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Cover photo: Sandra Kennedy and Tom Stephenson tag caribou calf at the Moose Research Center



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Study 1.45 Evaluation and Testing of Techniques for Moose Management

Study 1.45 Evaluation and Testing of Techniques for Ungulate Management

Editor's note: The study number stems from the earlier moose study.

Study 1.48 Influence of Selective Harvest Systems on Population Genetics of Alaskan Moose

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EVALUATION AND TESTING OF TECHNIQUES FOR MOOSE MANAGEMENT

By

Charles C. Schwartz Thomas R. Stephenson Kris J. Hundertmark Sandra L. Kennedy

Research Progress Report Federal Aid in Restoration Grant W-24-4 Study 1.45

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RESEARCH PROGRESS REPORT

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STUDY TITLE: Evaluation and Testing of Techniques for Moose Management

PERIOD: 1 July 1995–30 June 1996

SUMMARY

We designed an experiment to further investigate the role of bull moose in establishing a synchronous estrous period in cows. Seven cow moose were divided into 2 treatment groups. The control group was placed in a 4-ha holding pen with a mature bull in mid-September and allowed to breed normally. The treatment group was isolated from any bulls by a distance of at least 0.8km. Fecal samples were collected daily from both groups and frozen and analyzed later for progestagen (P4) concentrations. Progestagen profiles from control cows were similar to profiles observed during a normal estrous cycle, with a nadir (P4 < 0.7 mg/g) in P4 concentration occurring within 2 days of observed heat. Control females also had a nadir in P4 approximately 8-11 days before observed estrus. This nadir in P4 probably represented a "silent heat" in mid-September. Treatment cows also exhibited a nadir in P4 concentration when a "silent heat" should have occurred, followed by a second nadir during the normal breeding window (28 Sep - 12 Oct). Cows in the treatment group continued to cycle until sample collection terminated in mid November. The 2 primiparous females produced exceptionally high (>20 ug/g) P4 concentrations in mid to late October, making it difficult to interpret their P4 profiles. Data from 1995 trials were analyzed at 4-day intervals to determine approximate dates of the P4 nadir; additional samples are currently being analyzed to determine exact profiles. Preliminary analysis of the P4 data indicates cow moose exhibit a normal estrous cycle even when isolated from a bull.

We describe urine chemistry profiles for 4 healthy male moose fed an ad libitum or moderately restricted high protein, high energy diet, and for 1 male fed the same diet ad libitum but recovering from severe undernutrition. Urinary urea (U) and potassium (K) were expressed as ratios to creatinine (C) to account for differences in urine concentrations and to facilitate analysis of urine deposited in snow. Urea:C ratios did not differ among animals or over time. Mean K:C ratios for 10-day intervals varied over time and were correlated with allometric dry-matter intake for the previous 10-day interval, indicating that urinary K excretion reflects short-term variation in intake. These urinary metabolite ratios can be considered a baseline for moose fed supplementally during winter. Analysis of urine deposited in snow can be a useful indicator of population condition in moose, but a clear understanding of the physiological processes indicated by these profiles and their inherent limitations is essential to proper implementation.

Key words: Alces alces, feces, formulated ration, estrous cycle, intake, moose, potassium, reproduction, snow-urine, urea, urine.

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BACKGROUND

The Moose Research Center (MRC), with 32 confined moose and facilities to handle them, provides unique conditions for developing and testing techniques applicable to moose management. This study has been continuously active since 1969 when the MRC became functional. Four federal aid final reports covering the period 1968 through 30 June 1991 have been published (Franzmann et al. 1974, Franzmann and Schwartz 1982, Franzmann et al. 1987, Schwartz et al. 1993), in addition to more than 35 journal publications (see Schwartz et al. 1993).

In the past 2 decades, a wide variety of drugs have been used to immobilize moose (Franzmann and Arneson 1974, Gasaway et al. 1978, Franzmann 1982, Schmitt and Dalton 1987). Today, carfentanil citrate, a synthetic opiate used in combination with xylazine hydrochloride, a sedative analgesic, works well to immobilize moose (Haigh 1982; Franzmann et al. 1984, 1987; Franzmann and Schwartz 1984; Seal et al. 1985; Schmitt and Dalton 1987). The extreme potency of carfentanil, however, poses significant human safety problems; less than a drop can kill a human. Moreover, it can be absorbed through the skin and does not require a cut or wound for entry into the system. Accidental administration of carfentanil to humans during capture operations is a primary safety concern. Safety is especially important when darting animals in urban areas or when errant darts are not retrieved. As a consequence, it would be desirable from a human-safety perspective if moose could be immobilized effectively and reversed with an antagonist without requiring carfentanil.

