Bighorn Sheep/Selenium Study 2002

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The summer 2002 field research program was conducted with the purpose of maintaining data collection as part of ongoing Bighorn Sheep/Selenium Study initiated in 1998. Additions to the data base were proposed in five distinct areas of interest: 1) monitoring rainfall and subsequent chemical analysis for concentration of nitrate, sulfate and ammonium; 2) monitoring of bighorn sheep health, movement, and lambing success through five seasonal ranges; 3) monitoring experimental test plots on the alpine summer range to determine effects of varying amounts of water and nitrates on forage selenium uptake, and continuing to look at soil redox potentials relative to selenium speciation and availability; 4) a study of the local soil microbial populations for a possible role in selenium transformations and transport; and 5) an attempt to establish baseline data for forage selenium levels on other mountain sheep ranges for comparison to the Whiskey/Arrow/Middle Mountain forages where selenium responsive disorders (SRD) are suspected of threatening herd productivity and perhaps survival.

Although previously funded by The Wyoming Game and Fish Department, Foundation for North American Wild Sheep, U.S. Forest Service, Bureau of Land Management, and private groups and individuals, financial support for the continuation of this study in 2002 was made possible by grants from private sources (see acknowledgements) under management of the University of Wyoming. The establishment of the experimental test plots and training of a field crew was delayed until early July because of the lateness of obtaining necessary funding. Therefore we were not able to implement the experimental portion of the study in a way that replicated the previous year's work. In addition, the severe drought that continued throughout the summer made it difficult and in some cases impossible to replicate the study design. Consequently we have treated and interpreted the results of this part of the study with due caution and only in qualitative terms. Regarding the research budget for 2002, not all of the proposed equipment items were purchased and not all of the proposed research activities completed since project expenses greatly exceeded funds available.

RESULTS AND DISCUSSION:

1) **Precipitation:** Only ten rain events produced measurable amounts of precipitation compared with nineteen in 2001. Precipitation was measured using a standard rain gauge and all rain events, which produced sufficient quantities, were analyzed for $NO_{3,}$ SO_4 and NH_4 using a Hach spectrophotometer (see table for Water Chemistry Middle Mountain 2002). The entry for 70702 with an exceptionally high reading for sulfate (37mg/L) was run twice as a suspected contamination outlier. It is quite possible that these high levels could be the result of deposition by forest fires located upwind of the study area and active at the time of measurement. The nitrate concentrations are similar to those collected on the same site in 2001. The very small quantities of precipitation and an almost complete absence of fog resulted in a low overall nitrate deposition for 2002. As indicated in the table four of the ten rain events exceeded the low range mode of the spectrophotometer and were not reanalyzed in the high range. Therefore these nitrate levels may have been higher. Total nitrate in precipitation = > 11.352 mg/L (ppm): deposition = >

0.702 kg/ha NO_3: total for 2001 = 1.455 kg/ha NO_3. We reran some rain samples at a later date to determine if chemical levels and pH varied over time in storage (see yellow highlight in table).

2) **Sheep Migration and Health:** Drought also affected the winter snow deposition. The early melt encouraged early emergence of some forage species that was followed by early curing. During the migration from winter range to lambing grounds, the sheep bypassed the Lake Louise area (8,600 ft.) where most of the lambs were born in 1998. We assume this was because the forage at that time was sparse and dry. Vegetation was denser, more succulent, and more palatable on the higher historic lambing grounds of Middle Mountain (10,800 to 11,500 ft.) and at Goat Flat (11,500 to 12,500 ft.) where most of this year's lambing took place. Winter snowfields receded quickly and spring rains were nearly absent. This resulted in extremely low productivity of forage species above 11,000 ft., accompanied by early maturation and curing.

