The Effects of Disease, Stress, and Distribution on Bighorn Sheep Restoration in Nebraska

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Abstract: Twenty-two Rocky Mountain bighorn sheep (*Ovis canadensis*) were released in the southern Panhandle of Nebraska in 2001. Since the reintroduction, population size has fluctuated between approximately 30 to 60 animals, peaking at just above 60 in 2005. Due to the significant population fluctuations, research that investigates potential population limiting factors is crucial to long-term survivability of bighorn sheep in this area. The objectives of this study were to assess occupied and available habitat and investigate physiological stress response. Furthermore, all results were to be considered as they may have related to a respiratory disease epizootic that reduced the population by approximately 50% in early 2006. Observational data that was collected since the reintroduction was analyzed to identify primary occupied areas and illustrate changes in occupied habitat that may have been related to the respiratory epizootic. Fecal glucocorticoid metabolite assays were conducted to assess physiological stress response, and comparisons were drawn to evaluate differences in stress between occupied private properties and the Wildlife Management Area that served as the site of the reintroduction. On average, fecal glucocorticoid levels were significantly higher (t_{236} =2.92, P=0.004) in samples collected from sheep inhabiting private properties (33.60 ng/g) in comparison to sheep inhabiting the Wildlife Management Area (28.22 ng/g). These findings suggest that sheep occupying private properties may be exposed to a stressor that is not present within the Wildlife Management Area, such as livestock. When examined in the context of the affects of respiratory disease, these findings indicate that stress may have played a significant role in the outcome of the 2006 epizootic. Ultimately, this study suggests that careful management of occupied private properties may alleviate potential stressors that could contribute to disease.

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In March of 2001, the Nebraska Game and Parks Commission released 22 Rocky Mountain bighorn sheep into the Wildcat Hills of western Nebraska. The 22 sheep, consisting of twelve ewes, six lambs, and four rams, were captured and transported from the Pike's Peak area in Colorado and released in Cedar Canyon Wildlife Management Area (CCWMA). This release marked the first reintroduction of bighorn sheep in the southern panhandle of Nebraska. Prior to the reintroduction, the last documented sighting of bighorn sheep in Nebraska was in the early 1900s.

The first five years following the reintroduction were marked by successful growth of the population. By the end of 2005, the population had climbed to about 65 animals. Lamb production and survival rates were high and overall mortality rates were low. The population was on the rise, and a self-sustaining population was expected to be established in the Wildcat Hills within the next few years. However, the first few months of 2006 proved to be catastrophic for the population.

Initial concerns regarding the health of the population arose from observations of coughing sheep within CCWMA in the

spring of 2005. At this time, the population was spilt into two sub-herds; one that remained primarily with the boundaries of CCWMA and one that spent the majority of the time on neighboring private properties. The coughing appeared to escalate and spread amongst the sub-herd inhabiting CCWMA. Despite known interactions between the two sub-herds, the sheep occupying the private properties did not develop the cough until considerably later. Captures of two sheep on CCWMA property revealed infection of *Mannheimia haemolytica* as well as *Pasteurella trehalosi*.

Eventually, the coughing seemed to subside in the group of bighorn sheep within CCWMA. However, by early 2006, the sub-herd occupying the private properties was observed coughing, and by early February, pneumonia began to result in numerous mortalities within this group. In the end, the respiratory complex reduced the bighorn sheep population in the Wildcat Hills by about 50%. It was noted that all carcasses were found on private land, and no known mortalities occurred within CCWMA. The sheep that stayed primarily within CCWMA appeared to fend off early symptoms of respiratory irritation, while the majority of sheep inhabiting the neighboring private lands died of pneumonia.

Although it is widely known that *Pasteurella* causes respiratory distress in bighorn sheep and generally results in eventual death by pneumonia, the process of bacterial colonization of the lung is not well understood. It has been hypothesized that *Pasteurella* increases in number in the nose until an excess of organisms in the nose results in entry into the lungs (Grey and Thompson 1971). A healthy animal should be successful in clearance of the bacteria from the lungs, but the process may be inhibited by incidents of stress, concurrent viral infections, or environmental or climatic change (Boyce et al. 2004). These predisposing factors may compromise immunity of bighorn sheep, allowing for a shift from benign to lethal *Pasteurella* spp. infection or facilitating the establishment of highly pathogenic forms that would otherwise be controlled by immune system function (Monello et al. 2001). Factors that may result in suppression of the immune system and predisposition to pneumonia may include parasites such as lungworms (*Protostrongylus* spp.) or mites (*Psoroptes ovis*), nutritional deficiencies, periods of low forage quality and quantitiy, high predation or harassment, harsh weather conditions, inbreeding, or density dependent stress resulting from overcrowding (Risenhoover et al. 1988, Bailey 1990, Belden et al. 1992, Jones and Worley 1994, Frank et al. 2006).

