Bighorn Sheep Lamb Survival, Trace Minerals, Rainfall, And Air Pollution: Are There Any Connections?

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Abstract: A pneumonia outbreak during the winter of 1990/91 caused a 30% decline in the Whiskey Mountain Bighorn Sheep Herd near Dubois, Wyoming, USA. In subsequent years, lamb ratios on winter range were depressed well below the long-term pre-dieoff average of 37. Between 1997 and 2001, 56 ewes were marked and tested using Pregnancy Specific Protein (92% pregnant). During the summer of 1998, several key observations included: most lambs were sickly and exhibited rough coats, swollen eyes, coughing, congestion, nasal secretions, high respiration rates, slow growth, slumped shoulders and stiff-legged gaits. These were identical to symptoms of Nutritional Muscular Dystrophy known to occur in domestic lambs consuming a diet with < 20 ppb selenium on a dry matter basis. When these symptoms were displayed, selenium content of summer forage was 5 ppb dry matter. Selenium, a component of selenoproteins, is vital to healthy immune function, disease resistance, growth and milk production. Eighteen of 19 marked ewes were observed moving back and forth bimonthly between high elevation summer and fall range to lower elevation winter range in order to eat soil at natural mineral licks. Winter range forage was much higher in selenium because soils were derived from sedimentary rock. These findings suggested a selenium deficiency was occurring. Between 1998 - 2000, mortality rate for sick lambs was 4.7 X higher than healthy lambs (Chi-square = 8.35, $P = 0.004$). Lambs that survived suckled 50% longer per suckling event and had mothers with more "full" udders based on subjective observations. Beginning in 1999, mineral blocks containing 17 ppm selenium were placed on all seasonal ranges. This was increased to 60 ppm in 2000. Sheep readily found and consumed blocks. Sheep that had access to blocks during the summer and fall (i.e., Middle Mountain) ceased summer movements to lower elevation winter range and natural mineral licks, displayed dramatic improvement in lamb health and survival, and had ewes that shed earlier and lambs that weaned later. In contrast, sheep that did not have access to blocks during the summer and fall (Arrow Mountain) continued summer movements to lower elevation winter range and had lower lamb ratios ($P = 0.003$). Pure salt blocks (NaCl) on Arrow Mountain in 2001 stopped sheep movements to lower winter range. However, lamb survival was 67% lower than Middle Mountain. Between 1998 and 2001, lamb ratios correlated well with summer forage selenium ($r = 0.84$) on Middle Mountain. The lowest years of lamb ratios tended to occur in the wettest years. This suggested a possible connection between forage selenium and rainfall. We investigated possible factors effecting plant uptake of selenium including changes in soil pH from rainfall-derived nitrate and sulfate deposition, changes in soil redox potentials from differing levels of soil moisture and changes in microbial cycling of selenium from increased rainfall-derived nitrate deposition. We suspect that in granitic soils, wetter summers produce conditions unfavorable for selenium uptake by forage plants due to lowered soil redox potential thus converting selenite and selenate into chemical species (e.g., elemental selenium) unavailable for plant uptake. We also suspect that artificially enhanced nitrate deposition stimulates microbial decomposition processes including microbial transformation of selenite and selenate to unavailable forms of gaseous and elemental selenium. Ultimately, we suspect that wetter conditions result in less selenium uptake by bighorn sheep from forage growing on granitic summer range, thus lowering lamb health and survival.

Key words: Bighorn sheep, selenium, lamb survival, Whiskey Mountain, nitrates, nutritional muscular dystrophy.

The northern Wind River Mountains in Wyoming, USA support one of the larger herds of Rocky Mountain bighorn sheep (*Ovis canadensis*) in the world, numbering about 1,600 animals in the early 1990's. A primary wintering area is the Whiskey Mountain Wildlife Habitat Management Unit located near the town of Dubois. Due to a combination of land ownership, the unit and accompanying bighorn sheep are managed cooperatively by an interagency technical committee composed of US Bureau of Land Management, US Shoshone National Forest and Wyoming Game and Fish Department biologists. This herd is popular because wintering sheep are readily observable to the general public. The National Bighorn Sheep Interpretive Center is located in nearby Dubois, further attesting to the herd's attractiveness. In addition to its aesthetic value, the herd is an important game population and has served as a significant source of transplanted sheep (N ~ 1,900) for locations in Arizona, Idaho, Nevada, South Dakota, Utah and Wyoming.

During the winter of 1990/91, about 30% of the herd died in an apparent pneumonia outbreak (Ryder *et al.* 1992). Recruitment remained relatively poor in subsequent years based on an average winter ratio of 21 lambs:100 ewes since the dieoff. This was well below the long-term pre-dieoff average of 37 between 1958 - 1990. Consequently, the population has declined and is currently estimated at around 800 sheep. Beginning in 1997, we began to investigate the causes of low lamb ratios.

Trace minerals are chemical elements needed by higher animals in "trace" amounts, i.e. in the range of parts per million, billion or even less (Berger 1993). For many years, extensive research by agricultural scientists of trace mineral requirements has been conducted to enhance health and production of domestic animals (National Research Council 1983). Only in the last few decades has the importance of trace minerals to wildlife been investigated. Selenium is of particular interest, and plays an essential physiological role in higher animals, with deficiency maladies reported in domestic cattle, sheep and hogs (Underwood and Suttle 2000). Nutritional Muscular Dystrophy (NMD), also known as White Muscle Disease, is a degenerative disease of striated muscles associated with selenium deficiency known to occur in domestic lambs on selenium deficient forage at < 8 weeks of age (National Research Council 1985).

It was hypothesized that episodic selenium deficiency limits recruitment in this bighorn sheep population and is affected by soil properties, rainfall amounts and possibly nitrate deposition from anthropogenic (man caused) sources. The goals of this research were to document factors affecting recruitment, including pregnancy rates and lamb mortality, evaluate the effect of supplemental selenium on recruitment and to understand the environmental chemistry of selenium in the alpine environment.

STUDY AREA

The main study area was situated on summer range near the summit of Middle Mountain (3,350 m, 11,000 ft) in the Fitzpatrick Wilderness Area of the Shoshone National Forest, Wind River Mountains, Wyoming (Fig. 1). Elevations range from 2,271 m (7,450 ft) along Torrey Valley to 3,713 m (12,180 ft) on Torrey Peak. Average annual precipitation ranges from 41cm (16 in) in the lower portions to 102 cm (40 in) in the higher elevations. Temperatures generally range from -20 to 0^0 C (-4 to 32^0 F) in winter to 5 to 30^0 C (40 to 86^0 F) in summer. Middle Mountain geology is characterized by glacial deposits and granite outcrops. Soil is shallow, often only a few centimeters deep. Summer range consists of widespread fell-fields, intersected by alpine meadows associated with snow fields and runoff. Fifty percent or more of the total ground cover is exposed pre-Cambrian granite outcrops, boulder fields and expansive fell-fields. Vegetation in the fell-fields is dominated by thickets of *Geum rossii* and a variety of alpine cushion plants (*Silene acaulis*, *Phlox* spp. and *Trifolium nanum*) as well as a variable mixture of grasses and sedges, often dominated by *Poa alpina* and *Carex* spp. Above 3,350 m (11,000 ft) elevation, much of Middle Mountain is transitional fell-field/alpine meadow composition. The alpine meadows consist predominantly of grasses (*Deschampsia caespitosam*) with variable sedge (*Carex* spp.) and forbs (*Polygonum bistortoides*). The fell-field sites are mesic, but took on the appearance of xeric sites during 2001, following 2 years of severe drought.

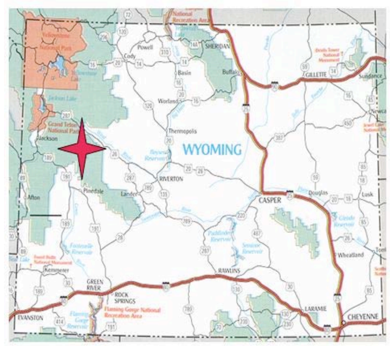

Fig. 1. Location of the Whiskey Mountain bighorn sheep herd.

Arrow Mountain (3,565 m, 11,693 ft) is separated from Middle Mountain by East Torrey Creek and served as a control area for experiments involving sheep treatment with mineral supplements. It is dominated by Gros Ventre-formation soil with pre-Cambrian granite. Summer range vegetation consists of approximately 80% alpine meadow and 20% fell-fields. The meadows are dominated by *Carex* spp., *Poa secunda, Calamagrostis purpurascens and Aster* spp.