Xylazine hydrochloride used alone or in combination with ketamine hydrochloride has been used as an effective immobilization agent on a wide range of domestic and wild ruminants (Franzmann 1982, Jessup et al. 1983, Hsu and Shulaw 1984, Seal et al. 1985, Kreeger et al. 1986, Takase et al. 1986, DelGiudice et al. 1989*a*, Doherty and Tweedie 1989, Golightly and Hofstra 1989, Garner and Addison 1994). Xylazine is a potent sedative-analgesic agent which may cause excessive depression of the central nervous system (Zingoni et al. 1982, Hsu et al. 1987) and respiratory distress characterized by tachypnea (Zingoni et al. 1982, Hsu et al. 1987) and hypoxema (Doherty et al. 1986, Waterman et al. 1987).

Xylazine sedation may cause animal immobilization for 3-6 hours or more (Mech et al. 1985). Therefore, an antagonist would be beneficial to safeguard the use of xylazine and reduce prolonged immobilizations. Xylazine has been reversed with yohimbine, tolazoline, and idazoxan in several ruminants, but effectiveness varies among species. In moose and caribou (*Rangifer tarandus*) as well as domestic cattle and sheep, yohimbine produces only partial reversal. According to Nolan et al. (1986), yohimbine usually is more effective in nonruminants than tolazoline in antagonizing the pharmacological effects of xylazine. In ruminants, however, tolazoline is more effective than yohimbine (0.8 mg/kg) has also been reported to be lethal in xylazine-treated sheep (Hsu et al 1987). Idazoxan seems effective in sheep (Hsu et al. 1989), moose, and caribou (Doherty and Tweede 1989), but is an experimental drug not available commercially. We tested 2 commercially available antagonists, yohimbine hydrochloride and tolazoline hydrochloride, as antidotes to xylazine in moose. We compare these results to moose immobilized with mixtures of xylazine and carfentanil antagonized with naltrexone.

Urine is a medium that contains metabolic by-products and has been used to assess nutritional restriction of captive cervids (Warren et al. 1981, 1982, Waid and Warren 1984, DelGiudice et al. 1987, 1990). Concentrations of urinary metabolites expressed as ratios to creatinine (C) are potential indicators of nutritional restriction of wild cervid populations, particularly the urea (U):C ratio (Mech et al. 1987, DelGiudice and Seal 1988, DelGiudice et al. 1989b, 1990, 1991a, 1991b). Expression of metabolite concentrations as ratios of C concentration correct for bias associated with urine concentration and allow this technique to be used with urine deposited in snow (DelGiudice et al. 1989b). As snow-urine is easily sampled, this technique has potential to be an effective tool for managers of moose and other species.

Urinary U:C ratios vary with nitrogen intake and energy balance. In undernourished deer, U:C ratios decline to relatively low values as individuals recycle urea for use in metabolic processes; whereas, in the late stage of undernutrition, catabolism of body protein is the primary source of energy and urinary U:C ratios rise dramatically (DelGiudice et al. 1987). Supplemental feeding of a high-protein diet can confound analysis of urinary U:C ratios because the source of urinary nitrogen (endogenous versus exogenous) cannot be ascertained. Of the other urinary metabolites described by DeGiudice et al. (1987), potassium (K) is the most informative and varies with intake. Thus, urinary K:C ratios can aid in the interpretation of U:C ratios. The usefulness of this technique has been the subject of recent debate (DelGiudice 1995, DelGiudice et al. 1995, Saltz et al. 1995, White et al. 1995a, 1995b). Central to the debate are age-related differences and intra-animal variability in metabolite ratios.