Most ewes left Middle Mountain lambing and nursery areas early in June and did not return except sporadically to utilize selenium mineral blocks placed at Three-Four Pass (located about the middle of Middle Mountain), and during the fall migration to Whiskey Mountain. Although sixteen ewes remained on Middle Mountain the bulk of the population scattered in small groups into higher remote regions far from the Middle Mountain study area and were nearly impossible to monitor.

Dispersal patterns of sheep as detected by radio telemetry where indicated south and west of Middle Mountain near Downs Lake, No Man's Pass, Spider Peak, Goat Flat, and the edges of Continental Glacier. Locations of a GPS collared ewe monitored by the Wyoming Game & Fish Department, downloaded to a topographic map (see map of GPS locations 2002) reflects the aberrant pattern sheep exhibited this year (the migration pattern representative of our study group is circled). They passed over Middle Mountain on well-established migration trails but unlike previous years they moved onto more remote summer ranges where residual mountain snows provided more favorable forage growth.

Therefore, in contrast to 2001, the GPS locations map for 2002 shows a lesser amount of foraging time (cluster of dots) spent on Middle Mountain and this was reflected in fewer numbers of observations and subsequent fewer lamb sightings (see maps of GPS locations 2001 and 2002). Middle Mountain was also the area where, in 1998 most of the ewe and lamb activity was concentrated, and symptoms of White Muscle Disease (WMD) were noted.

The 2002 summer range lamb/ewe ratio was 49 lambs/100 adult ewes. The ratio is based on a small number of sightings (n = 10) apparently due to the drought conditions. The sheep appeared healthy with no indications of WMD or any other illnesses. Lamb/ewe ratios in 2002 were higher than in 1998 (9 lambs/100 ewes). Despite the encouraging summer counts, recent winter range counts show a sharp decline in 2002 to 14 lambs/100 ewes. Additional observations are needed on the winter range to assess the reason for this disparity. Observations of symptoms of WMD (selenium deficiency) in sheep during July of 1998 (a high precipitation year) followed by the absence of observed symptoms in the following three dry years prompted us to conduct a comparative forage analysis to look for correlations between forage selenium and rainfall. Comparisons of periods of high and low rainfall over a thirty-year span indicated a loose inverse relationship between lamb recruitment and relative precipitation in the Middle Mountain area.

3) **Experimental Plots:** Preliminary test plot results from 2001 indicated that: a. increases in soil nitrate levels inversely effected selenium levels; b. sulfates had no effect on forage selenium and c. the addition of alkalinity to the soil had no effect on forage selenium. These plots were located in a typical felfield site.

Plans for 2002 included two test plot sites, one in typical felfield, and one in typical alpine meadow vegetation. Due to extreme drought conditions in 2002 at alpine elevations no xeric sites could be used for study, since the required quantity of vegetation for analysis could not be obtained. Thus the felfield test was abandoned.

Only one site with sufficient soil moisture for the mesic alpine meadow type was found. A covered test plot sequence of nine covered plots was established there with the addition of an uncovered control plot receiving no experimental treatments, but allowed to receive normal precipitation. Plots were 30 inches square, covered with a 48-inch square greenhouse fiberglass rain shield raised ten inches off the ground for airflow. Plots were selected to contain, as nearly possible, equal composition and density of vegetation while avoiding high percentages of nonforage species. The intent was to show the effects of systematic additions of both water and nitrates on selenium levels in alpine plant species utilized by bighorn ewes. An additional plot was added later to which NH_4OH was added at the rate of 3.5ppm/liter every second day. This became plot #10. The uncovered control plot became plot #11 (see Test Plot Schematic).

Since reduction/oxidation potentials are known to effect selenium availability in plants, redox probes were set in plots #4, #11, and a nearby felfield area for comparison (occasionally listed here as plot #12).

