Summer nutrition, disease, or predation? Quantifying causes of poor lamb survival in northwest Wyoming

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Being able to identify factors that are contributing to population dynamics of large ungulate populations are often seemingly intuitive, however, quantifying their magnitude of influence on populations remains a persistent challenge. For example, research has shown that climate, food availability, harvest, and predation can all play a role in limiting ungulate populations. Nevertheless, being able to conclude, with confidence, which factor is primarily responsible for suppressing population growth demands fitness-based, and mechanistic research. When food resources are abundant (i.e., population well below nutritional carrying capacity), populations have the potential to grow rapidly in size until density-dependent feedback heightens, competition for resources increases, nutritional condition declines, and productivity and population growth subsides (Forsyth and Caley 2006, Monteith et al. 2014). This pattern occurs as populations approach nutritional carrying capacity and their per capita resources decline, thereby reducing nutritional condition (Monteith et al. 2014). Indeed, allocation of body reserves to rearing of young is secondary to survival, meaning female ungulates will conserve their own survival at the sacrifice of investing in their offspring—a consequence of their conservative life-history strategy (Martin and Festa-Bianchet 2010, Monteith et al. 2013). Therefore, knowledge of the progression in demography and in particular, nutritional status of populations as they approach nutritional carrying capacity are key elements to identify which factors are primarily responsible for regulating population growth.

The entrance of epizootic pneumonia to bighorn sheep populations, however, muddles the already complicated processes underlying population dynamics and is often the culprit for massive crashes of sheep populations. Bighorn sheep populations often experience sporadic fluctuations in abundance with periods of rapid population growth followed by massive population declines (Monello et al. 2001, Cassaigne et al. 2010). Populations of bighorn sheep have been afflicted by respiratory disease for the past century (Grinnell 1928). Although a wealth of research on the effects of disease on bighorn sheep (Miller et al. 2012, Cassirer et al. 2018), pneumonia continues to be one of the most poorly understood diseases that afflict wildlife in North America (Plowright et al. 2013b). Although epizootic pneumonia caused by bacterial respiratory pathogens are known to be the underlying driver of the massive mortality events, the frequency and intensity of the dieoffs are inconsistent and infections are not always manifested in disease (Cassirer et al. 2013). Indeed, chronically infected animals continue to persist and appear to develop some level of immunity against pneumonia (Plowright et al. 2013a).

Therefore, dieoffs are likely dependent upon certain ecological or environmental conditions—understanding these interactions could yield management alternatives to help reduce the frequency of epizootics and dampen fluctuations in abundance. In an extensive analysis of pneumonia epizootics from 99 herds across bighorn sheep range, Monello et al. (2001) noted that 88% of pneumonia-induced dieoffs occurred at or within 3 years of peaks in population size. As density dependence increases with growth of populations, declines in food availability lead to reductions in nutritional condition. Pneumonia in bighorn sheep continues to be one of the most poorly understood diseases that threaten wildlife in North America (Plowright et al. 2013a). Consequently, unraveling the interaction among density, nutrition, and disease are necessary to understanding population dynamics in bighorn sheep populations (Monteith et al. 2018).

The debate among scientists as to the bacterial pathogen (e.g., *Mycoplasma ovipneumoniae*, *Mannheimia haemolytica*) responsible for dieoffs remains lively (Besser et al. 2012), in part because secondary infections contribute further to eventual death (Dassanayake et al. 2010, Cassirer et al. 2018). Furthermore, in Wyoming, many bacterial pathogens have been documented in nearly all herds across the state, some of which continue to thrive despite being infected with *M. ovipneomoniae* and other bacteria. It is puzzling why some herds undergo dieoffs while others appear to harbor the similar suite of pathogens with less observable effects. For example, bighorn sheep in the Dubois herds continue to exhibit suppressed lamb recruitment, compared with herds in Jackson and Cody. Although having knowledge of the primary infectious agent is important, a better understanding of the ecological and environmental conditions that prompt widespread mortality or suppressed populations are equally critical and likely will lead to the most fruitful approaches for managing the disease (Cassirer et al. 2013); yet little effort has been made to understand those relationships. Unfortunately the uncertainty of what factors are underpinning variable dynamics across herds has stymied the identification of management approaches to mitigate pneumonia dieoffs.