We examine urinary metabolite data from 5 captive moose on maintenance or ad libitum diets to determine urinary U:C and K:C ratios and associated measures of variability consistent with supplemental feeding and to discern temporal patterns associated with changes in intake. We also examine urinary U:C ratios from calves and adults collected in a separate study (Schwartz and Hundertmark 1993) to determine if age-related differences exist.

Mean (SD) length of the estrous cycle in moose ranges from 22 to 28 days (Schwartz and Hundertmark 1993). Work by Schwartz et al. (1990) demonstrated that bull moose do produce the male hormones androstenone and androstenal. In swine and red deer (*Cervus elaphus*), these hormones are responsible for stimulating estrus in the female. Before the studies of Monfort et al. (1993) and Schwartz et al (1995), the only reliable way to detect estrus in a female moose was by visually observing mounting by the bull. However, profiles of fecal progestagens (P4) indicate the nadir in P4 concentration is within ± 2 days of observed breeding. Consequently, profiles of P4 are useful in determining the approximate day of estrus in a cow moose in the absence of any visual mounting by the bull. With the development of this technique, we can now evaluate the role of the bull in stimulating estrus in the cow.

It has long been recognized that moose are difficult to keep in captivity (Speidel 1965): nonifectious disease and accidents account for about 67% of all reported mortalities (Schwartz 1992). The single greatest cause of deaths seems to be associated with poor nutrition and digestive upsets. Afflicted animals develop chronic diarrhea, eventually lose body condition, and become emaciated and dehydrated. Symptoms can last for weeks to many months, but inevitably result in death. The syndrome has been referred to as "chronic wasting." This term has also been used to describe similar but seemingly unrelated diseases in mule deer (*Odocoileus hemionus*), elk

(Cervus elaphus) (Williams and Young 1993), and moose (Rehbinder et al. 1991, Merza et al. 1994). Moose maintained in most facilities for more than a few years inevitably develop "chronic wasting" (Schwartz 1992), but the etiology is not understood.

"Chronic wasting" has been associated with the feeding of hay and/or crop residue to moose (Schwartz 1992). Eliminating these from diets of captive moose was one reason why a sawdustbased ration was developed in the late 1970s (Schwartz et al. 1980, 1985). Over the years there have been modifications of this ration; one involved the replacement of cereal grain with sucrose, additional molasses, and beat pulp as the energy sources (Schwartz 1992, Purina, Saint Louis, Mo). This diet was based on an untested hypothesis that moose cannot digest starch. Supposedly, undigested starch in the lower gut fermented causing chronic diarrhea. No quantitative data are available to support these claims, and the diet has not been on the market long enough for complete evaluation. We tested this hypothesis by quantifying concentrations of enzymes found in the pancreas and small intestine of 2 moose. We compared these values to a grain fed steer.

OBJECTIVES

- To test and evaluate techniques that are potentially useful for management of moose.
- To maintain and operate the MRC (Job 1)
- To test and evaluate immobilizing, tranquilizing, adjunct, and reversing drugs. (Job 2).
- To investigate the basic parameters of moose reproduction. (Job 5).
- To test miscellaneous techniques (Job 7).

METHODS

JOB 1. MAINTENANCE AND OPERATIONS

Construction of New Holding Facilities

Many of the handling facilities at the MRC were constructed in the late 1970s. Support posts for the woven wire fencing were beetle-killed white spruce (*Picea mariana*) trees treated with a wood preservative (Penta). Most of these posts are rotted or near rotten at ground level. As a consequence, we began an extensive renovation of the holding facilities.

JOB 2. DRUG TESTING

Use of Tolazoline Hydrochloride to Antagonize Xylazine Hydrochloride

We tested the potential effects of tolazoline hydrochloride to antagonize xylazine hydrochloride. Detailed methods were presented in a manuscript submitted to *Alces*.

JOB 5. REPRODUCTION STUDIES

Effect of the Bull on Reproductive Cycle of Cow Moose

Five cow (2 primiparous, and 3 multiparous females) moose were isolated (at least 0.8 km radius) from visual and olfactory contact with a male from late August until 15 November in 1994.

