to 0.381 (IQR from 0.233 to 0.50) in the years following die-off events. Desert bighorn sheep dropped from a median lamb to ewe ratio of 0.387 (IQR from 0.280 to 0.50) prior to die-offs to 0.352 (IQR from 0.233 to 0.433) following die-offs.

We addressed the influence of our hypothesized drivers of demographic responses on lamb recruitment by inspecting posterior credible intervals (pcis) on the coefficients in our state-space model. All coefficient estimates are presented in detail in Table 3.2 and summarized in Fig. 3.4.

Herd substructuring and density

Prior to die-offs, herd modularity for both rut and non-rut time frames did not have any influence on lamb recruitment (95% pci [-0.027, 0.087] and [-0.081, 0.037] respectively; Table 3.2). Following die-offs, increased modularity during the rut led to increased lamb recruit- ment (95% pci [0.086, 0.295]), while modularity outside the rut had a negative effect on lamb recruitment (95% pci [-0.272, -0.081]). We considered the effect of density for pre- and post-die-off periods together and found a small, positive effect on lamb recruitment (95% pci [0.00003, 0.0001]).

Subspecies

We contrasted lamb recruitment between the California/Rocky Mountain bighorn subspecies and desert bighorn sheep on their own. Our data suggested a reduced overall recruitment rate in desert bighorn sheep relative to the Rocky Mountain/California group ($\hat{\alpha}_{\text{subsp}}$=*desert*; 95% pci [-0.220, -0.121]). This trend was not significantly altered following die-off events.

Genetic diversity, *M. ovipneumoniae* status, and other pathogens

Lamb recruitment decreased with increasing values of Tajima’s D ($\hat{\beta}_{Taj}$; 95% pci [-0.236, -0.045]) prior to die-offs. Following a die-off, lamb recruitment increased with increasing values of Tajima’s D (95% pci [0.357, 1.048]). *M. ovipneumoniae* seroprevalence (95% pci [-0.708, -0.375]) had a negative impacts on lamb recruitment following die-offs. Prior to die-offs, seroprevalence has a positive impact (95% pci [0.061, 0.305]). The effect of EHD/BTV on lamb recruitment is negative prior to die-offs (95% pci [-0.427, -0.062]) but positive fol- lowing die-offs (95% pci [0.599, 1.038]). *Pasteurellaceae* had a negative impact on lamb recruitment prior to die-offs (95% pci [-0.236, -0.059]). This trend did not significantly change following die-offs.

Drought

We had insufficient variation within the dataset to model drought separately pre- and post-die-off. Over all years studied here, drought was found to have a positive effect on lamb recruitment (95% pci [0.015, 0.058]).

Follow-up analysis: Isolated effects of select covariates

We used a linear model to assess lamb to ewe ratios as a function of days of continuous drought alone in herd-years where herds were *M. ovipneumoniae* cELISA seronegative (*M. ovipneumoniae* titers were not detected in any animals tested). For every added 50 days of drought, lamb-to-ewe ratios decreased by 3.0% ($p = 0.0714$; 95% CI = [-0.001, 5.6X10−5], R2 = 8.3). We also used a linear model to assess lamb to ewe ratios as a function of seroprevalence alone in herd years not experiencing drought. For every 10% increase in *M. ovipneumoniae* seroprevalence, lamb-to-ewe ratios decreased by 4.5% ($p < 0.001$; 95% CI = [-0.703, -0.202], R2 = 33.0) (Fig. 3.5).

DISCUSSION

Herd-level responses of bighorn sheep after *Mycoplasma ovipneumoniae*-associated disease events are highly variable, and the factors associated with that variation are poorly understood. Here, we estimated the effects of a suite of hypothesized drivers including density, substructuring, genetics, host behavior and phenology, pathogen community, and environment, on lamb recruitment in tandem, using 2,900 population-years of bighorn sheep recruitment data from throughout the state of Nevada.

As we hypothesized, an increase seroprevalence of *M. ovipneumoniae* decreased lamb recruitment. Seroprevalence is likely a marker of carrier ewes within the population (Garwood et al. 2020; Plowright et al. 2017). If there are only one or two carrier ewes in a population, they could easily be missed in a sampling event. However, pathogen presence in the herd is likely based on elevated titer levels of all sheep in contact with the infected ewes, resulting in elevated seroprevalence.

We detected effects of other pathogens (both viral and bacterial) on recruitment. In aggregate, EHD and bluetongue had a strong detrimental effect on recruitment prior to die-off events. We speculate the positive effect seen post-die-off is due to lamb recruitment already being poor due to the presence of *M. ovipneumoniae*, leaving little room for additional negative influence. The presence of bacteria from the *Pasteurellaceae* family also had a negative impact on lamb recruitment prior to die-offs. The effect did not change post-die- off, indicating the effect of these bacteria do not seem to be amplified in the presence of *M. ovipneumoniae*.