Plots were watered every second day to simulate increasing nitrate deposition (horizontal axis, left to right), and increasing moisture (vertical axis, downward) through the growing season (July and August). In the initial phase plots were clipped and analyzed for selenium. Subsequently plots were clipped and analyzed once a month for two months. The effects of nitrates on forage selenium in alpine meadow vegetation type were inconclusive (see Test Plots 2002 Results).
There however was a direct correlation in July between available soil selenium (selenate and selenite) and forage selenium in the test plots ($r = 0.670$) based on soil analysis by Bruce Mincher at the Idaho National Engineering and Environmental Laboratory (INEEL). The correlation between available selenium and percent change in forage selenium between July and August was lower ($r = 0.409$).

Redox potentials generally were higher on dryer soils indicating as expected more available (oxidized) selenium species, and lower on wetter soils indicating less available (reduced) species as expected. Preliminary tests in 2001on Middle Mountain produced similar results (see Redox Values 2002).

4) **Soil Microbial Analyses:** It is possible that differences exist in the microbiota between felfield soils studied in 2001 and alpine meadow soils studied in 2002 affecting the availability of selenium differently in the presence of nitrates. Since fungal and or bacterial activity is suspected of playing a critical role in the mobility of selenium in the soil, microbial studies are crucial to an understanding of these data. Soil samples were collected at each monthly clipping and frozen for microbial analysis. Unfortunately our research budget is overspent and we do not presently have the funds to analyze these samples. Preliminary identifications made by Drs. Jack States and Martha Christensen in 2001confirm the presence

of selenium reducing fungi in Middle Mountain test plots. These fungi were found capable of reducing available selenite and selenate to dimethyl selenide or elemental selenium, both unavailable to vascular plants.

5) **Forage Selenium Analyses:** Included here is a list of forage selenium levels collected in 2002 from the seasonal ranges of the Whiskey Mountain herd (see table for Seasonal Range Forage Selenium). These confirm that forage on the winter range associated with the lower (Phosphoria) geologic formation has higher levels of selenium (mean = 0.152 ppm). These levels are well within the recommended range for health in domestic sheep (0.1 – 0.2 ppm) based on National Research Council (NRC) standards of recommended requirements (see table for Nutrient Levels Whiskey Mountain Seasonal Ranges). The high summer ranges located on granitic soils have levels well below these standards (mean = 0.043 ppm). If the hypothesis of an inverse relationship between rainfall (i.e. nitrate deposition) and forage selenium is correct these levels could easily drop into a critical health compromising range (< 0.20 ppm) in wet years. It is important to note that as yet no standards for forage selenium exist for bighorn sheep.

Species-specific selenium analyses were run on important forage species to help illuminate the variations in species uptake and availability of selenium to sheep in the habitat. Of special interest is the high nutritional level of selenium found in *Chrysothamnus nauseosus*, a shrubby plant on the winter range, often grouped with weedy species. It may prove to be nutritionally important because of its high selenium level in this range. The sheep have been known to show a particular appetite for this species (see table for Species Specific Forage Selenium Levels). An annual monitoring of key forage species for selenium is recommended.

Notably almost all other nutritional components tested fall within NRC standards with the exception of sodium and total digestible nutrients (TDN), which are below recommended levels while iron is high, approaching the toxic level in some samples.

The TDN are not low enough to be of concern. However sodium is very low (0.009 and 0.006) (recommended level = 0.360). The placement of sodium chloride blocks on Arrow Mountain in 2001 established that sheep migrations to mineral licks are controlled by sodium and can be altered by artificial sources. This herd has had salt available in the form of blocks since the 1950's and possibly since the 1930's. Because inappetence is the primary result of sodium deficiency, the deficient levels of forage sodium in the absence of supplemental blocks, could exaggerate other nutritional deficiencies such as selenium responsive disorders (SRD). Although not analyzed this year due to the expense of these analyses, sulfur and cobalt should also be analyzed in the future as they both influence selenium uptake and metabolism.