Cutting-edge research in the past decade has connected processes of immune function with nutritional condition, food availability, and physiological stress across numerous taxa (Stahlschmidt et al. 2015, van Dijk et al. 2015, Peck et al. 2016). For example, following nutritional limitation, North American elk and red deer exhibited suppressed nutritional condition which corresponded with an alteration of their immune function (Landete-Castillejos et al. 2002, Downs et al. 2015). Infection of *M. haemolytica* can be highly fatal to bighorn sheep but nonpathogenic to domestic sheep, which suggests differences in immune function between the two species (Dassanayake et al. 2009). Immune function plays an important, but often poorly understood and underappreciated, role in fitness of populations (Lochmiller 1996, Graham et al. 2010, Downs and Stewart 2014, Nussey et al. 2014, Downs et al. 2018). The potential interplay among density, nutrition, and immune function may hold fruitful avenues for understanding the ecological context for disease in sheep populations, but also has important implications for management in helping mitigate the effects of disease (Monteith et al. 2018).

What we've learned

Numerous bacterial pathogens exist across ranges occupied by bighorn sheep, and although the disease is polymicrobial, some have suggested that *M. ovipneumoniae* is the precursor to subsequent infections and clinical effects of the disease (Besser et al. 2013, Cassirer et al. 2018). Separation between domestics and wild sheep are a primary goal in avoiding exposure and subsequent epizootic dieoffs, but in many instances we now seek to manage herds that while exposure to domestics is eliminated or radically reduced, the pathogens responsible for epizootic dieoffs remain. Therefore, we seek to manage herds with chronically infected animals wherein epizootic dieoffs associated with pneumonia may still occur without any new exposure to domestics. Therefore, recurring dieoffs within chronically infected herds are likely dependent upon certain ecological or environmental conditions—understanding these interactions could yield management alternatives to help reduce the frequency of epizootics and dampen fluctuations in abundance.

Although much work has been conducted to understand the role of disease in dynamics of bighorn sheep, it would seem some of the underlying and fundamental principles of population ecology have been discounted. For example, even in the presence of disease, populations are still subject to density-dependent limitation in forage as populations grow, predation, and for that matter, interactions may exist among density dependence, animal nutrition, predation, and disease. Indeed, immune function and nutrition may well be tightly linked and thereby, may lend some ecological and environmental context to when epizootic dieoffs may occur in chronically infected herds.

Our aim is to explore the interface between nutrition and disease, while calibrating nutritional levels associated with animal-indicated nutritional carrying capacity (NCC) in bighorn sheep. In so doing, we will evaluate the proximity of 3 key sheep herds to NCC, and elucidate the interactions among density dependence, nutrition condition, chronic stress, demography, and disease susceptibility. Achieving these key objectives will aid in understanding how herd density, environmental conditions, and harvest management interact to affect proximity of populations to NCC and their susceptibility to pneumonia.

Since March 2015, we have employed a longitudinal design to data collection across the Jackson, Dubois, and Cody sheep herds in northwestern Wyoming to connect seasonal changes in nutritional condition, reproduction, survival, recruitment of young, presence of respiratory pathogens, and immunocompetence. Although efforts are still underway to analyze and process current data, a few meaningful and yet, intriguing patterns have emerged.

In brief:

1) Based on seasonal changes in nutritional condition, Dubois sheep are apparently nutritionally limited on summer range and yet experience adequate winter conditions. Meanwhile, Jackson summer ranges appear robust with winter ranges being less so, whereas Cody ranges fall out somewhere in between. Notably, in the absence of a longitudinal and dual-capture approach, these underlying patterns that have led to our current considerations would have remained entirely unseen.
2) Recruitment of young in Dubois during 2016 and 2017 was surprisingly low and costs of lactation (when they are successful) are higher in Dubois than the other ranges, providing further evidence for concerns of summer range. Thus, the shift in focus towards distinguishing factors contributing to poor performance on summer ranges in Dubois, especially in comparison with Jackson where summer ranges are superior.
    a. Nutrition alone appears inadequate to explain suppressed recruitment of lambs in Dubois, suggesting that potentially micronutrient limitation, predation, or disease are contributing to suppressed lamb recruitment.