Additionally, a treatment group of 4 cows (all multiparous) were isolated from September 15 until 15 November in 1995. Also, 3 and 4 adult females were put into a 15 ha holding pen in mid-September with a mature bull in 1994 and 1995, respectively, forming the control group. We collected fecal samples daily from 1 and 15 September through 15 November for the treatment group in 1994 and 1995, respectively. Feces were frozen at -20C in plastic bags until analysis. Fecal samples were analyzed for progestagens following the protocol described by Wasser et al. (1991) and Monfort et al. (1993) and modified by Schwartz et al. (1995). We replaced the tritium-based monoclonal P4 antisera with an iodine (¹²⁵I) based isotope of greater sensitivity. To generate progestagen profiles, we analyzed every 4th sample from the treatment group and daily samples for at least 5 days before to 5 days after observed estrus in the control group. Additional samples were analyzed to refine profiles as needed.

Nutritional requirements for Reproduction in Moose with Respect to Body Condition Thresholds

A literature review and proposed methods are described in a proposal in Appendix C.

JOB 7. MISCELLANEOUS PROJECTS

Carbohydrase Activity in Moose

We obtained tissue samples from 2 adult female moose in January of 1994. These samples were shipped on dry ice to the University of Kentucky, Lexington. Dr. David Harmon, from the Department of Animal Science, analyzed these tissues for concentrations of pancreatic and small intestinal enzymes. Details of the protocol are found in a manuscript accepted by the journal Alces.

Urine Chemistries of Supplementally Fed Moose

Four male moose, 3 calves (8 months old at the start of the study) and 1 3-year-old were housed in individual 3- x 10-m enclosures and were offered a formulated high protein-high energy ration (Schwartz et al. 1985) ad libitum for 49 days, beginning on 16 January 1989. Beginning on Day 50 and continuing for 40 days, the animals were offered a proportion of the mean ad libitum intake for their age group ranging from 75-100% (Table 1). Intake was measured daily by weighing amounts of feed offered and orts. Samples of feed and orts were dried in a forced air oven at 60°C to a constant weight, and amount consumed was expressed as dry-weight. Animals were weighed (to the nearest kg) at least once weekly. Three of the animals were removed from the study after Day 90, and the remaining calf was returned to ad libitum feed and monitored though Day 135, but its intake was not measured. On Day 71 a 10-year-old bull was added to the study when it became apparent it was experiencing severe undernutrition. This animal was offered ad libitum feed and monitored through Day 135, but its intake was not measured.

Urine from each animal was collected once every 2-3 days, beginning on Day 20, by means of plastic 10-cc vials attached to the end of a long pole and held in the urine stream during urination. Sampled urine was kept frozen until analyzed. Concentrations of urinary U (Talke and Shubert 1965) and C (Jaffe 1886) were ascertained via spectrophotometry (Abbott Laboratories 1984). Aliquots of urine were diluted 1:100 with a lithium diluent and K concentration was determined via flame photometry. Potassium ratios to C were expressed as K:C x 100 to maintain consistency

with other published reports. Estimates of U:C and K:C ratios were log-transformed and grouped into 10-day intervals for analysis.

Differences in U:C ratios among animals were tested using ANOVA with repeated measures. To meet the requirement for equal numbers of observations per cell, we did not analyze data collected beyond day 80 and censored 3 estimates among the remaining data. Two-way ANOVA was used to assess temporal differences within animals.

In a separate study of the efficacy of using hay as an emergency winter feed for moose (Schwartz and Hundertmark 1993), 2 groups of moose (4 calves and 4 adult females in each) were offered either grass hay or a balanced ration ad libitum for 11 weeks. Urine was collected at 3 intervals during the study, either as it was being voided or as snow-urine, and was analyzed for differences in U:C ratios attributable to diet. Urinary K was not measured for this study. These data were reanalyzed here to determine differences in U:C ratios attributable to age. Differences due to age were tested using ANOVA with repeated measures.

RESULTS AND DISCUSSION

JOB 1. MAINTENANCE AND OPERATIONS

Construction of New Holding Facilities

We dismantled much of the moose handling facility, particularly the holding, processing, and weighing area that had deteriorated as a result of rotting wooden posts. We designed and marked out new handling areas at the same location with numerous efficiency modifications. Furthermore, the redesign incorporates changes required because of the proposed use of self-feeding gates for feeding trials (see Appendix C). In the reconstruction, weather-resistant steel posts will replace the previous wooden posts.