The positive effect of density indicates a likely presence of a (small) Allee effect in bighorn sheep herds across the state of Nevada. That this effect persisted both pre- and post-die-off might suggest that a variety of factors (both disease- and non-disease-related) could be at play. This merits additional exploration going forward.

We did not see any effect of herd substructuring on lamb recruitment. This could be due to the small number of collars in each herd, with 65% of populations with collars having fewer than 10 individuals collared, limiting our ability to detect temporal differences in mixing dynamics, but might also be attributable to population- (as opposed to subherd-) level lamb counts. More intensive collaring efforts within herds and more years of collared data collection might help better understand the effects of herd modularity on pathogen spread amongst lambs. We did see a slight positive density effect.

Our analysis indicated that lamb recruitment in desert bighorn sheep was not reduced during periods of drought. This could be because desert bighorn sheep are adapted to arid environments, allowing them to better cope with the added burden of drought (Hoglander et al. 2015). The positive effect of lagged drought on lamb recruitment was seen in all subspecies and could be attributable to resource investment and reallocation in the ewes. Environmental stressors such as drought could lead a ewe to fail to enter estrus or reabsorb or abort her fetus during the time of stress, resulting in a reallocation resources that benefits her own survival. This means that she has a year of not lactating and rearing a lamb and directing all resources, regardless of how minimal they are, into her own health rather than towards the lamb (Berube et al. 1996; Blanchard 2005). This could lead her to actually improve her own condition in the following year, increasing that year's chances that she successfully rears a lamb.

Follow-up analyses showed that in isolation, drought, and *Mycoplasma ovipneumoniae* seroprevalence both decreased lamb to ewe ratios. This indicates that both metrics are drivers of bighorn sheep population dynamics. There are reasons to think that efforts to mitigate *M. ovipneumoniae* spillover risk will have more potential to benefit recruitment dynamics than efforts to alleviate consequences of drought. Once *M. ovipneumoniae* is in a population, the consequences will likely be felt consistently for many subsequent years (at least in California and Rocky Mountain bighorns; see Fig. 2.4). Drought is not always sequential, allowing for years of population recovery between drought events. This means *M. ovipneumoniae* presence has more potential for population decline and limited recovery than drought in most circumstances.

MANAGEMENT IMPLICATIONS

The best way to protect bighorn sheep populations is to prevent introduction of *M. ovipneumoniae* into populations, though that is not always feasible. Our goal is to help wildlife managers recognize bighorn sheep populations that will be most in need of mitigation and management efforts as agencies do not have unlimited resources. While these results are useful considerations for managers, further exploration is still needed. Recent work has considered the benefit of local translocations on substructuring (Lula et al. 2020). We also believe genetic diversity has the potential to be a useful metric for identifying populations that would most benefit from translocation efforts but more years of data need to be collected to accurately identify what genetic make-up in a herd prior to die-offs results in chronic lamb mortalities.

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TABLES AND FIGURES

Table 3.1. Number of *Mycoplasma ovipneumoniae* tests by subspecies used in analysis.

Pathogen Tests			
Subspecies	# of herds (# of herd-years)	# of ELISA tests (# of herd-years)	# of PCR tests (# of herd-years)
California	11 (600)	372 (12)	481 (49)
Desert	40 (2050)	787 (71)	1182 (158)
Rocky Mountain	5 (250)	51 (13)	93 (24)

Table 3.2. State-space model coefficient output values. Each variable is assigned a credible interval based on their influence to lamb recruitment. Pre-die-off variables which have credible intervals crossing 0 do not have a detectable influence on lamb recruitment, below 0 is a negative influence, above 0 is positive. Post-die-off effects are in relation to pre-die-off effects (i.e. a pre-die-off effect which is negative and a post-die-off effect that is neutral means the post-die-off effect does not change from the pre-die-off effect).

Coefficient Results for Lamb Recruitment Model			
Variable	Lower credible interval	Upper credible interval	Standard error
Offset	0.802	0.864	5.91E-05
Density	0.000	0.001	5.07E-07
Pre-die-off not rut modulatiry	-0.081	0.037	1.13E-04
Post-die-off not rut modularity	-0.272	-0.081	1.76E-04
Pre-die-off rut modularity	-0.027	0.087	1.03E-04
Post-die-off rut modularity	0.086	0.295	1.93E-04
Pre-die-off Tajima’s D	-0.236	-0.045	1.80E-04
Post-die-off Tajima’s D	0.357	1.048	6.48E-04
Pre-die-off desert	-0.220	-0.121	9.15E-05
Post-die-off desert	0.140	0.563	3.99E-04
Pre-die-off seroprevalence	0.061	0.305	2.35E-04
Post-die-off seroprevalence	-0.708	-0.375	3.16E-04
Pre-die-off	-0.227	-0.037	1.83E-04
Post-die-off	-1.405	-0.787	5.78E-04
Pre-die-off EHD/BT	-0.427	-0.062	3.25E-04
Post-die-off EHD/BT	0.599	1.038	3.98E-04
Pre-die-off *Pasteurellaceae*	-0.236	-0.059	1.61E-04
Post-die-off *Pasteurellaceae*	-0.019	0.598	5.92E-04
Drought	0.015	0.058	3.95E-05