3) Nutritional condition of animals in Jackson appears to be declining (both spring and autumn), which is connected with declines in pregnancy—evidence of a population approaching NCC and a potential dieoff?
4) Autumn condition of lactating females from the 3 herds falls around the point of animal-indicated NCC for sheep in the Sierra Nevada, with Dubois sheep consistently being below that level. Does this suggest that ranges in NW Wyoming exhibit comparable levels of NCC as those in the Sierra Nevada? Regardless, it lends optimism towards establishing animal-indicated NCC for our Wyoming sheep with potentially boosting the sample size with sheep from the Sierra Nevada. Knowledge of this anchor-point is fundamental to establishing nutritionally based strategies of population and habitat management, and for assessing whether populations are regulated by nutrition or extrinsic (i.e., predation) factors.
5) In March 2015, antioxidant levels differed across the herds. Although unclear as of yet what this indicates, it could be connected to differences in immune function across the ranges.
6) Although there are some differences across ranges, seasonal nutritional condition is strongly linked to pregnancy, recruitment of young, and seasonal change in condition.

Next Steps

Given current observations, our aim is to continue to unravel the processes underpinning the dynamics of these herds by maintaining our longitudinal study design and increasing our efforts to understand contributions of summer ranges, and role of pathogens and predation especially in Dubois and Jackson. Importantly, our current objectives are completely driven and informed by what we have learned in the past 3 years of research, and are explicitly aimed at better understanding the contributions of summer nutrition, predation, and disease on survival of young sheep.

Project objectives include:

1) Estimate nutritional carrying capacity (NCC) of bighorn sheep populations in Wyoming to assess the capacity of habitats to support sheep.
    a. Over the long term, a key goal of this effort is to calibrate nutritional models for bighorn sheep, by coupling data on nutritional condition, pregnancy, recruitment, adult survival, and ultimately, population growth to develop models of animal-indicated NCC for Wyoming sheep. This will provide managers with tools to assess the proximity of populations to NCC.
2) Assess survival and cause-specific mortality of adult female sheep in Jackson, Dubois, and Cody herds.
    a. We will assess factors that contribute to probability of survival (e.g., nutritional condition, body mass, age, reproductive status), and causes of mortality when it occurs (e.g., disease, predation, accident). To date, leading cause of mortality for adult females has been predation by mountain lions.
3) Assess survival and cause-specific mortality of newborn sheep in Whiskey and Jackson herds to provide a comparison of the relative roles of nutrition, habitat, predation, and disease on recruitment of young.
    a. We will determine survival and cause-specific mortality of neonatal sheep.
    b. We will assess factors that contribute to probability of survival of neonatal sheep, including but not limited to: birth mass, sex, birth date, litter size, habitat

conditions, maternal nutritional condition, presence of respiratory pathogens, and maternal age.

    c. We will evaluate factors that contribute to the cause of mortality (e.g., disease, predators, malnutrition, accident), including but not limited to: birth mass, sex, birth date, litter size, habitat conditions, maternal nutritional condition, presence of respiratory pathogens, and maternal age.
    d. We will quantify the effects of nutrition, predation, and disease on survival and recruitment of young to identify which is most limiting to Whiskey and Jackson bighorn sheep.

4) Evaluate intrinsic and extrinsic factors affecting vital rates (i.e., pregnancy, seasonal survival, recruitment of young).
    a. We will evaluate the relationships between nutritional condition, body weight, presence of respiratory pathogens, and age on pregnancy and fetal rate of individual females in spring.
    b. Similarly, we will evaluate factors that affect probability of recruiting young to autumn including, nutritional condition in March, which may indicate the potential carryover effects of winter habitat conditions on subsequent population performance.
    c. We will quantify nutritional condition of females during autumn to provide a representative measure of summer habitat conditions, somatic cost of reproduction, and seasonal precipitation.
5) Assess longitudinal changes associated with disease, nutrition, and immunocompetence.
    a. We will assess seasonal dynamics in nutritional condition and presence of respiratory pathogens to evaluate the interactive role between these two factors.
6) Evaluate diet, and forage quality (including micronutrients) and abundance during summer for animals in Whiskey and Jackson ranges.
    a. Jackson is a system where summer ranges appear to be of higher quality compared with Whiskey, yet similar pathogens exist in both herds. We will compare forage quality and quantity of sheep key forage species within diets between Whiskey and Jackson.
7) Evaluate the effects of naturally occurring browsing, intense browsing, and fire on forage quality (including micronutrients) and biomass of forage on summer range.
    a. We will install a series of small (1m x 1m) enclosures in representative habitat on summer ranges in Jackson and Dubois to assess the effects of foraging and fire on sheep habitat. In 3 separate treatments we will 1) excluding foraging, 2) simulate intense foraging via clipping and biomass removal, and 3) implement fire boxes as an assessment of a potential habitat treatment.