The respiratory disease complex in bighorn sheep is complicated by the fact that infected individuals do not always die and sometimes no harmful effects of the bacteria are observed. Because bighorn sheep infected with apparently pathogenic strains of *Pasteurella* sometimes show no clinical signs of respiratory disease, it is believed that certain ecological or environmental conditions play a role in the all-age die-offs (Festa-Bianchet 1988, Ryder et al. 1992). The stressors that may have played a role in the pneumonia epizootic in the Wildcat Hills are not well understood. Inclement weather, interactions with livestock, predators, human disturbances, movement patterns, and environmental factors are all variables that are worthy of investigation in light of potential stress as it relates to the respiratory disease complex. Of particular interest is the possibility that a stressor existing in the occupied private lands but absent within CCWMA may have played a role in the outcome of the all-age die-off.

Study Area

The primary research sites for this project included three properties: Cedar

Canyon Wildlife Management Area (CCWMA) located at N 41 45.930' W 103 45.927', the Hampton property located at N 41 42.374' W 103 50.190', and the Montz property located at N 41 46.716' W 103 55.207'. These three properties are situated in the southern panhandle of Nebraska in an area known as the Wildcat Hills. CCWMA is an 890 hectare state-owned property open to the public for hunting and recreation. The Hampton and Montz properties are privately owned ranches that are periodically grazed by cattle and encompass 3,520 hectares and 1,942 hectares respectively.

All three properties are located approximately 11 – 19 kilometers southwest of Gering, Nebraska. Although these areas are primarily within the boundaries of Scotts Bluff County, the southernmost portion of the Hampton property extends into Banner County. CCWMA was chosen for the release site of the reintroduction in 2001 based on a bighorn sheep habitat suitability assessment of the Wildcat Hills (Forbes 1999). The Hampton and Montz properties were colonized naturally by bighorn sheep following the reintroduction. These three properties were selected as primary research sites for this project based on previously collected observational bighorn sheep occupancy data.

Methods

Observational data was collected by field technicians since the reintroduction took place in 2001. Data included location, habitat, behavior, and distance from escape terrain, people, water, and livestock. The location data was used to determine occupied habitat and assess changes over the past years, and the additional variables were considered in relation to stress response as measured by fecal glucocorticoid assays and disease as it has affected the population.

Fecal samples were collected opportunistically beginning in November of 2006 and ending in December of 2007. Samples were collected from sheep inhabiting CCWMA, the Hampton property, and the Montz property for a total of 285 samples. To prevent disturbing of the bighorn sheep during collection, defecation was observed from a distance that did not alarm the animals, and pellets were picked up only after the sheep had moved a reasonable distance from the area on their own. Because the hormones may not be distributed evenly throughout the samples, pellets were collected from various segments of the entire sample.

The pellets were homogenized in freezer bags by thorough mixing and mashing. The freezer bags were marked with the date, time, location, and individual, and then stored in the freezer within two hours following collection. Fecal pellets were collected from ewes, rams, and lambs, and efforts were made to collect from several of the same radio-collared ewes as often as possible in order to establish baseline glucocorticoid levels for several individuals. Field notes were recorded during sample collection documenting weather and observations of any existing potential stressors (i.e. livestock in the immediate vicinity, presence of predators, human disturbances, observable changes in social structure or distribution of individuals, etc.).

Immediately prior to extraction, the frozen fecal samples were thawed and homogenized by thorough mixing. Plastic containers were labeled for each sample, and approximately 1 gram of each sample was placed in the appropriate separate container. The containers were then placed in an oven at 37° C for 18 – 24 hours to complete the desiccation process. Following desiccation, a mortar and pestle was used to grind each sample to a fine dust. Detritus including

sticks, hay, and other sizeable debris were removed from the sample and discarded.

From each of the finely ground samples, 0.2 grams of dried fecal material were placed in the bottom of appropriately labeled 15 ml extraction tubes. A solution of 90% ethanol (90:10 EtOH:distilled water) was added to the tubes in the amount of 2 ml. Tubes were placed on a shaker and shaken for 30 minutes and then centrifuged at 2000 x g, and 1 ml of ethanol was removed from each tube. The extracts were then capped and stored at -20° C until the assay procedure.