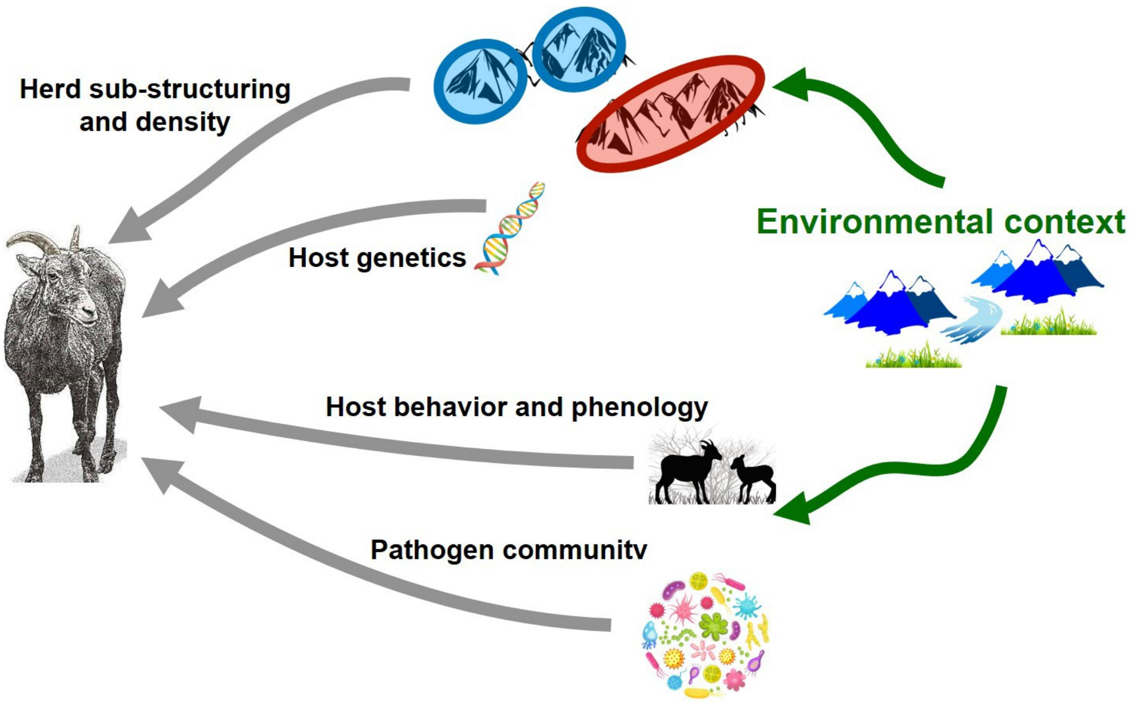

Figure 3.1. Drivers of host fate and population response. Proposed drivers of bighorn sheep population demography which are examined in this chapter.