METHODS

Bighorn sheep were captured on winter range during March 1998 – 2001 using immobilizing drugs administered from a

Dan-Inject pneumatic rifle (Wildlife Pharmaceuticals Inc., Fort Collins, CO). Sheep were approached by pickup with capture personnel situated in the bed. Hay was distributed from the bed while the truck was slowly backed away. Sheep generally approached the hay within a 10 minute period. Ewes that presented a clear target were randomly selected from the group. Sheep were darted using a 2 cc "cocktail" generally comprised of the following drug mixture: 3 mg carfentanil, 80 mg ketamine and 20 mg xylazine. Darted ewes became immobile within 5 minutes. Ewes were marked using combinations of neckbands, traditional radio-collars and/or GPS-collars. Blood and fecal samples were collected. Blood samples were tested for pregnancy specific protein (Bio Tracking, Moscow, Idaho), copper, iron, manganese, selenium, zinc, Vitamin A, Vitamin E and other constituents (Wyoming State Veterinary Lab, Laramie, Wyoming). Sheep were antagonized using a cocktail containing 200 mg naltrexone and 20 mg yohimbine.

Fecal samples were collected from captured ewes, cooled as quickly as possible and analyzed for lungworm (*Protostrongylus* spp.) and nitrogen levels. In addition, an observer collected fresh samples (< 10 minutes old) opportunistically throughout the summer and fall from both ewes and lambs. Two to ten pellets from each sheep were analyzed for lungworm using a modified Baermann technique (Samuel and Gray 1982) (Wyoming State Veterinary Lab). A second sample of 5 - 10 pellets was collected concurrently and analyzed for fecal nitrogen. Samples were air dried at room temperature for a minimum of 2 weeks, ground using a mortal and pestle and analyzed by the Analytical Services Lab, Wyoming Department of Agriculture in Laramie, using the Kjeldahl method (Horwitz 2000).

Forage samples consisted of a "bread bag" sized collection of forage from spring, lambing, summer, fall and winter ranges. For lambing, summer and fall ranges, samples mimicked the diet composition and proportion of plants and plant parts used by sheep based on close observations (< 15m, 50 ft). Samples from spring and winter ranges were collected randomly along historic production/utilization transects. Samples were oven dried and analyzed for total digestible nutrients, acid detergent fiber, nitrogen, 14 trace and macro minerals, and Vitamin A by the Analytical Services Lab, Wyoming Department of Agriculture in Laramie. Forage was also analyzed for selenium content. Values for 1998 - 2000 were measured at the University of Wyoming, Veterinary Science Laboratory using hydride generation atomic emission spectroscopy. For 2001, forage selenium measurements were made by Olson Biochemistry Laboratory (Brookings, SD) by derivitization and flourimetry. In both cases, samples were first prepared by oxidative digestion. Both laboratories have NIST-traceable quality control programs.

Soil oxidation-reduction potential was recorded versus time using an Ag/AgCl electrode immersed to a depth of 3 cm (1.2 in) in the soil, saturated with 0.005 Molar NaCl solution and exposed to room air at the surface. After 1 month, analysis of available selenium was obtained from a subsample of this soil collected from the probe depth in a nitrogen-filled glovebox to prevent any re-oxidation of reduced selenium.

Groups of ewes with lambs were followed daily or as frequently as possible between late May and late October in order to monitor visual signs of disease in ewes and lambs and record the number and length of suckling attempts by lambs. Suckling times were used as an indication of milk production in the mother (Horejsi 1972, Shackleton 1972, Thorne *et al.* 1979, Hass 1990, Hogg *et al.* 1992), with greater lengths of suckling inferring greater amounts of available milk. Lambs that displayed symptoms of disease (e.g. persistent coughing, eye swelling and infections, stiff legged gaits, general weakness, etc.) were considered "sick" in the analysis of lamb health versus survival. Survival of a lamb from a marked ewe was defined as a lamb that was observed alive at the end of October at which time observer monitoring ceased and is termed "Lambs That Survived." Mortality of a lamb from a marked ewe was defined as a lamb that disappeared prior to the end of October and is termed "Lambs That Died."

The Palmer Modified Drought Severity Index (PMDSI) (Heddinghause and Sabol 1991) was acquired from the National Climatic Data Center web site and represents the Wind River Basin, within which the study occurred. These data were acquired from the website www.ncdc.noaa.gov/oa/climate/onlineprod/drought/xmgr.html. PMDSI is calculated monthly and indicates the severity of a wet or dry spell. A range of 0 to -1 is normal, -1 to -2 is mild drought, -2 to –3 is moderate drought, -3 to –4 is severe drought, and anything below –4 is extreme drought. Conversely, positive numbers indicate wet spells with corresponding adjectives (i.e., +4 is extreme wetness). Satellite imagery was acquired from the Cooperative Institute for Research in the Atmosphere (http://goes-10-gems.cira.colostate.edu/). GOES-10 satellite images taken every 2 hours were copied into PowerPoint® and animated to show a summer's worth of weather patterns in several minutes. General air flow could then be readily assessed.

During summer of 2001, 9 plots measuring 51 cm X 51 cm (20 in X 20 in) were established on a 0.02 ha (0.05 ac) area on Middle Mountain to test the effects of various chemical additions and watering regimes on the uptake of selenium by alpine plants. Plots were selected to contain, as nearly as possible, equal composition and density of vegetation while avoiding high percentages of non-forage species. Two plots were covered with a rainshield constructed of 123 cm X 123 cm (48 in X 48 in) greenhouse fiberglass and mounted on an aluminum frame. The rainshield was raised above the ground 25 cm (10 in) at the back edge and 41 cm (16 in) on the front edge to allow for air flow. Select plots were watered every 4th day (Table 1). All forage was clipped in the entire plot at setup on June 18 and again following 30 days of manipulations on July 17. The percentage change in forage selenium was then calculated and compared between plots. The nitrate plot and sulfate plot had 0.3 ml of 1 Molar solutions of nitric and sulfuric acid, respectively, added to each liter of water to produce nitrate and sulfate ions. The additions were designed to simulate a pH of 3 – 4. A pH of 4 was the lowest rainfall pH recorded during the summer of 2000.

Individual rain events were collected in a US Weather Bureau All-Weather Rain Gauge and analyzed within 1 day on-site using a Hach® spectrophotometer. To ensure accuracy, standardized solutions of known nitrate concentrations were periodically tested prior to analyzing rainwater samples. A Hubbard-Brooke collector (Galbraith *et al.* 1991) (Fig. 2) captured rainfall over 2-week intervals. Samples were analyzed within 3 days off-site by the US Forest Service's Biogeochemistry Lab in Ft. Collins, Colorado, as well as on-site to allow

Table 1. Selenium forage test plots, Middle Mountain, Wyoming, 2001.

Plot Name	Type of water added	Additional Chemicals	Forage Selenium (ppb) for 1st Clipping (6/18/01) on Dry Matter basis	Forage Selenium (ppb) for 2nd Clipping (7/17/01) on Dry Matter basis	% Change Between 1st and 2nd Clippings	Total Water cm/month	Total NO3 in ka/ha/month	Total Water as compared to a "Normal" June rainfall	Total Water as compared to a "High" June rainfall	Total NO3 as compared to a "Normal" June rainfall	Total NO3 as compared to a "High" June rainfall
# 6 Covered Distilled Double Watering	distilled (0.34 mg/l NO3)	none	20.8	33.0	59%	16.43	0.56	153%	61%	19%	8%
# 3 Uncovered Sulfuric Acid	rain/snow	0.8 ml sulfuric acid	29.1	40.8	40%	12.09	1.07	113%	45%	37%	15%
# 4 Uncovered Sodium Bicarb	rain/snow	1.07 g Na bicarb	34.1	45.7	34%	12.09	1.07	113%	45%	37%	15%
# 1 Uncovered Control	rain/snow	none	34.9	44.9	29%	12.09	1.07	113%	45%	37%	15%
# 5 Uncovered Rain/Snow Double Watering	rain/snow	none	38.8	41.0	6%	20.31	1.26	189%	75%	44%	17%
# 8 Covered Distilled Matching Rainfall	distilled (0.34 mg/l NO3)	none	36.7	31.1	-15%	3.88	0.13	36%	14%	5%	2%
# 7 Uncovered Rainfall	none	none	40.6	33.9	-17%	3.88	0.88	36%	14%	31%	12%
# 2 Uncovered Nitric Acid[a]	rain/snow	0.8 ml nitric acid	48.1	39.1	-19%	12.09	18.38	113%	45%	638%	252%
# 9 Saturated by Streamside	none	none	84.1	27.7	-67%	saturated	0.88	saturated	saturated	30%	12%