A commercially available kit was used for the assay procedure. Specifically, the I^{125} corticosterone radioimmunoassay (RIA) kit (ICN #07-120103, ICN Biomedicals, Costa Mesa, California) was used to quantify the fecal glucocorticoid metabolite concentrations. This procedure has been determined to be effective for quantifying fecal glucocorticoid metabolite extracts in a wide array of wildlife species (Wasser et al. 2000). All samples were diluted 1:4 in a phosphate-buffered saline (PBS) and assayed in duplicate following the ICN protocol for the I^{125} corticosterone RIA.

A standard assay validation was performed to ensure that the assay could accurately and precisely measure fecal glucocorticoid metabolites in bighorn sheep. Parallelism was demonstrated through preparations of serial dilutions of a pooled sample that produced a displacement curve parallel to the standard curve. The pooled sample was measured in each assay performed to assess between-assay reliability. Based on the duplicate sample agreement (n=374 assay determinations), the intra-assay coefficient of variability was 4.7%. The interassay coefficient of variation (n=6 assays) was 14.4 %. The range of standards was 12.5 – 500 picograms per tube.

Fecal glucocorticoid metabolite concentrations were analyzed by calculating the mean and range for several different groups of samples. An Analysis of Variance (ANOVA) was conducted to evaluate differences in glucocorticoid metabolite levels between the groups. These groups included ewes, rams, and lambs, as well as samples divided by location of collection. The glucocorticoid concentrations of the samples collected within CCWMA and surrounding areas were compared with those collected on the Hampton and Montz properties. Because there were no sheep inhabiting the Hampton or Montz properties during December through February, samples collected during these months were removed from the CCWMA group to form an adjusted group that was representative of samples collected over corresponding time periods. Additionally, differences across seasons were analyzed and samples collected from five adult ewes were compared. The individual ewe data included samples collected from three ewes primarily inhabiting CCWMA as well as two ewes primarily inhabiting the Montz property. The Hampton property was rarely occupied throughout the duration of this study.

Results and Discussion

Results of the glucocorticoid metabolite assays suggest that while sex and age class do not significantly affect stress as measured by corticosterone concentration, there are significant differences across seasons and the areas occupied by the sheep of this particular population. Additionally, an analysis of fecal glucocorticoid metabolite concentrations from samples collected from five adult ewes revealed no significant differences between individuals. Overall, the average concentration of corticosterone for the 285 samples collected

was 27.58 ng/g of feces. Results ranged from a minimum of 7.13 ng/g to a maximum of 77.85 ng/g with a standard deviation of 13.41 (Table 1).

Table 1. Overall corticosterone concentration data.

	AVERAGE	MAX	MIN	MEDIAN	STDEV	TOTAL SAMPLES COLLECTED
OVERALL	27.58	77.85	7.13	25.23	13.41	285
RAMS	27.02	35.01	13.97	26.08	6.22	155
EWES	28.31	70.69	7.13	24.15	18.01	75
LAMBS	28.17	77.85	12.47	24.46	14.18	55

The observational data collected since the release in 2001 emphasized the failure of the bighorn sheep to re-colonize the Hampton property following the respiratory epizootic that decimated the sub-herd which had previously occupied that area. The additional variables that were recorded revealed that bighorn sheep in the Wildcat Hills typically occupy areas greater than 100 meters from water, livestock, and people, and they are generally found within 25 meters of escape terrain.

Perhaps the most interesting findings of the study were the glucocorticoid assay results which revealed that bighorn sheep occupying the Montz and Hampton properties experience significantly increased levels of corticosterone in comparison to sheep occupying CCWMA and areas in the immediate vicinity (Table 2). A two-sample *t* test assuming equal variances was performed and the Montz and Hampton group yielded significantly (t_{236} = 2.93, P = 0.004) higher corticosterone concentrations than the adjusted CCWMA and nearby areas group.

Table 2. Corticosterone concentrations by location.

	Average	Max	Min	STDEV	Median	Total Samples Collected
CCWMA and Nearby Areas	25.46	77.85	7.13	12.56	22.89	211
Montz and Hampton	33.60	76.74	15.58	13.99	29.4	74
Adjusted CCWMA	28.22	77.85	7.13	12.68	25.84	165

The difference could be attributed to numerous variables, and should be discussed with careful consideration of the respiratory disease epizootic of 2006. One of the variables that could have contributed to the increased corticosterone levels is livestock. The Montz and Hampton property are both periodically grazed by cattle, while CCWMA is generally not grazed, and

was never grazed throughout the duration of the study. Although numerous variables may have contributed to the increased corticosterone levels, the grazing is the most obvious difference between CCWMA and the Montz and Hampton properties.