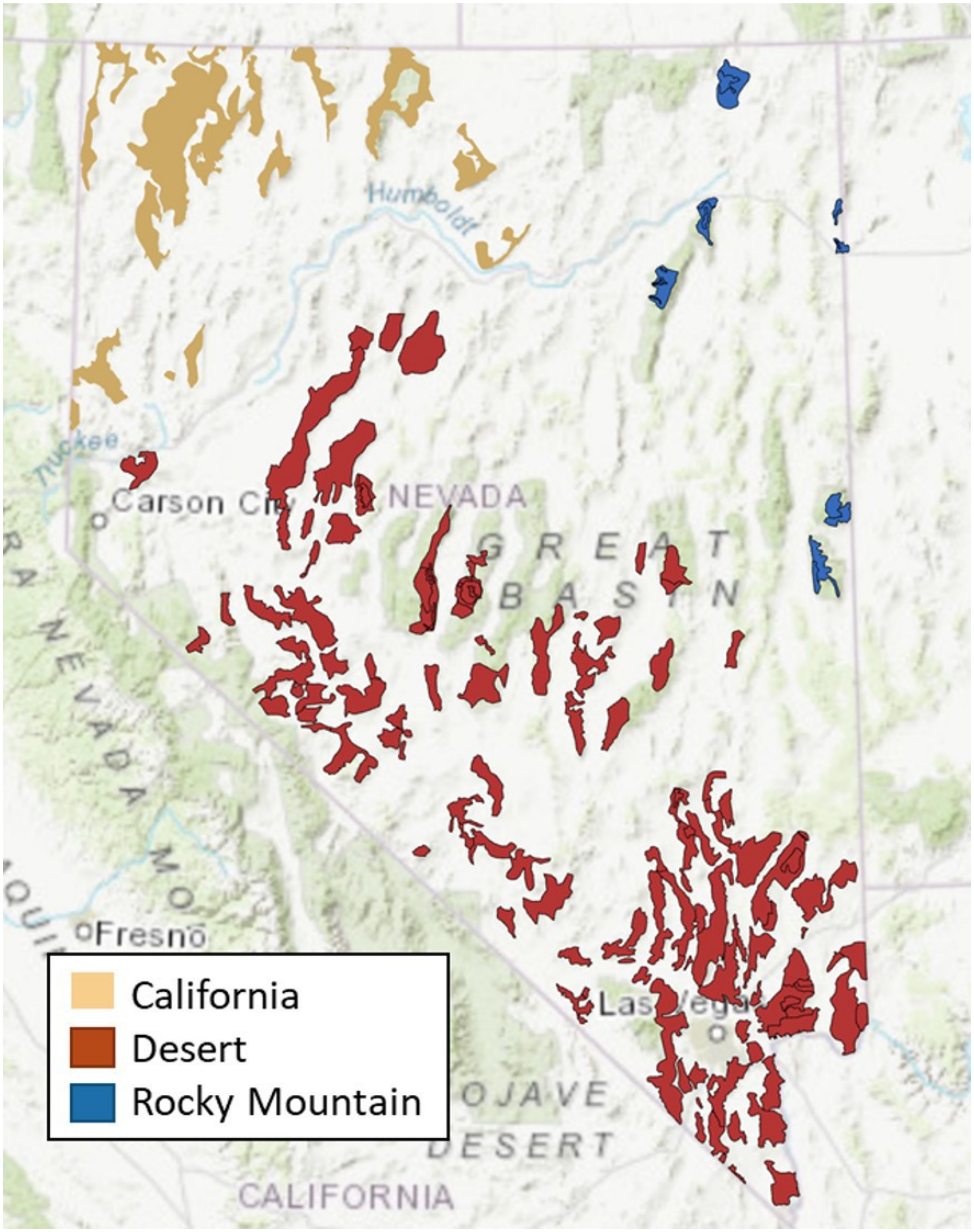

Figure 3.2. Nevada herd distribution map. Distribution of bighorn sheep herds across Nevada colored by subspecies. California sheep are herds in gold, desert in red, and Rocky Mountain in blue.

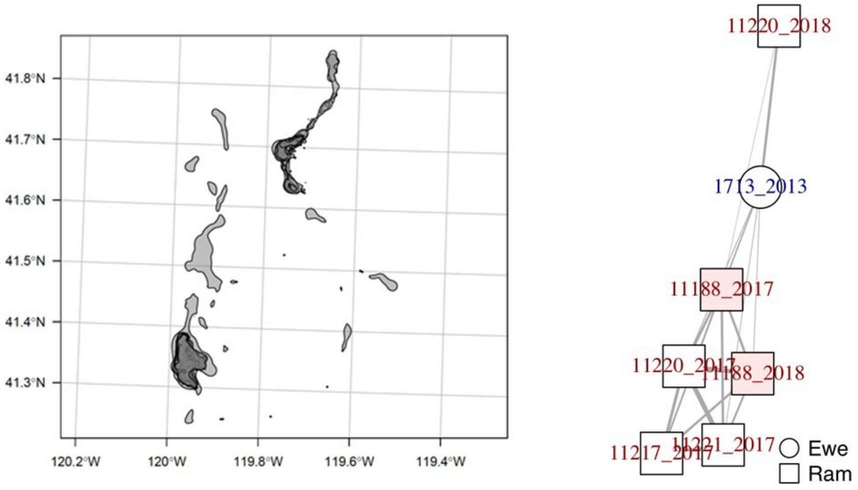

Figure 3.3. Modularity networks. The first panel represents the Brownian bridge home range output of one herd. The second panel is an example of modularity between individuals, with line weights increasing as connectedness increases.