Notably, our objectives correspond directly to needs outlined in The Wyoming Plan by the State-wide Bighorn/Sheep Domestic Sheep Interaction Working Group (2004). Specifically, that group expressed needs to monitor habitat selection and habitat NCC (pg 10), evaluate if poor nutrition contributes to disease susceptibility (pg 10), and determine if habitat improvements influence nutritional status of populations and thus influence NCC (pg 15). In addition, the group indicated that knowledge of carrying capacity was necessary to ensure sheep do not exceed the capacity of their habitat, and that understanding how habitat improvements modify nutrition and subsequently influence performance is necessary for sound management (Appendix K).

Study areas

We will initiate this facet of the project in the Jackson herd (HA 7), Cody herds (HA 1–4), and Dubois herd (HA 10)—all of which are core, native sheep herds within Wyoming, and are key herds within the Statewide Surveillance Program. Large-scale pneumonia dieoffs have never been documented in Cody herds. The Jackson herd has undergone two pneumonia dieoffs in the past 15 years, but has recovered relatively quickly following good lamb survival and recruitment. In contrast, the Dubois herd underwent a pneumonia dieoff over 20 years ago and continues to exhibit chronically low lamb recruitment. By contrasting these three herds, we hope to gain insight into why each has responded differently to the same bacterial pathogens.

The Jackson region is located east of Grand Teton National Park, near Jackson, WY. Summer and winter ranges are located in the Gors Ventre Wilderness and Wind River Range. Seasonal ranges in the Whiskey region are located south and east of Dubois, WY and extends into the northern end of the eastern front of the Wind River Range, and over to the western foothills of the Wind River Range for components of the herd.. Seasonal ranges in the Cody region occur throughout the Absaroka Range, east of Yellowstone National Park. This region extends from the northern Wyoming border to the southern extent of the Absaroka Range, ending north of the Buffalo Valley near Moran, WY.

Approach:

Our aim is to take a multi-pronged approach to address multiple causal factors contributing to population dynamics of bighorn sheep in northwest Wyoming, and how that broadly will aid in future management of chronically infected sheep herds. Specifically, we will quantify the relative contributions of nutrition, disease, and predation on population performance, and assess the current state of forage on summer ranges. Our approach is to continue our longitudinal monitoring of adult females from each of the 3 herds, which will yield valuable information on nutritional status and reproduction as females transition from one season to the next. We will link data on nutrition and reproduction to patterns of pathogen presence over time. With this next phase of the work however, we apply increased efforts to understand contributions of summer nutrition and disease to lamb survival by monitoring summer diet, forage quality, and survival, and cause-specific mortality of lambs in the Dubois and Jackson herds.

Adult capture and monitoring.—We will strive to maintain a sample of 58 adult (≥1 yr old) female bighorn sheep across the 3 herds, including a marked cohort of 18 animals in Jackson, 25 animals in Whiskey, and 15 animals in Cody herds to capture variation in habitat conditions and population densities among the herds. Desired samples in each region reflect in part, the goal of having at least 15 pregnant animals in Jackson each spring, and 20 pregnant animals in Dubois each spring. Therefore, estimated need also reflects projections for expected pregnancy rates, which with our work to date has ranged from 80 to 100%. Radiocollars will remain on animals that survive the duration of the experiment (3-5 years). When mortalities occur, collars will be recovered and placed on new, unmarked animals during the following capture effort. Our relative distribution of samples across the three regions reflects in part the size of the populations, accessibility of the animals, and feasibility. Therefore, our power in this project comes from combining sheep from 3 different regions that are exposed to different climate and habitat effects, disease presence, and density. With our approach to long-term monitoring among the herds and the potential change in disease frequency and its effect, we hope to be able to elucidate the potential interactions among density, nutrition, and disease.

We will continue to recapture the same radiomarked females during both autumn and late winter to allow assessment of seasonal ranges, and provide links to seasonal pulses in mortality commonly observed with pneumonia (i.e., breeding and lactation seasons). Animals in Jackson and Dubois will be recaptured via helicopter netgunning (Barrett et al. 1982), and given logistical circumstances, will be captured via ground darting in Cody (Smith et al. 2015). We will fit each sheep with an Iridium satellite GPS collar programmed to obtain a location at least every 1 hr throughout the year. In addition, the Iridium collars will be programmed to transmit location data and status (live or in mortality) remotely via the satellite system at least every 3 days, or in the instance of a mortality, immediately. Sheep in the Jackson and Whiskey regions will be fit with Iridium GPS collars (825g; ~1% body weight) equipped with a communication UHF system to allow remote monitoring of vaginal implant transmitters and fawn collars by mom's collar. Because the radiocollars are live satellite collars, all animals will be monitored live relative to survival, and mortalities will be recovered as quickly as possible following death in an effort to ascertain cause of death.