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JOB 2. DRUG TESTING

Use of Tolazoline Hydrochloride to Antagonize Xylazine Hydrochloride

Results of these studies were presented at the 32nd North American Moose Conference. A copy of the abstract is presented in Appendix A.

JOB 5. REPRODUCTION STUDIES

Effect of the Bull on Reproductive Cycle of Cow Moose

Because we changed the bioassay for fecal progestagens (¹²⁵I vs. ³H), it was necessary to compare the 2 techniques. We did this by running a duplicate assay on one data set collected in 1992 (Schwartz et al. 1995). Comparisons indicate the 2 techniques provided similar trends in P4 profiles (Fig. 1), but on average, absolute concentrations for ¹²⁵I technique were slightly less (x =1.65 + S.D. 0.89 mg/g) than ³H (x = 1.74 + S.D. 1.05 mg/g). Regression analysis of the 2 methods was highly correlated (Fig. 2). The concentration of P4 on the day of estrus was below 0.7 mg/g. Estrus occurred 2-3 days before the nadir in P4 (Fig 1). Additional data collected in 1994 and 1995 are being analyzed to determine the predictive value of P4 concentrations to estimate the day of heat.

Visually, profiles of fecal progestagen excretion from treatment cows in 1994 could be divided into 3 general profiles: 1) control animals maintained with the bull, (adults Sony, Lara, and Allye), 2) adult females in isolation (Satorine, Deshka, and Luna), and 3) primiparous females in isolation (Satorine and Sue). Cows from 1994 represented a fourth group.

Group 1. Animals with the Bull and with a Known Day of Heat

Allye presents a typical P4 profile (Fig. 3). She exhibited overt estrous 1 day before her nadir in fecal progestagen concentration. This profile was nearly identical to that presented by Schwartz et al. (1995). Profiles for Sony and Lara were equally as clear. Heat occurred at the nadir for Sony (Fig. 4) and 2 days before the nadir for Lara (Fig. 5). All 3 animals also show a nadir 11 days before observed estrus that probably represents a silent heat because no mounting was observed.

Group 2. Adult Females Held in Isolation

Luna and Deshka (Fig. 6 and 7) present progestagen excretion profile similar to that described by Schwartz et al. (1995) for moose cycling multiple times in the presence of a vasectomized male. They show a nadir in fecal progestagen concentration in mid to late September (Sept. 18 and Sept. 21-27), followed by a second nadir about 8-11 days later. This short cycle probably represents a silent heat which has been documented in moose (Backstrom 1952, Edwards and Ritcey 1958, Markgren 1969, Simkin 1974), domestic cattle (Robinson and Shelton 1991), and sheep (Lindsay 1991). The second nadir (Sep 29 to 1 Oct and 2 to 6 Oct) occurred during the normal breeding season at the MRC (28 Sep-12 Oct) (Schwartz and Hundertmark 1993). The next nadir occurred 22-26 days later, within the normal bounds for a typical estrous cycle (22-28 days, Schwartz and Hundertmark 1993). The pattern for Satorine (Fig. 8) was less clear. Her fecal P4 values were below 0.7 mg/g from the beginning of collection on 7 September. They remained low until 28 September and then increased to 1.4 mg/g. A second nadir (0.6 mg/g) occurred on 7 October and probably represents her first estrus. We cannot establish the day of her silent heat. Her profile indicates a second estrus on 28 October when her P4 concentration declined below 0.7 mg/g.

Group 3. Primiparous Females Held in Isolation

It is difficult to interpret these P4 profiles because of gaps in the data (Fig. 9 and 10). Unusually high progestagen concentrations in late October and early November made profiles difficult to interpret. Additional samples have been sent to the lab for analysis.

1995 Data

Preliminary profiles from 1995 studies look promising. Because P4 estimates are only on 4-day intervals, we sent additional samples to the lab for analysis to refine profiles at suspected locations for each nadir.

JOB 7. MISCELLANEOUS PROJECTS

Carbohydrase Activity in Moose

Results of this study were submitted to the journal *Alces* and have been accepted for publication in Volume 32. An abstract of the manuscript is presented in Appendix F.