The existence of competition between cattle and bighorn sheep is debatable. Because bighorn sheep prefer steeper slopes in comparison to the gentle slopes typically used by cattle, ranges generally do not overlap spatially. It has been suggested that bighorn sheep prefer slopes > 30% in contrast to the ≤ 30% slopes favored by cattle during the winter (Lauer and Peek 1976). Although earlier studies did not reveal competition between cattle and bighorn sheep (Halloran and Blanchard 1950, Couey 1959, and Arellano 1961), it has been argued that bighorn sheep have not used certain gentle slopes due to the grazing of cattle on these areas (Barmore 1962).

The analysis of data collected since the reintroduction in 2001 revealed that bighorn sheep were documented at distances greater than 100 meters from livestock 99.51% of the time. From these results, it could be inferred that bighorn sheep generally avoid close contact with cattle. However, this data may not be adequate to accurately reflect the complex relationship between bighorn sheep and cattle in the Wildcat Hills. The data does not exemplify occurrences of cattle in areas generally used by bighorn sheep or the observed utilization of resources that have been determined to be important to the sheep. Detailed observations about cattle and sheep including recorded occurrences of cattle in primary areas of sheep habitat, even in instances when the sheep are not in the area, would improve knowledge about the dynamics between bighorn sheep and cattle in the Wildcat Hills.

Although very few direct interactions between cattle and bighorn sheep have been documented within the Montz or Hampton properties, it is plausible that the Hampton property is no longer used by sheep due to an avoidance of cattle. It has been suggested that although bighorn sheep may not compete directly with cattle for space or resources, they often exhibit a level of social intolerance for cattle (King and Workmann 1984), and numerous studies support the argument that bighorn sheep avoid areas grazed by cattle (Barmore 1962, Albrechtsen and Reese 1970, Ferrier and Bradley 1970, Dean 1975, and Gallizioli 19977). In certain instances, this avoidance could also be attributed to overgrazing and a resulting lack of adequate bighorn sheep forage in grazed areas.

Based on this available information about the relationship between cattle and bighorn sheep, it could be speculated that the increased corticosterone levels found in fecal samples collected on the Montz property were at least partially influenced by the presence of cattle in the area. It was suggested that an outbreak of respiratory disease in Aravaipa Canyon, Arizona, that reduced the desert bighorn population by 52% was a result of livestock related viral disease compounded by stress related to nutrition (Mouton et al. 1991). Although the culprit of the all-age die-off in the Wildcat Hills was a bacterial infection that was more likely to have originated from domestic sheep, the pneumonia epizootic may have been related to stress and nutrition associated with cattle in ways similar to such an instance.

However, there are numerous other variables to consider in the investigation of potential causes of elevated corticosterone levels in samples collected on the Montz property. One important consideration is the number of sheep occupying the Montz property in comparison to the number occupying CCWMA throughout the study. Throughout the duration of the study, only a small group of sheep inhabited the Montz property. For the majority of the time, this group included two adult ewes, a yearling ewe, two lambs, and several young rams that tended to come and go on a regular basis. This group was significantly smaller than the group or groups inhabiting CCWMA. Typically there were over a dozen adult ewes comprising a group at CCWMA in addition to the majority of yearlings and lambs of the population and rams that were frequently observed with the herd.

The small size of the group inhabiting the Montz property during much of the study period could be directly related to the increased corticosterone levels due to variables such as alertness and foraging efficiency. A relationship between increased foraging efficiency and increased group size at least up to five sheep has been demonstrated, and it has been determined that sheep in small groups were more likely to momentarily cease foraging activity to scan their surroundings in alertness than were sheep in larger group (Risenhoover and Bailey 1985). Similar studies revealed that sheep foraging efficiency increased with group size up to 20 animals, and solitary sheep were three times more alert than individuals in groups of 20 animals (Berger 1978 and Simmons 1982). Despite the individual increased alertness in small groups, the combined alertness of sheep foraging in groups of 20 animals afforded nearly seven times the number of alert postures per minute, and therefore it could be said that the ability to detect predators or other threats increases with group size. Considering this correlation between group size and increased foraging efficiency and predator detection ability, it could be inferred that sheep in smaller groups naturally experience higher levels of stress in comparison to individuals comprising large groups. This could contribute to an explanation for the higher levels of corticosterone in the samples collected from the Montz property compared with the CCWMA samples.