We will determine age of each female via incisor replacement patterns and horn annuli. We will measure body weight using an electronic platform scale, and assess relative size of animals using morphometric measurements. We will conduct field ultrasonography with a 5-MHz transducer to determine nutritional condition of each captured animal using standard protocols developed for bighorn sheep (Stephenson et al. in prep). During late-winter captures we will again use ultrasound and transabdominal scanning with a 3-MHz transducer to determine pregnancy status, after shaving the left-caudal abdomen (Stephenson et al. 1995).

Upon completion of ultrasonography in March, pregnant female sheep will be fitted with VITs. Chemical immobilization is not necessary to fit females with VITs (Bishop et al. 2007), thus all animals will remain physically restrained during processing. We will insert a VIT using the technique described in detail by Bishop et al. (2007), which has become the standard in studies utilizing VITs to identify birth sites and capture neonatal ungulates (Monteith et al. 2014, Smith et al. 2014b). We will sterilize all VITs in chlorhexidine solution prior to installment. We will insert VITSs using smoothed and sterilized plastic tubing and wooden dowels. The plastic tubing is approximately 20cm long, with 2.5-cm diameter (Bishop et al. 2007). We will place tubing and dowel in chlorhexidine solution between use. To insert a VIT, we will fold the silicone wings of the VIT together and place the VIT in the end of the tubing. We will apply liberally nonspermicidal lubricating jelly to the tubing, and will insert it into the vaginal canal until the tip of the wings of the VIT are pressed firmly against the cervix. We will then use the dowel, which extends through the tubing, to firmly hold the VIT in place while the tubing is gently pulled out from the vagina. The transmitter antenna is usually flush with the vulva, but sometimes extends outward in smaller animals. Fitting pregnant animals with VITs takes <1min to complete and will be coincident with other data collection and monitoring of our animals during capture. Therefore, fitting females with VITs will not increase our processing time more than 1 min. We will use standard VITs that have been sufficiently described in detail elsewhere (Bowman and Jacobson 1998, Carstensen et al. 2003, Johnstone-Yellin et al. 2006, Carstensen et al. 2009, Smith et al. 2014b), with the exception that ours will have the addition of a new chipboard with technology to communicate with the dam's Iridium collar. The new chipboard comes at no additional weight and does not alter the shape of the VIT.

VITs have flexible, silicone wings that induce pressure against the vaginal wall to retain the transmitter, thus eliminating sutures and facilitating a quick, nonsurgical insertion process

(Bishop et al. 2007). Vaginal implant transmitters have been employed without any reproductive problems or effects on female survival (Bowman and Jacobson 1998, Carstensen et al. 2003, Bishop et al. 2007, Carstensen et al. 2009, Monteith et al. 2014, Smith et al. 2014b) and are a practical technique for locating birth sites and subsequently, the capture of neonates (Carstensen et al. 2003, Johnstone-Yellin et al. 2006, Bishop et al. 2007, Monteith et al. 2014, Smith et al. 2014b). In a study focused on animal welfare, Johnson et al. (2006) found that VITs caused minimal tissue irritation and did not influence reproductive performance. Antennas of the VIT's will be approximately 9.5 cm in length and the tips are encapsulated in a resin bead to avoid fraying of the antenna. Vaginal implant transmitters will be equipped with a temperature-sensitive and light-sensitive switch that will increase pulse rate from 40 pulses to 80 pulses per minute when the temperature decreases below 32° C representative of the VIT being expelled by the sheep, a signal that will be received by the mothers Iridium GPS collar, activating an email to be sent indicating birth has occurred along with the current location. Alternatively, we will monitor VITs via aerial or ground-based telemetry. We will then recover the VIT immediately to determine if the location the VIT was expelled was indicative of a birth site, and thereby begin searches for the neonate(s). All neonate capture, handling and monitoring is described below.