[a] Since no high elevation analysis of nitrate concentrations in rainwater had been done near the study area previously, we were uncertain as to what levels of nitrate we should add to our plots. Hence, several plots had nitrate levels lower than what fell during the summer of testing and 1 plot that was much higher.

comparisons of methods. The Lab followed protocol established by the National Atmospheric Deposition Program (NADP). Because these collection devices were continuously exposed to the atmosphere, both wet and dry depositions were collected. Due to the remoteness of Middle Mountain, monthly precipitation data had never been collected previously. Therefore, estimates of “normal” and “high” June precipitation were extrapolated from rainfall data acquired at the Gypsum Creek NADP site located upwind and 32 km (20 mi) southwest of Middle Mountain and precipitation isopleths as detailed in Gibson (1990).

Fig. 2. Hubbard-Brooke rain collector, Middle Mountain, Wyoming, 2001.

A 5 cm X 2 cm (2.0 in X 0.8 in) soil core was extracted from each forage plot using a metal core borer and returned to the lab for analysis. Samples were diluted and spread on agar petri dishes. Bacterial colonies were counted after 72 hours of incubation at room temperature. Samples were amended with potassium nitrate (as 0.25%) and selenate to test for effects on bacterial colonies.

Soil samples were collected just below the detritus layer, at a depth of 7 - 15 cm (2.8 - 5.9 in). The principal sample site (MM#1) on Middle Mountain summer range was a relatively level, damp depression, with alpine meadow vegetation where sheep had previously been observed foraging. A nearby fell-field (MM#2) was also sampled. Soil sampling increased with time as it became apparent that there was a possible link between forage selenium and lamb recruitment. Arrow Mountain (AM) and Goat Flat (GF) summer range was also sampled. Natural licks used by bighorns were sampled at Torrey Valley (TV) and Beck’s Bridge (BB#1 and BB#2) at 2,270 m (7,445 ft). Samples were spread and air dried in an aluminum tray then sieved to 50 mesh. Total selenium was measured by hydride-generation atomic absorption spectrophotometry (AA). Samples were oxidatively digested by boiling 1 g in concentrated nitric acid to near dryness, followed by the addition of hydrogen peroxide. This was heated to dryness and the NO_x-free ash was redissolved in hot concentrated hydrochloric acid then diluted to 4 – 6 Molar for AA. The absolute detection limit was approximately 2 ppb (parts per billion), with repetitive analyses resulting in a relative standard deviation of 15%. The addition of ferric iron and/or nitrate did not suppress the generation of H_2Se and are not regarded as interferences. Samples were also analyzed for pH using a standard combination electrode after 45 minutes of stirring at a ratio of 6 g of sample to 35 ml demineralized water.

Available selenium was defined as the sum of freely soluble and readily exchangeable selenium. Soluble selenium (probably selenate) was extracted from 1 g samples using 10 ml of a non-complexing salt solution (0.01 Molar $CaCl_2$). Exchangeable (probably surface-bound selenite) selenium was next determined by extracting from the same 1 g sample with a pH 7, 0.25 Molar phosphate/0.20 Molar citrate buffer solution.

Two-tailed t-Tests for the equality of 2 population means for normal populations with unequal variance were used to analyze fecal lungworm, fecal nitrogen levels, length of lamb suckling events, lamb ratios and PMDSI values. A Paired t-Test was employed to compare lamb ratios between Arrow Mountain and Middle Mountain over time and nitrate concentrations in rainfall on Middle Mountain and at the Gypsum Creek NADP site. Pearson Correlation was used to correlate forage selenium with lamb ratios. A 2 X 2 contingency table with Yates' Corrected Chi-square was used to compare lamb health to lamb survival. Statistix7 software provided all results.

RESULTS

Ewes were marked on winter range during March 1997 – 1998, and March and May 2001 using neckbands (N = 34), neckbands with radio-collars (N = 22) and neckbands with GPS-collars (N = 3). Whole blood selenium levels of ewes captured in late March (N = 40) and early May (N = 3) were 0.13 ppm (parts per million) (SD = 0.025) and not deficient when compared to domestic sheep (Underwood and Suttle 2000) or other bighorns (Puls 1994). Iron and zinc were ~30% below recommended levels. There were no significant differences in any blood constituents between ewes with Lambs That Survived and ewes with Lambs That Died. Pregnancy Specific Protein testing showed that 92% of 62 ewes tested were pregnant during March 1997 – 1998, and March and May 2001. Some ewes were tested in multiple years. Observations on lambing range revealed that lamb drop occurred normally.

In mid-July, 1998, the majority of lambs (N ≥ 30) observed on Middle Mountain became ill and displayed the following symptoms: unthrifty coats, swollen eyes, coughing, nasal secretions, high respiratory rates, slumped shoulders and a stiff-legged gait (Fig. 3). They also exhibited general weakness and poor growth, diarrhea and secondary infections. Abnormalities observed in ewes included small udder size, poor milk production, early weaning of lambs in August, late shedding of winter coats and periodontal abnormalities (observed in 10 ewe skulls found on winter range during winter of 1998/99). Complete recovery of the sickest, most debilitated lambs followed movements to natural mineral licks. All of these symptoms have been displayed by domestic sheep and goats that were severely deficient in dietary selenium and developed NMD (National Research Council 1985, Smith 1994, Underwood and Suttle 2000). Selenium content of summer forage was 5 ppb dry matter (Table 2). Domestic sheep consuming forage with < 20 ppb selenium dry matter typically develop NMD (National Research Council 1985, Underwood and Suttle 2000).

More specifically, sick lambs appeared hyperactive with associated muscular weakness, uncoordination and/or apparent "sag" of the triceps brachii muscle from the normal 35 - 45º to < 0º with respect to a horizontal plane. In very severe cases this was associated with a marked protuberance of the cleidobrachial muscle from the point of the shoulder down into the muscle mass where it joins the radius, on both right and left forelegs equally. Lambs displayed severely stiff muscle movement in the shoulder and hind quarters that created an unstable wobbly appearance when walking or standing.

Table 2. Analysis of bighorn sheep forage from the Whiskey Mountain area near Dubois, Wyoming, on a dry matter basis during 1998 - 2001. Foraging sheep were observed closely to determine composition and proportion of plants in the diet. Samples were then collected to reflect diet composition and proportion. Additional random samples were collected for selenium analysis. Samples are compared to the National Research Council's (1985) recommendation for maximum production in domestic sheep.