Ultimately, further research is necessary to determine the cause of the increased fecal glucocorticoid metabolite concentrations in samples collected from the Montz property. Cattle and small group size are two variables that could serve as focal points for future research projects aiming to assess stress in this bighorn sheep population.

In addition to comparisons of samples collected across different areas, fecal glucocorticoid metabolite results were grouped and compared by season (Table 3). An Analysis of Variance (ANOVA) revealed significant differences in corticosterone concentration across seasons ($F = 61.22$, $F\ crit = 2.64$). Measurements were highest in the summer with an average of 35.64 ng of corticosterone/g of feces and lowest in the winter with an average of 15.23 ng/g. Samples collected in the spring averaged 34.12 ng/g, while samples collected in the fall averaged 24.91 ng/g.

Table 3. Average corticosterone concentrations by season.

	Winter	Spring	Summer	Fall
Ewes	17.06	35.37	31.3	22.04
Rams	16.4	34.05	44.26	19.37
Lambs	14.01	30.18	38.4	24.03
Overall	15.23	34.12	35.64	24.91
Total Samples Collected	76	69	80	60

The results of this project largely support another study (Goldstein et al. 2005) which demonstrated that bighorn sheep glucocorticoid levels appear to reflect a cyclical pattern based on the seasons, with the highest levels measured in the summer and the lowest levels measured in the winter. One important consideration however, is that according to the results of this project, corticosterone levels of ewes are actually slightly higher in the spring than in the summer months. It can be inferred that this is due to the lambing season. Ram corticosterone levels, on the other hand, were higher in summer than spring. Overall, it does appear that there is a natural fluctuation of fecal glucocorticoid metabolites based on the seasons. It is plausible that these fluctuations are more a result of normal seasonal metabolic rhythms than a result of actual seasonal changes in the degree of physiological stress experienced by the animals. Therefore caution is urged in the interpretation of the seasonal comparisons of fecal glucocorticoid metabolite measurements used in this study.

This project did not reveal significant differences in corticosterone levels of rams compared to ewes, nor did it reveal significant differences in the comparison of adults and yearling with lambs. Therefore age class and sex of bighorn sheep may have little effect on the concentration of corticosterone found in fecal samples, suggesting that males and females, as well as adults and lambs, all face similar stressors and respond to them in similar physiological ways. The project also revealed no significant difference between average corticosterone levels of five adult ewes from which samples were collected throughout the duration of the study. Further research that involves very consistent sampling from individuals over extended periods of time would be necessary to draw solid conclusions about differences in individual corticosterone levels. For purposes of this study, the individual ewe results provide preliminary baseline data illustrating corticosterone measurements for several adult ewes in this bighorn population.

A final aspect of this project worthy of consideration is the necessary caution in interpretation of the glucocorticoid metabolite results. While fecal glucocorticoid metabolite assays provide a noninvasive alternative to traditional stress assessment methods that involve capture of wildlife for collection of blood samples, careful consideration of all of the existing variables is essential to drawing accurate conclusions from the results. Specifically, caution is urged in consideration of what the results might mean for the animal in terms of acute versus chronic stress.

The release of glucocorticoid hormones in response to a stressful event initiates numerous physiological reactions that are critical to survival in the presence of a stressor (Von der Ohe and Servheen 2002). In the case of acute stress, this release of hormones is healthy and advantageous to the animal. However, in the instance of prolonged stress and extended periods of high glucocorticoid levels in the bloodstream, these hormones can also have a deleterious effect on the health of the animal. In instances of elevated circulating glucocorticoids over prolonged periods of time, the immune system of the animal may be compromised, resulting in increased susceptibility to disease. Direct results of prolonged elevation of glucocorticoids may include inhibition of enzyme production, delayed processing of antigens, and quantitative reduction of immune system responses (Kiecolt-Glaser et al. 1984, Golub and Gershwin 1985).

Ultimately, the findings of this project raise questions about the differences in corticosterone levels experienced by the reintroduced sheep inhabiting private properties in Nebraska in comparison with sheep inhabiting Cedar Canyon Wildlife Management Area, as well as the reason behind the changes in primary areas occupied, specifically the failure to re-colonize the Hampton property, that followed the epizootic. Further research aimed at identifying differences between the Montz and Hampton properties and CCWMA would be of value. Understanding these differences could have implications for disease prevention and bighorn sheep management in the Wildcat Hills of western Nebraska.

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