Fecal samples will be collected during capture from each individual and subsequently analyzed for cortisol levels to relate to nutritional condition and infection. As females arrive to winter range each autumn, and before our autumn capture efforts, we will determine recruitment status (i.e., number of young reared) of each radiocollared female by hiking or driving to within visible range (<200m) of each female using radio telemetry.

Lamb survival and monitoring.—During the springs, and the beginning of the parturition season (approximately 1 June), adult females and VITs will be monitored remotely via UHF and satellite technology that links mom's GPS collar with the VIT. The technology will take advantage of the Iridium satellite system and Iridium GPS collars to uplink data that correspond to the status of the VIT and neonate collars directly. Therefore, most monitoring will be conducted remotely via email, or in lieu of that technology, will be completed daily via telemetry. Nevertheless, to ensure the success of the project in the event that the technology does not work seamlessly, we will be prepared to conduct daily fixed-wing flights during spring and summer to monitor animals via radio telemetry. Upon evidence of an expelled VIT, we will use field crews and ground-based telemetry to locate the VIT and the radiocollared female as quickly as possible. The location of the VIT, and location and behavior of the female will be used to identify search areas for the newborns. When searches fail to produce neonates, personnel will evaluate whether the location of VIT was an actual birthsite and confirm that supposition by observing pregnancy status and behavior of female (Monteith et al. 2014, Smith et al. 2014a, Smith et al. 2014b). If the female appears to have undergone parturition and if personnel are available, we will attempt to observe the female from a safe distance to avoid disturbance (>500m if habitat and topography allow) and utilize postpartum behavior of female to locate neonates (Carstensen et al. 2003, Monteith et al. 2014, Smith et al. 2014b).

We will capture neonatal sheep by hand and place them in a cloth bag containing local vegetation to facilitate restraint, although added restraint is rarely necessary, and to minimize scent transfer, although that will have little influence on potential abandonment (Livezey 1990, Carstensen et al. 2003, Monteith et al. 2014, Smith et al. 2014b). We will determine sex of each neonate and acquire a measurement of new hoof growth using dial calipers (Robinette et al. 1973, Brinkman et al. 2004, Monteith et al. 2014). We will measure length of hind foot, and total body length using a cloth tape (Sams et al. 1996), and determine the body mass of each neonate within the cloth bag to the nearest 0.1 kg using a hand-held electronic scale (Smith et al. 2014b).

Disease monitoring.— Our disease sampling protocol replicates that which has been part of a broader effort between Tom Besser (veterinary microbiologist, Washington State University College of Veterinary Medicine), Hank Edwards and Jessica Jennings-Gaines (Wyoming Game and Fish Department wildlife disease specialist), and Jennifer Ramsey and Neil Anderson (Montana Fish Wildlife and Parks) to derive standardized methodologies to allow comparison of results across a broader range of bighorn sheep populations in the tri-state area. The extensive and standardized methodologies stem from a broad and detailed collaboration to develop the most robust sampling design for bighorn sheep (Butler et al. 2017).

We will collect a suite of nasal (n = 2), throat (n =2), and ear swabs (n = 2) to culture and identify the various potential pathogens associated with pneumonia. Nasal and throat (pharyngeal) bacteriological swabs will be collected using sterile polyester tipped applicators and placed immediately in Hardy glycerol-buffered broth, and frozen as soon as possible. We take care to not contaminate samples by contacting non-target areas. We will enhance ability to collect tonsil swabs using a lighted extender and tongue depressor to sample the tonsillar crypts. Nasal swabs will be collected by simply rotating swabs inside each nostril. A replicate will be collected and included in a cryovial to be frozen. All culture and pathogen identification work will be conducted by the Veterinary Health Laboratory of the Wyoming Game and Fish Department using their standardized protocols. Nasal swabs will be cultured specifically for *Mycoplasma ovipneumonia* with a PCR assay used to determine if this pathogen is present in each sampled population. An index of the relative exposure of each sampled population to *Mycoplasma ovipneumonia* will also be obtained by submitting a small volume of serum for anti-*M. ovipneumoniae* antibody detection by ELISA. Similarly aerobic culture of throat swabs will be performed to determine presence of typical Pasturella pathogens including *Mannhemia haemolytica, Bibersteinia trehalosi, and Arcanobacterium pyogenes.* If *Mannhemia haemolytica* is detected in culture we may also perform leukotoxin gene testing.

We will evaluate whether previous experiences of individual animals (i.e., nutritional condition, reproduction, etc.) affects their probability of becoming infected, becoming symptomatic, and the subsequent influence of infection on vital rates.