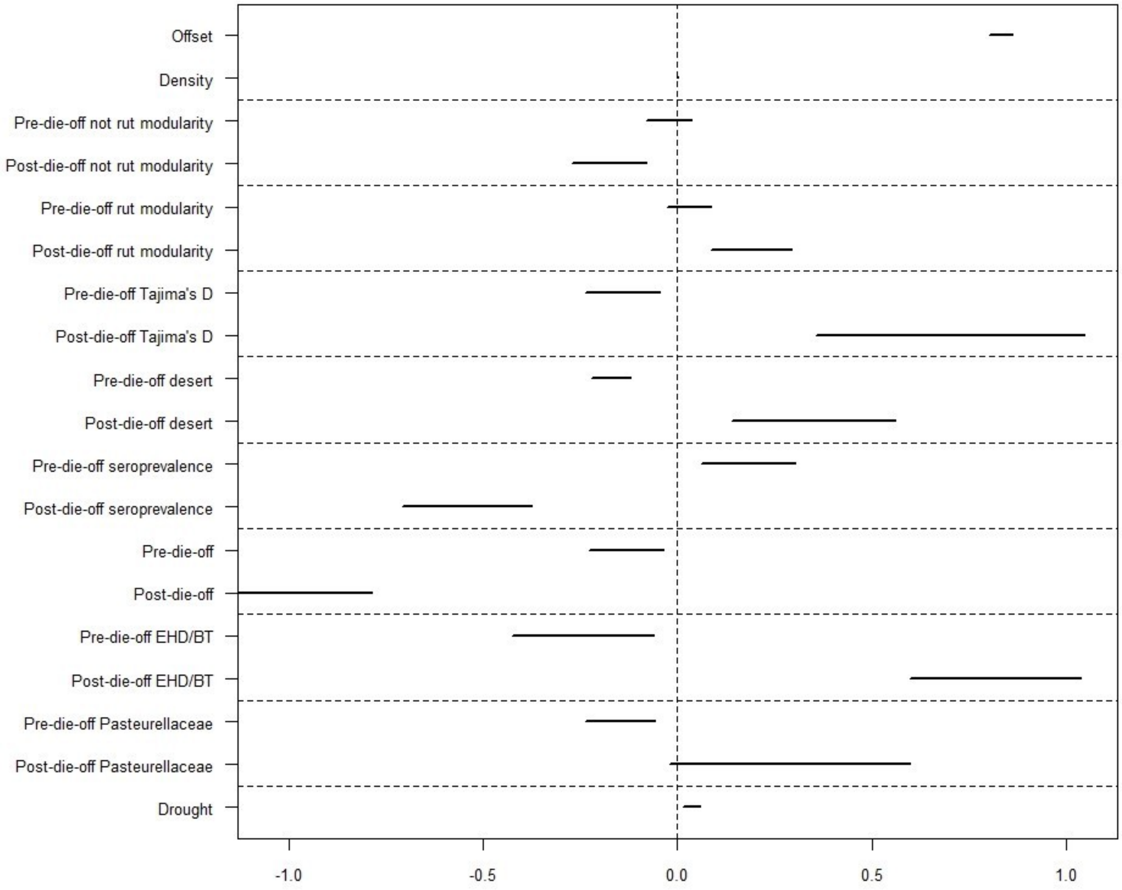

Figure 3.4. Results from model indicating effect of drivers on lamb recruitment. Names along the y-axis indicate the driver. Lines falling across 0 have no effect, those entirely above 0 have a positive effect, those below have a negative effect. Post-die-off effects are in relation to pre-die-off effects (i.e. a negative pre-die-off effect and neutral post-die-off means that variable's effect post-die-off does not change from pre-die-off). The effect of density was positive but is small enough to not be evident on this plot.

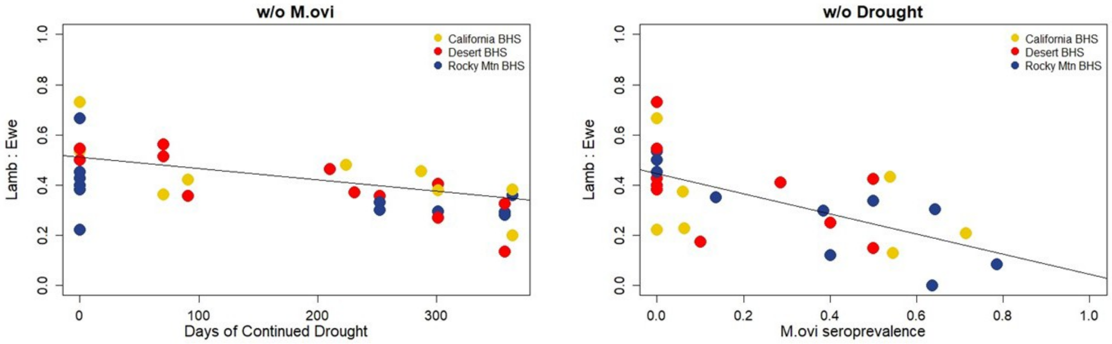

Figure 3.5. Patterns of drought and *M. ovipneumoniae* seroprevalence on lamb-to-ewe ratios in Nevada. The first panel indicates lamb to ewe ratios as a result of drought pressure, measured by days of drought duration, in the absence of *M. ovipneumoniae*. The second panel is lamb to ewe ratios with positive seroprevalence and in the absence of drought. In both panels, points are colored based on subspecies: gold for California, red for desert, blue for Rocky Mountain.

CHAPTER 4

CONCLUSION

Summary

Across the Intermountain West, bighorn sheep population recovery is an ongoing concern for managers. Bighorn sheep that are carrying *Mycoplasma ovipneumoniae* asymptomatically are playing a critical role in continued poor population performance following pathogen introduction events. Identifying drivers of population trends is necessary for sustaining bighorn sheep populations. Here, we utilized a temporally and spatially broad dataset which revealed unique insights into bighorn sheep population trends following pneumonia die-off events.