	Domestic Lamb NRC[a] Requirement	Lambing Range (June: Lake Louise, Middle Mtn)				Summer Range (June, July & August: Middle Mtn, Arrow Mtn, Goat Flat, Torrey Peak)				Fall Range (September & October: Whiskey Mtn, Osborn Mtn, Fremont Glacier, Middle Mtn)				Winter Range (March, May, September, October, November & December: Torrey Rim, Sheep Ridge, BLM Ridge, Trail Lake Meadow)			
		N	Mean	SD	Range	N	Mean	SD	Range	N	Mean	SD	Range	N	Mean	SD	Range
ADF[a] %		4	32.5	1.24	31.1 - 34.4	13	32.9	6.22	23.7 - 42.6	6	35.5	2.09	33.1 - 38.0	12	46.4	4.38	38.8 – 53.3
Calcium %	0.82	4	0.52	0.075	0.42 - 0.60	13	0.62	0.170	0.41 - 1.00	6	1.01	0.173	0.70 - 1.20	10	0.37	0.085	0.28 – 0.54
Carotene ppm[a]		4	165	20.8	140 - 190	13	175	52.4	110 - 270	6	90	23.2	55 - 113	10	23	18.9	0 - 61
Copper ppm	Mo < 1, then Cu 7-8; Mo > 3 , then Cu 17-21	4	18	7.6	12 - 28	13	16	6.0	8 - 29	6	12	2.7	8 - 15	10	16	6.7	8 – 29
Iron ppm	30 – 50	4	157	30.3	121 - 190	13	181	182.0	98 - 780	6	237	67.4	180 - 370	10	185	118.1	88 – 510
Magnesium %	0.12	4	0.15	0.033	0.11 - 0.19	13	0.18	0.049	0.13 - 0.29	6	0.18	0.033	0.14 - 0.23	8	0.09	0.008	0.08 – 0.10
Manganese ppm	20 - 40	4	92	21.4	70 - 121	13	140	80.1	35 - 360	6	71	23.4	41 - 110	10	40	18.0	20 – 78
Molybdenum ppm	> 0.50	4	1.5	0.50	0.9 - 2.1	13	0.9	0.52	0.3 - 2.3	6	1.0	0.52	0.6 - 1.9	10	1.2	0.21	0.9 – 1.6
Phosphorus %	0.38	4	0.33	0.057	0.26 - 0.40	13	0.19	0.035	0.12 - 0.24	6	0.10	0.017	0.08 - 0.13	10	0.13	0.076	0.06 - 0.30
Potassium %	0.5	4	2.0	0.31	1.7 - 2.4	13	1.7	0.21	1.3 - 2.0	6	0.91	0.131	0.72 - 1.10	10	0.81	0.577	0.20 – 2.20
Protein %	12.8	4	17.1	1.22	15.9 - 18.1	15	16.6	3.97	10.7 - 23.6	6	8.0	1.23	6.1 - 9.3	12	6.3	3.61	2.8 – 14.0
Selenium ppb[ab]	100 – 200	3	51[a]	41.4	25 - 99	49	42[a]	21.4	5 – 128	15	52[a]	29.0	19 – 109	14	150[b]	121.0	63 – 479
Sodium %	0.3600 – 0.4700	1	0.0049			1	0.0019			1	0.0008						
Sulfur %	0.140 – 0.180	1	0.145			1	0.113			1	0.098						
TDN[a] %	65.0	4	63.1	0.88	62.0 - 64.0	13	63.0	4.91	55.2 - 70.0	6	61.0	1.70	59.0 - 63.0	12	52.2	3.38	47.0 – 58.0
Vit A IU/kg[a]	2,380	4	66,000-116,100		57,200 - 134,600	13	69,100 - 121,100		44,700 - 185,000	6	35,900 - 63,300		21,700 - 79,600	10	32,600	33,100	0 – 100,300
Zinc ppm	33	4	39	2.6	36 - 41	13	41	10.7	22 - 58	6	19	5.1	12 - 25	9	20	5.0	12 - 27

[a] NRC = National Research Council's (1985) national standards of recommended requirements for domestic sheep. ADF = Acid detergent fiber is the most indigestible portion of fiber and is comprised primarily of cellulose. Ppm = Parts per million. One ppm is equivalent to 0.0001%. Ppb = Parts per billion. One ppb is equivalent to 0.0000001%. TDN = Total digestible nutrients is the most digestible portion of the sample. IU = International Units. Results for Vit A are a range. No SD given.

[b] Significant differences between means were tested only for selenium using 2-tailed t-Test with unequal variances. Means with a different letter are significantly different at $P<0.05$.

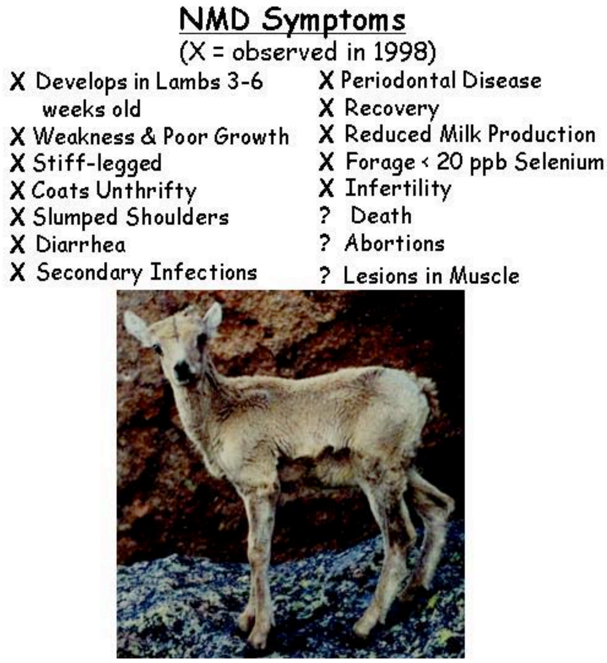

Fig. 3. Sick lamb and NMD symptoms, Middle Mountain, 1998.

The above symptoms were strikingly sudden and ubiquitous in lambs. Some lambs subsequently developed bacterial infections such as apparent pneumonia and eye infections. Though lambs displayed sickness (primarily coughing) in subsequent years (1999 - 2001), 1998 was the only year in which lambs displayed the symptoms described above.

During 1998, 18 of 19 marked ewes were observed between June 15 and September 30 moving back and forth between high elevation summer and fall range to lower elevation winter range in order to eat soil at natural mineral licks located on Torrey Valley winter range (Fig. 4). Typically, several marked ewes and their lambs were accompanied by 10 - 20 other unmarked ewes and lambs. Licks were visited nearly continuously throughout the summer, suggesting that the entire Torrey Rim contingent of wintering sheep (N = 150 - 180 sheep) were utilizing the natural licks. Licks were located on soils originating from glacial sediments higher in available selenium for plant uptake. The 13 km (8 mi) one-way-trip involved a 792 m (2,600 ft) drop, then a 701 m (2,300 ft) climb and finally a 900 m (2,955 ft) drop in elevation and was done on a bimonthly basis. At 2 locations along the route, observations of mountain lions (*Felis concolor*), lion sign and sheep remains in lion scat were noted. On several occasions (N < 5), ewes displayed "lost-lamb behavior" (i.e., regular bleating associated with extreme nervousness and agitation), at both of these locations (Akenson and Akenson 1992). The ewes' lambs were not observed again indicating mortality. Though determination of exact cause of death was not possible, these observations indicated that lambs were susceptible to lion predation at these locations. The sickest lambs lagged behind increasing the likelihood of predation.

Fig. 4. Summer migration of bighorn sheep from Middle Mountain to natural mineral licks, Wyoming, 1998.

In response to this, we placed mineral blocks that contained 78% salt (NaCl), 2% magnesium, 0.4% potassium, 17 ppm selenium and 17 ppm cobalt on lambing, summer and fall range. Sheep readily found and consumed blocks. Sheep that had access to blocks during the summer and fall (i.e., Middle Mountain) ceased summer movements to lower elevation winter range and natural mineral licks entirely and displayed dramatic improvement in lamb health and survival. Also, ewes shed winter coats ~1 month earlier and weaned lambs ~1 month later. No evidence of mountain lion predation was observed. In contrast, sheep that did not have access to blocks during the summer and fall (i.e., Arrow Mountain) continued summer movements to natural licks on lower elevation winter range and had lower lamb survival. A paired t-Test showed lamb ratios at the end of October in 1999, 2000 and 2001 were significantly greater ($P = 0.003$) for sheep on Middle Mountain (Mean = 27, SD = 10.1) than Arrow Mountain (Mean = 16, SD = 10.0) (Table 3). No significant difference in forage selenium was found between mountains.

In 2000 and 2001, selenium and cobalt content of blocks was increased to 60 ppm. This was done to provide a multiple-day dose since sheep were visiting blocks only once every 10 to 14 days. Similar differences between groups of sheep with and those without blocks were observed both years. In addition, we placed pure salt blocks (NaCl) on Arrow Mountain in 2001 to help assess the effect of salt alone on migration and lamb survival. Sheep from Arrow Mountain discontinued movements to lower winter range and had a fall lamb ratio that was 67% lower than Middle Mountain.

Between 1998 and 2001, lamb:ewe ratios on Middle Mountain correlated well with summer forage selenium concentration ($r = 0.84$). When data were considered for years 1998 - 2001 on both Middle Mountain and Arrow Mountain, this correlation decreased to $r = 0.64$. A loss of lambs during September 2001, most likely unrelated to forage selenium, confounded results. A wet summer in 1998 and low forage selenium was in sharp contrast to dry summers in 1999, 2000 and 2001 and higher forage selenium (Table 3). The 1998 forage selenium level of 5 ppb falls well below the 99.9% lower confidence interval of 31 ppb for the combined years of 1999 - 2001 on Middle Mountain. This suggested a possible connection between forage selenium and rainfall.