Demography.—We will evaluate intrinsic and extrinsic factors related to vital rates (i.e., pregnancy, seasonal survival, recruitment) using appropriate statistical analyses. Assessing pregnancy and recruitment status each year will allow us to identify factors affecting survival of young, with specific emphasis on late-winter nutritional condition, age, and evidence of infection. In addition, we will evaluate how successfully recruiting young affects nutritional status and reproduction in subsequent seasons, as well as potential for interactions between reproductive success and susceptibility to disease.

We will monitor radiocollared adults and lambs again using the Iridium satellite system for continuous remote monitoring, and with fixed-wing or ground-based telemetry. When mortalities are detected, we will use ground telemetry to locate carcasses as quickly as possible (<8 hrs). We will examine carcasses to estimate date of death based on decomposition and condition of the animal. We will evaluate and record the location and arrangement of the carcass, presence and position of tooth marks, ante- and post-mortem bleeding or bruising, fractures, and remaining organs when present. We will identify other physical evidence of predation including tracks and feces (Elbroch 2003), take pictures, and collect hair for confirmation of the predator responsible (Moore et al. 1997). When the cause of death cannot be ascertained, the carcass will be taken from the field to the laboratory to be necropsied; field necropsies will be performed when distance or a precarious location hinders transport of the carcass from the field.

Nutritional carrying capacity.—Over the long term, a key goal of this effort will be to calibrate nutritional models for bighorn sheep, by coupling data on nutritional condition, pregnancy, recruitment, adult survival, and ultimately, population growth. This will provide managers with tools to assess the proximity of populations to NCC, much like that which has been developed for mule deer (Monteith et al. 2014). Doing so will yield innovative and empirically based tools to be able to assess when resources are overtaxed and potentially when population crashes are imminent or susceptibility to pneumonia dieoff increases. Interestingly, our nutritional condition levels relative to population performance appears to align to some extent with that of bighorn sheep in the Sierra Nevada (Stephenson et al. in prep), perhaps indicating some similarities in nutritional dynamics with population performance across systems for bighorn sheep.

Forage and diet monitoring.— To quantify quality of diets relative to habitat composition of female sheep on summer range in Jackson and Dubois, we will take advantage of the satellite uplink collars that provide real-time locations of radiocollared females. Based on the GPS locations from each female on summer range, we will determine short-term utilization distributions of their summer range, and then will grid search the area to collect fresh fecal samples. We will assume fecal samples occurring within each summer polygon were deposited by the individual female or a female residing with her in the area. We will strive to collect 10 samples within each female's temporary range, which will then be dried, blended, and composited to form one sample per individual female per sampling period. Because of changes in plant phenology and potentially diet throughout the summer, we will attempt to visit each female's summer range once during June (immediately following parturition), July (peak of lactation), and August (summer dry down and heavy lactation) to provide 3 composited fecal samples from each female's summer range during each year (2019–2021). Fecal samples will be prepared and analyzed via DNA metabarcoding to index diet composition (Kartzinel et al. 2015).

To assess forage quality across summer ranges, using a stratified-random approach to select sampling locations, we will establish transects to assess plant composition and biomass. We will assess habitat composition via point intercept transects, as well as biomass assessments via double-sample methodology. Once at every site, we will collect (clip) plant biomass by species to calibrate field-estimated biomass with actual (clipped) biomass. Clipped samples will be dried and weighed to convert field estimates (wet mass) to dry mass, which will be averaged across all plots at each site.

We will collect representative forage samples to determine crude protein content and in-vitro dry matter digestibility (IVDMD; carbohydrate content) by collecting portions of unbrowsed plants (i.e., new growth) from multiple plants and compositing them (>10 plants from same genus) (Hanley et al. 1992). In addition, from the same samples, we will assess micronutrient and plant secondary metabolites (Robbins 1983, Hanley et al. 1992). Plant samples will be collected and analyzed during summers 2019 and 2020 following identification of important forage items from the diet during a preliminary assessment during summer 2018. Finally, we will implement a series of small enclosures on representative summer ranges to assess the effects of naturally occurring herbivory, intensive herbivory, and fire on forage quality and biomass (White and Currie 1983, Hobbs and Spowart 1984, Greene et al. 2012, Holl et al. 2012, Whitford and Steinberger 2012).