Urine Chemistries of Supplementally Fed Moose

The 3 calves gained from 1-6% body weight during the first 49 days of the study while the adults lost 6%. During the second phase, 1 calf gained weight while the other 3 animals maintained or lost weight (Table 1). Dry matter intake for each animal varied prior to restriction. Initial values were low, particularly for the adult, due to voluntary reduction because of stress of confinement. Intake increased as animals became acclimated to their confinement; by Day 45 intake was normal. During restriction, intake was constant for animals on restricted diets with the exception of 2 periods of a few days each during which intake dropped slightly. These were associated with chemical immobilization of the moose for another study. Intake for the animal left on ad libitum feed varied greatly, primarily due to the effects of immobilization after which intake declined to nil and increased to normal over a period of about 5 days. These periods of anorexia caused the mean intake for this animal to approach those for intake-restricted animals.

Mean U:C ratios did not differ among animals (P = 0.89) or among 10-day intervals within animals (P = 0.27). Coefficients of variation of U:C ratios within animals ranged from 3.3-58.4%. The highest values were associated with ad libitum feeding, and the lowest values belonged to those animals on the most restrictive diets. Of the 3 animals on restricted rations, 2 exhibited a decrease in variance of U:C ratios during restriction, and the third exhibited low variance throughout the trial. For these 3 animals, the pooled variance for the mean U:C ratio was greater during unrestricted feeding than for restricted feeding ($F_{28,37} = 1.90$, P = 0.034). Coefficients of variation for K:C ratios (range 3.6-39.6%) were similar to those of U:C ratios but did not exhibit a consistent trend among the calves. For the 3 animals offered restricted rations, the variances of the pooled mean K:C ratios did not differ between restricted and unrestricted phases of the trial ($F_{37,28} = 1.31$, P = 0.231).

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Urinary K:C ratios declined slightly for the 2 calves on restricted rations and declined dramatically for the adult (Fig. 11). The calf fed ad libitum began the trial with low K:C ratios, again probably due to voluntary intake reduction, but these ratios increased throughout the trial. By the end of the trial, this animal's intake was increasing due to a seasonal increase in appetite (Schwartz et al. 1984).

Temporal variation in K:C ratios indicates a time lag between reduced intake and reduced K excretion. Intake for all animals was minimal at the start of sampling before increasing to maximum by Day 45, yet mean K:C ratios did not increase until the next 10-day period (Fig. 11). Additionally, as intake was reduced for 3 moose on Day 50, mean K excretion did not show a corresponding decline until the day 60-69 interval. The single animal for which sampling continued after being returned to ad libitum feed continued to excrete reduced amounts of K for more than 10 days before ratios returned to levels associated with ad libitum intake. Mean log K:C ratios were correlated with mean log allometric intake (expressed as g dry-matter intake/kg BW^{0.75}) for the previous 10-day interval (r = 0.56, P = 0.02), but intake was not correlated with K:C ratios measured during the same interval (r = 0.009, P = 0.97). K:C ratios for animals on restricted rations remained level or declined (Fig. 11), despite relatively constant intake. This trend indicates the conservation of serum K by reabsorption in the kidney (Gans and Mercer 1984).

The 10-year-old male exhibited U:C ratios that did not differ from those of the other animals in the trial, although by Day 102 he had lost 30% of his peak fall weight. He continued to lose weight (29 kg) for 1 month after being offered ad libitum feed, after which he began to gain weight. Initial urinary K:C ratios for this animal were below 50 and increased steadily over the next 60 days to approach levels exhibited by animals fed ad libitum.

For animals fed the high-quality ration, mean U:C raios for calves (N = 4) tended to be greater than those of cows (N = 3), but were not significant $(F_{1,5} = 2.58, P = 0.17, Table 3)$. Repeated measures ANOVA was not conducted on the U:C ratios of animals consuming hay because of missing cells due to very dilute snow-urine, but a similar trend was evident (Table 2).

As urinary concentrations of metabolites are most informative when assessed temporally (Delgiudice 1995), they should be compared with reference values for meaningful interpretation. We believe the data presented herein represent reference values for urinary U:C and K:C ratios for moose consuming high-quality forage in winter. The mean U:C ratios for these moose were similar to those of supplementally fed, free-ranging white-tailed deer (*Odocoileus virginianus*) in winter (DelGiudice et al. 1989b). This similarity between species is fortuitous and implies that urinary concentrations of metabolites in cervids respond similarly to nutritional restriction.