PMDSI was analyzed for 42 of 44 years between 1958 - 2001 and compared to lamb ratios. Two years of lamb ratios were not available. Generally, poor lamb ratios were associated with wetter years (Fig. 5), although not correlated well ($r = -0.43$) year-to-year. In a different approach, the 42 years were sorted by PMDSI into the lower (drier) and the higher (wetter) half. The PMDSI for the years with the lowest values was significantly lower (Mean = -3.48, SD = 1.49, $P = 0.000$) than the higher half (Mean = +1.30, SD = 1.84). The corresponding lamb ratios were significantly higher ($P = 0.005$) in the drier half (Mean = 39, SD = 12.7) than the wetter half (Mean = 28, SD = 12.1). This process was repeated with the lowest 10 (Mean = -4.67, SD = 0.67, $P = 0.000$) and highest 10 years (Mean = +2.95, SD = 1.37) of PMDSI. Comparison of corresponding lamb ratios showed even greater separation with 37 lambs:100 ewes for the driest 10 years (SD = 13.2) and 23 lambs:100 ewes for the wettest 10 years (SD = 12.6, $P = 0.021$).

Of 41 lambs monitored closely between June - October 1998 - 2000, 27 were Lambs That Survived and 14 were Lambs That Died. Of the Lambs That Survived, 20 were healthy and 7 were sick. Of the Lambs That Died, 3 were healthy and 11 were sick. For

Table 3. Lambs:100 bighorn ewes at the end of October, summer forage selenium in parts per billion, presence/absence of supplemental mineral blocks, and soil conditions on Middle Mountain (MM) and Arrow Mountain (AM), Wyoming, 1998 - 2001.

	1998	1999	2000	2001[a]
MM lambs:100 ewe	12	38	26	18
MM forage Se (ppb)	5 (N = 1)	58 (N = 2, SD = 18)	34 (N = 10, SD = 13)	43 (N = 22, SD = 16)
Blocks present	No	Yes (17 ppm Se)	Yes (60 ppm Se)	Yes (60 ppm Se)
AM lambs:100 ewe	No data	26	16	6
AM forage Se (ppb)	No data	58 (N = 2, SD = 17)	20 (N = 3, SD = 0)	38 (N = 10, SD = 17)
Blocks present	No	No	No	Yes (NaCl only, no Se)
Soil condition both sites	Very wet	Dry	Dry	Dry

[a] An additional, undetermined source of mortality affected lambs at both locations.

healthy lambs, 3 of 23 died (13%) while for sick lambs 11 of 18 died (61%). Therefore, the mortality rate was 4.7 X higher for

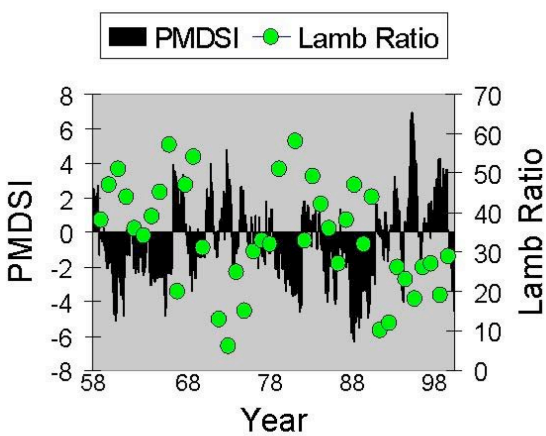

Fig. 5. Palmer Modified Drought Severity Index for the Wind River Basin, Wyoming, and lamb:ewe ratios from Whiskey Mountain, Wyoming 1958 - 2001.

sick lambs (Chi-square = 8.35, P = 0.004). Between June - August 1998 - 2000, lamb suckling averaged 17.0 seconds (N = 49, SD = 10.2) per attempt for Lambs That Survived versus 11.3 seconds (N = 29, SD = 11.2) for Lambs That Died (P = 0.030). Lambs That Survived generally had better overall suckling history and mothers with more "full" udders based on subjective observations. Fecal lungworm levels varied greatly between samples. However, there was no significant difference between the mothers of Lambs That Survived and Lambs That Died in 1998 (P = 0.214), 1999 (P = 0.358), 2000 (P = 0.531), or when all years were combined (P = 0.305) (Table 4). Fecal nitrogen levels also showed no significant difference between the mothers of Lambs That Survived and Lambs That Died in 1998 (P = 0.692), 1999 (P = 0.690) or when all years were combined (P = 0.887) (Table 5). The lambs' mothers were used to infer fecal lungworm and fecal nitrogen levels because data were insufficient for the lambs themselves.

Two lambs that died on summer range were necropsied within 36 hours of death. Ultimate cause of death was pneumonia. Selenium content in liver tissue was not

Table 4. Comparison of *Protostrongylus* spp. larvae per gram of feces in marked ewes with lambs that survived to late October and those whose lambs did not, 1998 – 2000. Samples were collected between March 15 - November 15 and were collected on Torrey Rim, Middle Mountain, Arrow Mountain, Goat Flat, and Whiskey Mountain, Wyoming, from sheep associated with the Torrey Rim wintering area.

Year	Status of lamb	Mean LPG	Std. Dev.	Range	No. of ewes	No. of samples	% of samples Infected (>0 LPG)	P[a]
1998	Survived	149	179	0 - 470	4	13	85	0.214
	Died	264	365	0 - 1,247	9	23	87	
1999	Survived	240	360	0 - 1,613	12	54	81	0.358
	Died	334	412	0 - 1,604	4	22	82	
2000	Survived	41	128	0 - 533	8	17	65	0.531
	Died	20	35	0 - 109	4	10	70	
All years	Survived	186	311	0 - 1,613	14	84	79	0.305
	Died	247	365	0 - 1,604	12	55	82	

[a] Means are compared using a 2-tailed t-Test with unequal variances.

deficient. These lambs died in 1999 and 2000 during which the mean summer forage selenium was 11 and 7 X higher, respectively, than 1998 when NMD was suspected. Mineral blocks supplemented with selenium were also available on summer range in 1999 and 2000.

Forage analysis for 17 parameters indicated that summer range forage was low in selenium and averaged 42 ppb (N = 49, SD = 21.4) between 1998 – 2001 (Table 2). This was not significantly different from lambing or fall range, but was significantly lower (P = 0.005) than the average winter range forage of 150 ppb (N = 14, SD = 121.0) where natural licks utilized by sheep were situated (Torrey Valley). In addition to selenium, phosphorus was low and sodium was very low on summer range when compared to domestic sheep recommendations. Iron, manganese and potassium were above recommended levels, but were below the maximum tolerable limit for domestic sheep (National Research Council 1985).

Selenium test plots that had nitrate depositions that were less than the "expected" deposition during June with "normal" rainfall showed increases in forage selenium (range of 6 - 59%). None of these plots had nitrate added artificially. In contrast, the plot with 638% of the "expected" nitrate deposition (and 252% of what would theoretically fall during a very wet June, as in 1998) showed a 19% decrease (Fig. 6). On this plot, nitrates were

Table 5. Comparison of percent fecal nitrogen in marked ewes with lambs that survived to late October and those whose lambs did not, 1998 – 2000. Samples reflect the primary lactating period from June 1 - August 31 and were collected on Torrey Rim, Middle Mountain, Arrow Mountain, Goat Flat, and Whiskey Mountain, Wyoming, from sheep associated with the Torrey Rim wintering area.

Year	Status of lamb	Mean Fecal Nitrogen	Std. Dev.	Range	No. of ewes	No. of samples	P[a]
1998	Survived	3.09	0.40	2.48 - 3.57	4	6	0.692
	Died	3.17	0.48	2.40 - 4.14	8	18	
1999	Survived	3.39	0.26	2.78 - 3.73	11	19	0.690
	Died	3.28	0.50	2.77 - 3.74	3	4	
2000	Survived	3.01	0.18	2.75 - 3.23	3	5	0.026
	Died	3.55	0.51	2.42 - 4.05	4	8	
All years	Survived	3.26	0.32	2.48 - 3.73	12	30	0.887
	Died	3.28	0.50	2.40 - 4.14	12	30	

[a] Means are compared using a 2-tailed t-Test with unequal variances.

added. The aforementioned plots had precipitation levels between 113 - 153% of a “normal” June (Table 1). Conversely, plots that received sulfate and sodium bicarbonate showed increases in forage selenium of 40% and 34%, respectively. These plots had identical levels of watering as the nitrate plot.