Collections were made this summer of forage from other bighorn sheep ranges (see table for Forage Selenium Values of Various Wild Sheep and Goat Ranges) in an attempt to begin the process of establishing baseline data for selenium levels on historic sheep ranges. It is interesting to note that selenium levels are similar (double digits ppb) in three locations where sheep populations are known to be declining – Wind River's in Wyoming (mean = 0.0717 ppm), Lemhi's in Idaho (mean = 0.0040 ppm), the Fraser River herd in British Columbia (mean = 0.0568 ppm), and one range where rocky mountain goats have been confirmed to have WMD in the Purcell Mountains of British Columbia (Hebert 1971) (mean = 0.0612 ppm). All forage collections were made this summer and were analyzed at the same lab, Olson

Biochemistry Laboratory, South Dakota State University at Brookings, to avoid differences in analytical procedures. The herd listed under the heading British Columbia only recently began feeding on this forage so herd status cannot be assessed (Dr. Helen Schwantje, wildlife veterinarian - Biodiversity Branch Ministry of Water, Land, and Air Protection, Victoria, B.C. - personal communication).

Forage selenium levels are almost identical for the Whiskey Mountain summer range in the northern portion of the Wind River Mountains, and the Lemhi Mountains where the sheep population (originally transplanted from Whiskey Mountain) has dwindled from 41 animals to 6 in the last twenty years – Northern Wind River Range (mean = 0.043 ppm); Lemhi Range (mean = 0.040 ppm). These levels are below the NRC standards for domestic sheep.

The Gros Ventre Range herd has also experienced a recent population decline (winter/spring of 2002). Here the opposite situation of selenium levels may be involved. An examination of this forage availability revealed elevated selenium levels, which under winter conditions of crusted snow cover, may be well into the toxic range due the ubiquitous presence of the selenium converter plant, *Astragalus bisulcatus*. This, however, is beyond the scope of this study.

The remaining ranges with known sheep populations did not exhibit population declines. Forage selenium is within the NRC recommended levels – Absorokas (0.126 ppm); Owl Creeks (mean = 0.225 ppm); Sierra Nevadas (mean = 0.284 ppm [species specific analysis]).

There is a significant difference ($p = 0.042$) between the selenium levels on summer range forage samples where sheep populations are declining ($n = 19$; mean = 0.60 ppm; SD = 0.061 ppm) and forage of summer ranges where populations are stable ($n = 7$; mean = 0.237 ppm; SD = 0.181 ppm). This suggests that population size may be influenced by forage selenium levels.

Although many factors affect population dynamics, it is possible that lamb recruitment data can be used as an indicator of low selenium availability. The fact that selenium plays a pivotal role in so many physiological functions in ungulates from immune response, muscle growth, milk production, to body temperature regulation, it is not unreasonable to suspect that population size and lamb survival may be effected by a critically low availability of this nutrient.

RECOMMENDATIONS:

To realize its full significance the investigation of the bighorn sheep/selenium/atmospheric nitrogen deposition relationship needs to be extended to additional sheep ranges. It is recognized that the subject of selenium responsive disorders, particularly in the purview of this hypothesis involving the interactions of nitrates, soil chemistry, microbial conversions, plant assimilations, and animal heath is affected by a multitude of environmental variables which are not yet fully understood.

Lab based research under controlled conditions may be necessary to eliminate many of the variables found in the field. The geochemical aspect of selenium availability is of great importance and needs further study. Atmospheric deposition parameters need to be delineated in much greater detail especially in regard to the affect of elevation on deposition. Also, the backtracking of weather patterns is crucial to understanding the wide fluctuations in nitrate concentrations in precipitation. Seasonal blood draws from ewes and lambs for Glutathione peroxidase analysis should be instituted to monitor variations in blood selenium seasonally and year to year. Most importantly it is critical to establish a clinical diagnosis of WMD in lambs within the study area. Many of these goals would be most readily achieved during high rainfall years. A plan of action contingent on the appearance of a wet spring and summer is suggested. A captive bighorn-feeding program could establish exact levels of selenium and related nutrients required to produce SRD and clinical WMD.

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