Preventing *Mycoplasma ovipneumoniae* from entering bighorn sheep populations is the most effective tool for managing bighorn sheep herds. However, wildlife managers do not have unlimited resources and prevention is not always possible. Because of this, recognizing drivers of bighorn sheep demographic trends following introduction is important. We have found drivers representing environment, density, substructuring, behavior, host genetics, and pathogen community can all play a role in lamb recruitment both prior to die-off events and in the years following die-offs. Our results suggest tracking seroprevalence in populations is important for identifying herds that may have asymptomatic carriers. We found an unusual positive density dependence, suggesting a small Allee effect. While follow- up analysis shows drought has a negative impact on lamb to ewe ratios, lagged drought in our model actually had a positive effect. As drought can vary year to year, this suggests that populations frequently have a time to recover from the impact of drought. In contrast, once *M. ovipneumoniae* is in the population, the pathogen is often there to stay for years, causing a continuous burden.

Moving forward, we will be adding in more hypothesized drivers of population demography, such as *M. ovipneumoniae* strain and normalized difference vegetation index, to increase our knowledge of critical drivers in this system. Improving modularity and genetic diversity metrics will be necessary for increased inference on these metrics. Ideally, we would like to broaden the dataset to include more Rocky Mountain herds to draw stronger inferences about driver effects on this subspecies.

APPENDICES

APPENDIX A

Nevada Bighorn Sheep Data

Table 1: Nevada bighorn sheep herds used in analysis.

Nevada Bighorn Sheep Herd Info

Location	**Hunt Unit**	**Sub-species**	**First Year of Counts**	**Last Year of Counts**	**Total Years of Counts**	**Years Since DieOff**	**Modul-arity**	**Years of cELISA Tests**	**Years of PCR Tests**	**EHD/ BT Testing (#)**	***Pasteure-llaceae***
Massacre Rim/Hays Canyon Range	11/13	California	1990	2017	22	NA	Yes	1	1		
Calico Hills	12	California	1990	2017	25	NA	No	2	5		
Granite Range	14	California	1985	2017	30	NA	No	1	1		
Petersens/Virginia Mountains	21/22	California	1991	2017	21	NA	No	0	0		
Pine Forest Range	32	California	1986	2017	28	NA	Yes	3	6	Yes(1)	
Guano Rim	33	California	1970	2017	40	NA	No	0	2		
Black Rock Range	34	California	1993	2017	23	NA	No	1	3		
Jackson Mountains	35	California	1986	2017	28	16	No	0	4		
Sahwaves	41	California	1993	2017	19	NA	No	1	1		
East Range/Stillwater Range	44/182	Desert	1984	2017	30	NA	Yes	1	3	Yes(2)	
Tobins	45/152	Desert	2004	2017	14	NA	Yes	1	3		
Santa Rosa/Mt. Moses	51	California	1980	2017	33	13	No	4	7	Yes(2)	Yes
Snowstorms	66	California	1991	2017	19	5	Yes	4	5	Yes(2)	Yes
Sheep Creek Range	68	California	1992	2017	23	NA	Yes	2	5	Yes(1)	Yes
Badlands	74	Rocky Mountain	1992	2017	23	16	Yes	3	5	Yes(2)	Yes
Leppy Hills	91	Rocky Mountain	1991	2016	18	5	No	1	1		
Ruby Mountains	102	Rocky Mountain	2009	2012	3	15	Yes	1	2		
North Snake Range, Mt. Moriah	114	Rocky Mountain	2006	2017	12	NA	No	1	5		
South Snake Range, Great Basin NP	115	Rocky Mountain	2013	2017	5	NA	No	4	4	Yes(1)	Yes
Duckwater Hills/White Pine Range/ North Pancake Range	131/164	Desert	2004	2017	14	NA	No	1	3		Yes
Grant Range	132	Desert	2007	2017	11	NA	No	2	4		Yes