Precipitation and associated soil wetness were also varied on select selenium test plots (Fig. 7). The saturated plot showed a 67% decrease in forage selenium. Conversely, plots receiving 113 - 153% of normal precipitation showed increases of 29 - 59%. These plots had nitrate levels between 19 - 44% of a “normal” June.

Analysis of rainfall chemistry was done on 5 Hubbard-Brooke samples and 19 individual rainfall events from Middle Mountain. Monitoring period was June 18 to August 28, 2001. Results from the on-site Hach® spectrophotometer and off-site Biogeochemistry Lab agreed well. In addition, an independent study concurrently documenting rainfall chemistry corroborated our findings. This study site was located 48 km (30 mi) southwest of Middle Mountain

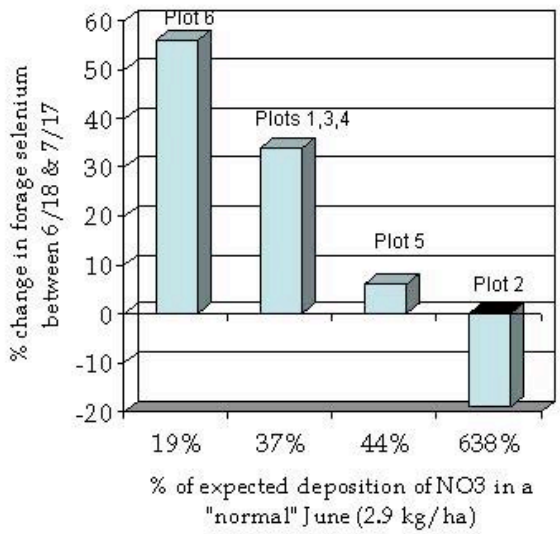

Fig. 6. Percent change in forage selenium as related to nitrate deposition on selected plots, Middle Mountain, Wyoming, 2001.

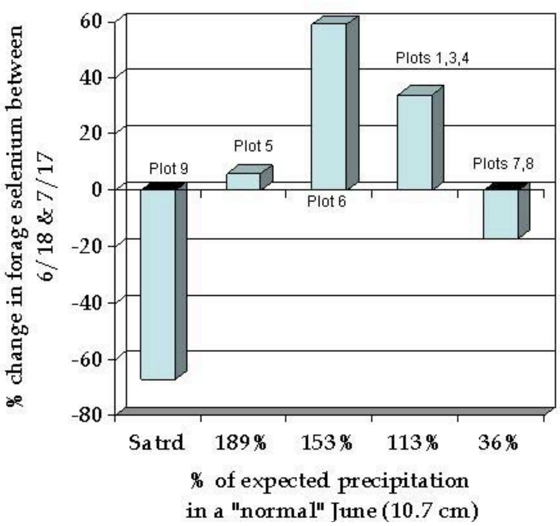

Fig. 7. Percent change in forage selenium as related to precipitation on selected plots, Middle Mountain, Wyoming, 2001.

at a similar elevation within the same mountain range and used the same protocol. Precipitation amounts from Middle Mountain and nitrate concentrations as determined by the Biogeochemistry Lab were compared to the Gypsum Creek NADP levels from June - August 1985 – 2001 (Table 6). Amount of precipitation, nitrate concentration and nitrate deposition were all higher on Middle Mountain when compared to Gypsum Creek in 2001 and the 1985 - 2001 long-term means. Paired t-Tests showed significant differences in deposition only ($P = 0.076$). Deposition is a function of nitrate concentration in rainwater and amount of precipitation. Our data suggested that deposition rates might be multiple times higher on Middle Mountain than at lower elevation sites because of higher nitrate concentrations and higher amounts of rainfall.

Preliminary tests of lab-cultured microbes from Middle Mountain soils indicated that addition of nitrate increased growth of actinomycetes, which accounted for 52% of the microbial population. Addition of selenate to the colonies resulted in volatilization to dimethyl selenide and a pinkish coloration of some colonies. Pinkish coloration was indicative of microbial conversion to elemental selenium (States 1966). Both would result in a net loss of selenium for plant uptake.

Summer range soil from Middle Mountain (MM#1) was black and contained 25.2% (SD = 10) by weight organic material and 2.8% by weight iron as Fe_2O_3 (Table 7). It was acidic, ranging in pH from 5.29 (MM#2) to 6.88 (GF). Values as low as 4.7 pH have been recorded from other locations in the Wind River Mountains (Clayton *et al.* 1991). In contrast, mineral lick soils were alkaline, ranging from a pH of 9.20 (TV) to 10.25 (BB#1). Total selenium content of the soils ranged from a low of 58 ppb (SD = 34) at the TV mineral lick to a high of 1,072 ppb (SD = 116) at BB#1 lick. This range of values brackets those of summer range with a low of 155 ppb (SD = 43) at MM#2 to a high of 640 ppb (SD = 51) at MM#1. A single value for soil from the control area at

Table 6. Comparison of nitrate concentration (ppm) and deposition (kg/ha) in rainfall between Middle Mountain, elevation 3,292 m (10,800 ft) and Gypsum Creek NADP site, elevation 2,439 m (8,000 ft) near Dubois, WY, 2001. Gypsum Creek NADP site was located 32 km (20 mi) upwind of Middle Mountain.

		Gypsum Creek NADP Site					Middle Mountain	
Type	Month	1985-2001 Mean Precip (cm)	1985-2001 Weighted Mean	1985-2001 Range	2001 Mean Precip (cm)	2001 Weight-ed Mean	2001 Mean Precip (cm)	2001 Weight-ed Mean
Concentration (ppm)	June	4.00	0.67	0.21 – 1.86	1.01	0.21	1.15[a]	2.69[a]
	July	3.14	1.10	0.02 – 4.37	0.85	2.23	3.07	2.42
	August	3.04	1.35	0.55 – 3.08	1.63	1.23	2.36	1.72
	Total	10.18	1.01		3.49	1.18[b]	6.58	2.22[b]
Deposition (kg/ha)	June		0.26	0.02 – 0.59		0.02		0.31[a]
	July		0.34	0.01 – 1.07		0.19		0.74
	August		0.37	0.15 – 0.75		0.20		0.41
	Total		0.97			0.41[b]		1.46[b]

[a] Data from June 18th-30th only.
[b] Paired t-test showed no significant differences for 2001 weighted mean concentrations (P = 0.280) between Gypsum Creek and Middle Mountain. Deposition, however, was nearly significant (P = 0.076).

Arrow Mountain (399 ppb) and the mean value for summer range at nearby Goat Flat (Mean = 209 ppb, SD = 75) fell within the range found for the MM sites. However, summer range soils were found to have no measurable soluble selenium content. In contrast, the BB lick soils contained from 4 - 13% of total selenium in the soluble form, presumably as selenate. Summer-range soils contained 11 - 30% phosphate-extractable selenium, while lick soils contained 11 - 67%. This fraction presumably represents available selenite. These values for air-dried soil represent those to be expected in the field during dry years.

The effect of water saturation on the redox potential for 3 MM soil samples showed a rapid drop in potential. Redox potential is a measure of the oxidizing power of the soil and is related to the amount of available oxygen. As soils become wetter, gaseous oxygen is replaced with water in the soil pores. Presumably, microbial action also depletes oxygen. Consequently, redox potential drops and anaerobic conditions prevail in a matter of hours. When a sample of MM#1 soil was maintained under these conditions for 1

Table 7. The total selenium concentration in ppb and % selenium availability for selected study area soils, Wind River Mountains, Wyoming, 2001.