Net benefits

Respiratory disease has afflicted populations of bighorn sheep for the past century and, despite substantial research on the topic, pneumonia continues to be one of the most poorly understood diseases that afflict wildlife in North America. Although we have learned much in recent years, most research has been myopically focused on identifying the primary infectious agent associated with pneumonia. Nevertheless, evidence continues to support multiple primary and perhaps secondary infectious agents, and in most instances, we now manage herds that are chronically infected as opposed to those subject to new exposure. Moreover, fundamental components underlying any large ungulate population including, habitat quality and quantity, and density dependent interactions remain operational and yet, are often neglected when considering disease dynamics. Our work to date has demonstrated that indeed, infected populations are not immune from fundamental nutritional dynamics and instead, suggests that nutrition may well be a key explanatory factor, along with disease, of the disparity in performance across sheep herds in northwest WY.

Our work seeks to understand how we can more effectively manage chronically infected populations. In doing so, we have the opportunity to more effectively manage sheep and their habitat through science. Moreover, implicit with our continued work is calibrating models of animal-indicated NCC for Wyoming sheep, which will increase the toolset for managers to understand how habitat, density, and extrinsic factors such as predation or perhaps disease are regulating these and other populations.

Timeline

This project began in 2015, with the goal of maintaining longitudinal data collection through 2021. Currently, we aim to enter phase II of this work as outlined in the proposal herein during spring 2019. In this scenario, we aim to recruit 2 technicians during the summer of 2018 that will conduct thorough reconnaissance work on summer ranges in Dubois and Jackson to determine feasibility, access, logistics, and collect preliminary diet and forage data to inform how the lamb survival work will proceed during summers 2019–2021. Lamb survival and intensive sampling of summer ranges would occur during summers 2019–2021, with data analyses and writing occurring thereafter.

Year	Time	Task
2018	Summer	Preliminary fecal collection and reconnaisance work
	Autumn	Recruitment surveys
	December	Recapture adults
2019	March	Recapture adults--fit VITs
	Summer	Neonate capture and monitoring
	Summer	Fecal and veg collection and assessment
	Autumn	Recruitment surveys
	December	Recapture adults
	Ongoing	Lab and data analyses
2020	March	Recapture adults--fit VITs
	Summer	Neonate capture and monitoring
	Summer	Fecal and veg collection and assessment
	Autumn	Recruitment surveys
	December	Recapture adults
	Ongoing	Lab and data analyses
2021	March	Recapture adults--fit VITs
	Summer	Neonate capture and monitoring
	Summer	Fecal and veg collection and assessment
	Autumn	Recruitment surveys
	December	Recapture adults
	Ongoing	Lab and data analyses
2022+	Ongoing	Analyses, presentations, publications, outreach

Projected Budget:

Description	FY2019	FY2020	FY2021	FY2022	FY2023
Radiocollars					
58 Satellite uplink GPS collars at $2400/collar	139,200	0	0	0	0
Satellite uplink for GPS collars ($500/collar/year)	29,000	29,000	29,000	10,500	0
35 Expandable GPS lamb collars at $700/collar	24,500	24,500	24,500	0	0
35 Vaginal implant transmitters at $275/transmitter	9,625	9,625	9,625	0	0
58 Rebattery GPS collars at $400/collar	23,200	23,200	0	0	0
Sheep Captures					
Spring helicopter capture 45 sheep at $750/per	33,750	33,750	33,750	0	0
Autumn helicopter capture 45 sheep at $750/per	33,750	33,750	33,750	33,750	0
Ground captures 30 sheep per year at $100/per	3,000	3,000	3,000	1,500	
Lab Analyses					
Plant quality analyses, 35 spp, 3 sampling bouts (Jackson & Dubois)	1,500	36,750	36,750	36,750	0
Fecal DNA metabarcoding, 3 sampling bouts (Jackson & Dubois)	2,000	9,600	9,600	9,600	0
Necropsy-related analyses	2,000	2,000	2,000	0	0
Personnel, Travel, Supplies					
Field equipment and supplies	32,000	20,000	15,000	15,000	500
Travel expense	21,000	21,000	21,000	12,000	6,000
MSc Student (n = 2)	50,400	51,912	53,469	55,073	0
Field Technicians (n = 6)	33,600	33,600	33,600	15,000	0
Accounting and technical support	30,697	23,218	21,353	13,242	945
Publications and outreach	0	0	0	0	7,000
	469,222	**354,905**	**326,397**	**202,416**	**14,445**
			Total Projected Cost:		**1,367,385**

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