Nevada Bighorn Sheep Herd Info											
Location	**Hunt Unit**	**Sub-species**	**First Year of Counts**	**Last Year of Counts**	**Total Years of Counts**	**Years Since DieOff**	**Modul-arity**	**Years of cELISA Tests**	**Years of PCR Tests**	**EHD/ BT Testing (#)**	***Pasteure-llaceae***
Pancake Range	134	Desert	1987	2017	21	5	Yes	1	3		Yes
Toquima Range	161	Desert	1984	2017	21	NA	Yes	0	2	Yes(1)	
Hot Creek Range	163	Desert	1987	2016	15	NA	No	2	3	Yes(1)	Yes
Toiyabe Range	173	Desert	1983	2017	21	NA	Yes	0	1	Yes(1)	
Monte Cristo Mountain	181	Desert	2006	2019	9	NA	No	2	4		Yes
Sand Springs Range	181	Desert	2006	2019	9	NA	No	2	5		Yes
Slate Mountain	181	Desert	2006	2019	9	11	No	3	6		Yes
Clan Alpine Mountains	183	Desert	1987	2017	29	NA	Yes	2	4		
Desatoyas	184	Desert	1987	2017	27	NA	Yes	1	3		
Virginia Range	195	Desert	2014	2017	4	NA	Yes	0	0		
Wassuk Range	202	Desert	2004	2017	12	NA	Yes	1	5		
Pine Grove Hills	204	Desert	1994	2017	16	NA	No	1	2		Yes
Gabbs Valley Range	205/207	Desert	1985	2017	31	NA	No	2	3	Yes(1)	
Excelsior Mountains/Candelaria Hills	206/208	Desert	1987	2017	26	NA	Yes	2	4	Yes(2)	
Silver Peak Range/Volcanic Hills/Lone Mountain	211	Desert	1994	2017	13	NA	No	1	4		Yes
Lone Mountain	212	Desert	1984	2016	22	NA	Yes	1	4	Yes(2)	
Monte Cristo Range	213	Desert	1980	2016	23	NA	NA	1	2		Yes
North Hiko Range	223	Desert	2005	2015	4	NA	No	1	1	Yes(1)	
Delamar Mountains	241	Desert	2005	2015	4	NA	Yes	1	1	Yes(1)	
Meadow Valley Mountains	243	Desert	1987	2017	21	NA	Yes	1	2	Yes(1)	
Arrow Canyon Range	244	Desert	1983	2016	16	NA	No	0	0	Yes(1)	Yes
Mt. Irish/Pahranagat Range	133/245	Desert	1978	2016	17	NA	No	0	1	Yes(1)	Yes
NNSS	252	Desert	1978	2017	23	NA	Yes	4	4	Yes(1)	Yes
Bare Mountains	253	Desert	1994	2016	11	NA	Yes	4	3	Yes(2)	
Last Chance Range	261	Desert	1990	2016	16	NA	No	2	2		Yes
Spring Mountains	262	Desert	1982	2016	23	?	Yes	3	2	Yes(2)	Yes

Nevada Bighorn Sheep Herd Info

Location	**Hunt Unit**	**Sub-species**	**First Year of Counts**	**Last Year of Counts**	**Total Years of Counts**	**Years Since DieOff**	**Modularity**	**Years of cELISA Tests**	**Years of PCR Tests**	**EHD/ BT Testing (#)**	***Pasteurellaceae***
McCullough Range	263	Desert	1983	2016	27	2	No	2	3	Yes(1)	Yes
Newberry Mountains	264	Desert	1978	2016	17	NA	No	0	0		
Eldorado Mountains	265/266	Desert	1976	2017	23	NA	No	1	3	Yes(1)	
Black Mountains/Muddy Mountains	267/268	Desert	1986	2017	31	NA	No	0	3		
River Mountains	269	Desert	1987	2016	28	?	No	7	5	Yes(3)	
Mormon Mountains	271	Desert	1987	2017	27	36	No	1	3	Yes(1)	
Virgin Mountains	272	Desert	1987	2017	17	NA	Yes	1	1	Yes(1)	
Spotted Range	280	Desert	2000	2017	17	NA	No	0	1	Yes(1)	
Pintwater Range	281	Desert	1986	2017	28	NA	No	0	0		
Desert Range	282	Desert	1986	2017	32	NA	No	0	5		
Las Vegas Range	286	Desert	1986	2017	28	NA	No	0	0		

APPENDIX B

Covariate Table

Model Covariates

Covariate	**Process**	**Construction**	**Level**	**Standardized**
Subspecies	Host Physiology and Behavior	NDOW data	Herd	No
Die-offs		NDOW count and health data	Herd, Year	No
Density	Herd Density and Substructuring	Lagged population size determined from aerial counts preformed by NDOW	Herd, Year	No
Herd Modularity	Herd Density and Substructuring	Brownian Bridge home ranges and overlap weights using GPS locations from collared individuals	Herd	Yes
Tajima's D	Host Genetics	Differences between nucleotide diversity and Watterson's estimator	Herd	Yes
Mycoplasma ovipneumoniae cELISA	Pathogen Community	WADDL results with results reported as % inhibition and considering positive results as those with inhibition 25.2%	Herd, Year	Yes
Mycoplasma ovipneumoniae PCR	Pathogen Community	WADDL results reported as cycle threshold and considering positive results as those with a threshold smaller than 38	Herd, Year	Yes
Drought	Environment	Days of continued drought within a calander year	Herd, Year	Yes

APPENDIX C

Integrated Population Model

Pneumonia-related die-offs have detrimental long-term effects on bighorn sheep (*Ovis canadensis*) populations. Like many species in the Intermountain West, bighorn sheep were already suffering from over hunting and habitat degradation (Spraker et al. 1984). Introduction of the pathogen *Mycoplasma ovipneumoniae* from domestic sheep have caused high mortality rates in many populations (Cassirer et al. 2018) and can limit a populations ability to recover in the years following by continued lamb moratlities (Cassirer and Sinclair 2007; Manlove et al. 2016). Understanding demographic drivers of bighorn sheep populations is critical for recovery.