Site	N	Total Mean Se ppb (SD)	pH	% Soluble Se	% Exchangeable Se	Total Available for Plant Uptake
MM #1	5	640 (51)	5.49	<1	11	11
MM #1R				<1	5	5
MM #2	4	155 (34)	5.29			
AM	1	399	6.67			
GF	2	209 (75)	6.88	<1	30	30
TV[a]	2	58 (34)	9.20	0	67	67
BB #1[a]	2	1,072 (116)	10.25	13	14	27
BB #2[a]	2	213 (19)	10.18	4	11	15

[a] Natural mineral licks used by bighorn sheep and located on winter range in Torrey Valley.

month in the laboratory (MM#1R), a 50% decrease in the amount of available selenium was found. This is the value to be expected during a wet field season. In 2001, 4 redox probes were installed on Middle Mountain. Soils had a mean redox potential of 511 mV (N readings = 68, SD = 53). During saturated conditions, redox potential dropped to a low of 350 mV. Redox potentials this low are at the threshold of where selenite (available to plants) would be converted to elemental selenium (unavailable to plants) at the pH of these soils (Geering *et al.* 1968).

DISCUSSION

Lamb survival is affected by many factors including forage quality and quantity (Festa-Bianchet 1988, Dunbar 1994), nutritional and health status of mother and lamb (Thorne *et al.* 1979, Festa-Bianchet 1984, Festa-Bianchet 1991), inbreeding (Hass 1989), weather (Wehausen *et al.* 1987, Ryder *et al.*1992) and predation (Hass 1989, Wehausen 1996). Survival of lambs in this study appeared to be highly related to health, as mortality rate for healthy lambs was nearly 1/5 that of sick lambs. Health of wild and domestic lambs is intimately related to adequate nutritional intake obtained from mother's milk (Horejsi 1972, Shackleton 1972, National Research Council 1985). This was confirmed in our study by the fact that Lambs That Died had much higher rates of poor health and lower intake of milk as inferred by average suckling time and milk production (i.e., from subjective qualification of udder size).

The shedding of lungworm larvae through the intestine and measured in feces has been used as an inference to the potential level of stress in bighorns and impacts on reproduction and lamb survival (Ellenberger 1976, Thorne *et al.* 1979, Samuel and Gray 1982, Festa-Bianchet 1989, Festa-Bianchet 1991). Festa-Bianchet (1984) reported that ewes with lambs that survived until October had statistically lower lungworm densities (mean = 642 l/g in 1982, mean = 455 l/g in 1983) than ewes with lambs that died (mean = 967 l/g in 1982, mean = 1,130 in 1983, $P < 0.03$ using Mann-Whitney U test). Festa-Bianchet (1984) implied that the density of lungworm may not have been the cause of lamb loss but that higher levels may be simply an indication of ewes in poorer condition and thus not as capable of producing healthy lambs. In contrast, our means were much lower and showed no statistical differences. Consequently, we believe that lungworm loads inferred by fecal lungworm counts did not have an effect on lamb survival. Data were insufficient to compare lambs directly thus the lambs' mother was used.

Fecal nitrogen levels have been utilized as an indicator of animal health and forage quality (Hebert *et al.* 1984, Kie and Burton 1984, Leslie *et al.* 1989, Irwin *et al.* 1993, Hodgman *et al.* 1996, Kucera 1997). We used fecal nitrogen to infer protein intake between mothers with Lambs That Survived and mothers with Lambs That Died. Protein intake is important for milk production and growth (National Research Council 1985). If protein intake as reflected by fecal nitrogen were effecting lamb survival due to impairment of milk production and lamb growth, then we expect lower fecal nitrogen levels in mothers with Lambs That Died. Our data did not support this as there were no significant differences in fecal nitrogen levels between groups in 1998, 1999 or 1998 - 2000. Means for all 3 years combined were identical. We expected to find no difference since both groups occupied identical summer ranges. A significant difference did occur in 2000. However, mothers with Lambs That Died had the higher mean, counter to the reasoning stated above.

Selenium levels in whole blood were not deficient in ewes captured during March or May on winter range. Winter range, comprised of alkaline soils derived from sedimentary rock, produced forage that had adequate levels of selenium (mean = 150 ppb dry matter). Ewes were not deficient simply because they had been feeding for 5 months on this forage.

Selenium, a component of selenoproteins, is vital to general health and proper immune function (Berger 1993, Underwood and Suttle 2000), disease resistance, growth (Langlands *et al.* 1990), milk production (Smith 1994) and regulation of body temperature (Underwood and Suttle 2000) of domestic sheep and goats. Stabel *et al.* (1989) stated that domestic calves from mothers fed a diet marginally deficient in selenium (30 - 50 ppb dry matter) showed an increased susceptibility to *Pasteurella hemolytica* than calves from mothers fed 100 ppb selenium dry matter and injected with sodium selenite every 60 days. Donald *et al.* (1993) found an increase in survival of newborn domestic lambs from 61% to 91% once selenium supplementation was provided.

Selenium is also an essential constituent of several variants of the blood enzyme glutathione peroxidase. The glutathione peroxidases act as selenium storage and as antioxidants that help protect cells from oxidative damage as well as damage from heavy metal poisoning (Rosenfeld and Beath 1964, National Research Council 1983, Underwood and Suttle 2000). As muscles utilize oxygen, peroxides are produced and if not removed result in the destruction of lipids, including subcellular membranes. In extreme cases, breakdown of cellular tissue will result in the degeneration of muscle resulting in NMD. NMD has been common in places throughout the world, but especially in New Zealand and Australia, accounting for dozens of publications related to its study (National Research

Council 1983, National Research Council 1985, Underwood and Suttle 2000).

Subclinical deficiency may exist in wild animals, with apparent symptoms resulting only after stress (Robbins *et al.* 1985). For example, mountain goats (*Oreamnos americanus*) in Canada did not develop symptoms until after being captured (Hebert and McTaggart-Cowan 1971). Further, fawn survival was improved from 32 to 83 fawns:100 does for symptomless black-tailed deer (*Odocoileus hemionus columbianus*) provided with selenium supplements in an area of northern California (Flueck 1994). Increases in glutathione peroxidase activity due to selenium supplementation have been demonstrated for mountain goats and bighorn sheep (Robbins *et al.* 1985, Samson *et al.* 1989), white-tailed deer (*Odocoileus hemionus*) (Brady *et al.* 1978) and black-tailed deer (Flueck 1991).

We believe that lambs on Middle Mountain had NMD during the summer of 1998. Forage selenium was only 5 ppb dry matter and lambs:100 ewes was 12 at the end of October. In subsequent years (1999 - 2001), lambs did not display NMD symptoms. Forage selenium values were > 34 ppb dry matter and lamb ratios ranged from 18 - 38. In addition, mineral blocks supplemented with selenium were available on summer range and readily used by ewes and lambs. Lamb survival during these subsequent years was still low to moderate and may have been affected by a subclinical deficiency (i.e., sheep may have been deficient and were adversely affected by this deficiency, but were not deficient enough to develop full-blown NMD).

Although the exact concentration of forage selenium required by bighorns is unknown, an extensive review of New Zealand publications showed NMD in grazing domestic lambs occurred where selenium content of spring pastures was < 20 ppb dry matter (National Research Council 1985). Deficiency resulting in symptoms similar to those of livestock has been reported for mountain goats using forage species of which most contained < 45 ppb dry matter (Hebert and McTaggart-Cowan 1971). Caution must be used when using forage concentrations of selenium as the only indicator of possible deficiency. Selenium is affected by many other variables including general health of animal, level of stress, diseases present in animal, digestive absorption as a function of the form of selenium intake, and dietary intake of sulfur, sulfur-containing amino acids, and Vitamin E (Rosenfeld and Beath 1964, National Research Council 1985, Underwood and Suttle 2000).

Robbins *et al.* (1985) rightly expressed caution when using domestic livestock standards as guidelines for wildlife, as many wildlife species have evolved in low selenium habitats and consequently have developed compensatory mechanisms. Though selenium deficiency affecting wildlife production may have been present for thousands of years and hence wildlife species have adapted to it, Flueck (1991) contends, that "…there is growing evidence that anthropogenic (man caused) manipulation of ecosystems can rapidly alter selenium cycling and availability or requirements of free-ranging herbivores." Impacts might result from acidification of soils, exposure to heavy metals and deposition of sulfur and nitrogen (Flueck 1991).