We used herd level demographic, genetic, and pathogen data and individual demo- graphic, pathogen, and fate data in an integrated population model to better understand the drivers of post-die demographic dynamics in bighorn sheep populations. We considered both lamb recruitment and ewe survival as critical vital rates determining post-die trends.

METHODS

Underlying Markov process

Underlying system dynamics were described using a female-only Markov process model containing both demographic and disease status. Model states were Ewes_{S}, Ewes_{I}, Ewes_{R},

Lambs_S, Lambs_I, and Lambs_R. This structure is similar to one employed by Cahn et al. (2011) in a previous study examining propagation of bighorn sheep pneumonia. The current effort extends that model by empirically estimating state-transition rates, and allowing those rates to respond to covariates characterizing density, environmental condition, and herd health history. State-transitions were implemented through the likelihood of the integrated population model.

Biological process models for each vital rate

We investigated whether vital rates varied with die-off events, herd subspecies, herd-level *M. ovipneumoniae* PCR and cELISA prevalence, median positive seroprevalence levels, yearly drought values, and several interactions through rate-specific generalized linear models. For example, where Cahn et al. treat fecundity as constant, we treat it as a function of covariates:

$$\text{Fecundity}_{\text{Sewes}}[i,t] = \frac{exp(\beta_0 + a_1[die - off[i,t]] + a_2[subsp[i]] + \beta_1 * drought_{i,t})}{(1 + exp(\beta_0 + a_1[die - off[i,t]] + a_2[subsp[i]] + \beta_1 * drought_{i,t}))}$$

We included several interactions in our model. First is an interaction between cELISA seroprevalence and the median titer values of positives. We included this term as a measure of pathogen presence and virulence in a herd. Next was an interaction between drought and cELISA prevalence. The assumption here was drought in addition to high seroprevalence may have a stronger affect. Next, we looked at the additive effect of drought with seroprevalence and median titer values of positives. We then looked at the effect of drought where cELISA tests were not preformed. Finally, we looked at cELISA prevalence and median positive titers by subspecies.

To account for over-dispersion, we rescaled the data by subtracting the mean value from each herd-year value and dividing it by the standard deviation. We preformed this rescaling on drought, cELISA titer values, and median positive cELISA values.

Likelihood for proportion of ewes in "susceptible" and "infected" states

We estimated the proportion of "susceptible" (seronegative and PCR-negative), "infected" (seropositive and PCR-positive), and "recovered" (seropositive and PCR-negative) animals in a population-year from aggregated test results in years when animal handling occurred.

$$\pi_{Infected}[i,\ t] \sim \mathrm{Uniform}(0,\ 1)$$

Likelihood for vital rate effects

In the state-space model of population counts, we linked observed counts, y_{ewe}, to true abundance, N_{ewe}, through a Binomial distribution. Observed counts were taken from herd-level counts done every 1-2 years in 41 populations.

Next, we utilized known-fate data from collared individuals. We linked observed fates, $y_{indivIid}$, with true fates, $z_{individ}$, using a Bernoulli distribution. We had known fates on 100 individuals encompassing all three subspecies and 15 herds from 2005 to 2019.

Observation model for population count data

We used a state-space framework to account for imperfect population count data. We linked observed ewe counts, $y.ewe_{i,t}$, with true abundance, $N.ewe_{i,t}$, using a Normal distribution.

$$y.ewei,\ t \sim Normal(N.ewei,\ t,\ 0)$$

We utilized survival data from collared individuals through a known-fate model that linked observed, $y.individ_{i,h,t}$, and true, $z.individ_{i,h,t}$, survival probability through a Bernoulli distribution,

$$y.individ_{i,h,t} \sim Bernoulli(z_{i,h,t})$$

Incorporation of missing covariate data

For various reasons, much of our covariate data had many missing values, thus limiting how many population year results we were able to include in the model. To make use of more data, we incorporated a method suggested by Kery and Royle (2015) which estimates hyperparameters of a specified prior in order to predict missing values.

Priors

We used uninformative or minimally informative priors wherever possible. For our categorical predictors, subspecies and die-offs, we used Normal priors with means of 0 and standard deviations of 10. For all other predictors, we used Normal distribution with means of 0 and standard deviations of 100. The standard deviation for the observational model was created with a Uniform distribution running from 0 to 25.

Model implementation

We implemented our models using Nimble (de Valpine et al. 2017) in R (R Core Team, 2018). MCMC sampling was conducted as follows

- We ran three chains, each consisting of 50,000 steps. 25,000 of these were treated as burn-in and excluded.
- Poor mixing and slow convergence in early fits led us to replace the default Nimble samplers with block updates for all parameters describing individuals in a particular states (i.e., all coefficients affecting survival probabilities among PCR-positive lambs were updated as a block).
- We assessed sampler convergence using standard Gelman-Rubin diagnostics, as well as effective sample size. We sampled until a minimum of 500 effective samples were obtained for each inference-associated parameter in the model.