Nitrate deposition does appear to be increasing in the Wind River Mountains based on the Gypsum Creek NADP site. Our analysis of rainfall chemistry indicated that the study area received multiple times more deposition than the Gypsum Creek site in 2001. Analysis of animated cloud movements from GOES-10 satellite images indicated most air masses from which summer precipitation fell on the upper Wind River Mountains during 2000 and 2001 first traveled through major urban and pollution centers in Mexico, Arizona, southern California and Utah. Potential upwind local sources (< 450 km, 275 mi) of nitrates included the Salt Lake Valley, extensively developed oil/gas fields in southwestern Wyoming, Interstate Highways, fertilizer, gas and trona processing plants, and coal-fired electric generating plants. Williams and Tonnessen (2000) documented the adverse affects of nitrate deposition on alpine environments near Denver, Colorado. This publication showed that Lincoln County in southwestern Wyoming, located upwind of the Wind River Mountains, was among the top counties in Arizona, Colorado, New Mexico, Utah and Wyoming for nitrate emissions in 1990. They state, "…nitrate emissions associated with this energy development might well have effects on nitrogen loading to Wilderness Areas in both northern Colorado and southern Wyoming."

In our study, forage plots on Middle Mountain that received less than "expected" nitrates and "normal" precipitation showed increases in forage selenium of 6 - 59% during 1 month of the growing season. In contrast, plots that received heavy additions of nitrate or were saturated decreased in forage selenium. Extremely dry plots also showed a decrease in selenium. This may be due to the roots difficulty in absorbing soil moisture (and hence, soluble forms of selenium) since there was so little soil moisture present. The % decrease was greatest under saturated soil conditions. The heaviest loaded nitrate plot showed a 19% decrease in forage selenium. The level of nitrate deposition on this plot would be about ½ of what would theoretically fall during a very wet summer, such as the summer of 1998 when heavy rainfall resulted in boggy, saturated conditions across Middle Mountain. Lab analysis of Middle Mountain soils showed a 50% decrease in available selenium under saturated conditions. All of these results supported our hypothesis. However, no plots showed less than 28 ppb forage selenium. Thus, our manipulations did not reproduce conditions found in 1998 during which there was only 5 ppb forage selenium on summer range. The reductions in forage selenium on the plots would not likely be low enough to cause NMD, but may be low enough to create subclinical deficiency capable of affecting lamb survival. Other as-yet unknown factors may have influenced such low levels in 1998. Further research is needed to confirm the effects of soil wetness and nitrate deposition on selenium availability.

Certain soils are susceptible to producing forage low in selenium. In Scotland, low blood selenium levels in domestic sheep and cattle were attributed to a combination of granitic soils, high elevation, high rainfall and slightly acidic soils (Anderson *et al.* 1979, Arthur *et al.* 1979). Forage selenium concentrations are not related to total soil selenium concentration, but rather to the fraction of total soil selenium in the available form. Alkaline, dry soil conditions are thought to favor available selenium (Mincher *et al.* in preparation). Factors which lower either pH or redox potential of soil can theoretically change the form of selenium from available (selenite SeO_3^- and selenate SeO_4^{-2}) to unavailable (selenide or elemental selenium) (Geering *et al.* 1968). Available forms are water-soluble and hence absorbable by plant roots. Elemental selenium (Se^0) and selenides (Se^-) are not readily water-soluble and thus not easily absorbed by plants. (Fisher *et al.* 1987).

Lamb recruitment data from Whiskey Mountain support the hypothesis that years of higher precipitation are related to lower lamb recruitment. Higher precipitation would cause wetter soil conditions, lowering soil redox potential and resulting in less available selenium. Also, higher precipitation would result in greater biomass of forage creating a "dilution" effect (i.e., available selenium in the soil would be absorbed by roots, but assimilated into more biomass above ground causing lower concentration of forage selenium). Greater precipitation would also result in greater amounts of nitrate deposition. Nitrates would likely fertilize plants, further exacerbating the "dilution" effect. Preliminary tests of lab-cultured microbes from Middle Mountain soils indicated that addition of nitrate increased growth of soil microbes. Soil microbes appeared to convert available forms of selenium into elemental selenium and to volatilize dimethyl selenide (States 1966, Holzinger-Love 1974). Both would result in a net loss of selenium for plant uptake. Although elemental selenium may convert back to available forms under certain conditions, volatilized selenium leaves the ecosystem.

Sheep behavior may also affect selenium uptake. We observed bighorns selecting for the most succulent forage. This forage tended to grow in the wettest places, likely resulting in lower forage selenium for the reasons listed above. It is likely that the most succulent forage would also be the most digestible and hence have the shortest passage time in the gut. As a result, trace minerals like selenium would have less time to be absorbed in the digestive tract (Ron Dean, pers. comm.). Figure 8 details factors that may influence selenium assimilation in bighorns.

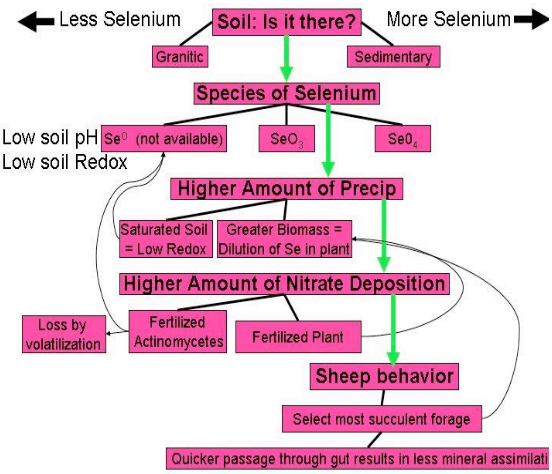

Fig. 8. Factors affecting selenium uptake by bighorn sheep.

In 2002, work conducted by Mionczynski (2002) showed additional support for a possible link between herd status and forage selenium. Summer range forage samples were collected from declining herds (Wind River Mountains in Wyoming, Lemhi Mountains in Idaho and Fraser River in British Columbia) and compared to stable/increasing herds (Owl Creek Mountains and Absoraka Mountains in Wyoming and Sierra Nevada Mountains in California). Mean forage selenium was statistically lower ($P = 0.042$) for declining herds (mean = 60 ppb dry matter, $N = 19$, $SD = 61$) as compared to stable/increasing herds (mean = 237 ppb dry matter, $N = 7$, $SD = 181$).

In summary, the connection between dietary selenium and lamb survival cannot be proven by this study. However, numerous facts point toward its connection and include: (1) mineral cravings by ewes and huge expenditures of energy to acquire mineral soil containing selenium at natural licks (2,270 m, 7,450 ft elevation) located 13 km (8 mi) away from summer range (3,354 m, 11,000 ft elevation); (2) near absence (5 ppb dry matter) of selenium in summer forage in 1998 when lambs were critically ill and exhibited all 9 external symptoms of NMD displayed in domestic sheep. Recovery from symptoms occurred following visits to natural licks; (3) summer range forages in other years that were 20 to 50% of the National Research Council's (1985) lowest recommended level for domestic sheep (100 ppb dry matter); (4) the marked relationship between health of bighorn lambs and increased survival. Selenium is critical in maintaining health of domestic lambs; (5) increased survival of lambs following placement of supplemental selenium blocks on summer range; (6) higher survival and overall health of lambs on sites with blocks as compared to sites without blocks; (7) greater time spent suckling for Lambs That Survived inferring greater milk production. Selenium deficiency can impair milk production in domestic livestock; and (8) lack of relationship between lamb survival and other important factors like dietary intake of protein as inferred by fecal nitrogen and lungworm infestation as inferred by fecal lungworm loads.

We suspect that in granitic soils, wetter summers produce conditions unfavorable for selenium uptake by forage plants due to lowered soil redox potential, thus converting selenite and selenate into chemical species (e.g., elemental selenium) that are unavailable for plant uptake. We also suspect that artificially enhanced nitrate deposition stimulates microbial decomposition processes including microbial transformation of selenite and selenate to unavailable forms of gaseous and elemental selenium. Ultimately, we suspect that wetter conditions result in less selenium uptake by bighorn sheep from forage growing on granitic summer range, thus lowering lamb health and survival. A possible selenium deficiency is not the only factor affecting lamb survival at Whiskey Mountain, however. Poor lamb recruitment still continues even with mineral supplementation. Monitoring of marked sheep, additional analysis of rainfall chemistry, expansion of plot treatments, feeding trials of captive ewes and lambs on low and high selenium diets, sampling blood from sheep on summer range, and necropsy of lambs displaying NMD should be done to further expand current knowledge of the relationship between bighorns and selenium.

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