The time lag observed between intake levels and urinary K:C ratios supports the conclusion of Parker et al. (1993) that urinary metabolite ratios reflect short-term changes in nutritional restriction rather than represent an index to long-term changes in body composition. We conclude that K:C ratios reflect intake of the animal during a period sometime prior to sampling, indicating that substantial changes in the diet of wild moose would not be reflected in urinary K output for approximately 10 days. Our data provide no insight concerning a similar trend in urinary U output, but such information would be vital to correct interpretation of urinary metabolite ratios. If a time lag exists in U:C ratios, then K:C ratios would discriminate between animals excreting endogenous U versus those excreting dietary U. Otherwise, the usefulness of K:C ratios is in doubt. DelGiudice et al. (1990) demonstrated a simultaneous increase in urinary U:C and K:C ratios in white-tailed deer 2 weeks after they were returned to a high protein, high energy diet after being on a restricted diet for 12 weeks, indicating U:C ratios respond at least as quickly to changes in intake as do K:C ratios. As they reported their data in 2-week intervals, however, the specific temporal responses of U:C and K:C ratios within those intervals are unknown. Although our data for the severely undernourished bull might indicate that U:C ratios respond more rapidly than K:C ratios, we hesitate to draw that conclusion because his intake was not measured, and we have no urinary metabolite estimates before his being placed on supplemental feed.

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RECOMMENDATIONS

Although our data indicated no significant difference between U:C ratios of calves and adults fed the same diet, a trend toward higher values in calves was apparent. As our sample size was relatively small (4 calves, 3 cows, 3 observations per animal), we recommend sampling be based on the assumption of age-related differences and that sampling be stratified by age. Significant differences in urinary U:C ratios between calves and adults were reported for nutritionally restricted mule deer (*Odocoileus hemionus*, Saltz et al. 1991b) and elk (*Cervus elaphus*), White et al. 1995b).

Midwinter snow-urine assays would be useful for determining if wild populations would benefit from supplemental feeding, as suggested by Saltz and White (1991*a*). However, by the time U:C ratios are increasing rapidly due to endogenous protein catabolism, it may be too late for supplemental feeding to save individuals from starvation (Saltz and White 1991*b*). Serial sampling of populations in years of different winter severity would yield insight into the chronology of undernutrition. However, the natural variation in certain indices (DelGiudice et al. 1989*b*, White et al. 1995*a*), and the variation associated with age (Saltz and White 1991*a*, White et al. 1995*b*) and sex (White et al. 1995*b*) must be accounted for in the sampling design and data analysis. Also, when using this technique to compare condition of disparate populations, it is imperative to interpret the results based upon a knowledge of local conditions to determine causes of observed differences. Additional research investigating the relationships between urinary metabolites and body composition in moose and other cervids, particularly in extremely undernourished animals, would aid in our understanding and interpretation of these values.

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We plan to continue to evaluate new drugs and related products as they become available. We will continue to investigate various components of moose reproduction as outlined in the new proposal. Handling facility reconstruction and redesign will continue.

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Fig. 1. Comparison of fecal progestagen (P4) concentrations from a female moose determined with a tritium- $({}^{3}H)$ and iodine- $({}^{125}I)$ based monoclonal antisera. Arrows indicate the day the animal came into heat based on observed mounting by a bull.



Figure 2. Relationship between fecal progestagen (P4) concentrations from an adult cow moose determined with a tritium- $({}^{3}H)$ and iodine- $({}^{125}I)$ based monoclonal antisera.



Figure 3. Progestagen concentrations (P4) for a typical estrous cycle in the cow moose "Allye." Her day of estrus was 7 October. The nadir that appears on 26 September is 11 days prior to her overt estrus and probably represents a silent heat.

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Fig. 4. Fecal progestagen (P4) profile for adult cow moose "Sony." Her day of estrus was 9 October. The nadir that appears on 28 September is 11 days prior to her overt estrus and probably represents a silent heat.



Fig. 5. Fecal progestagen (P4) profile for adult cow moose "Lara." Her day of estrus was 7 October. The nadir that appears on 26 September is 11 days prior to her overt estrus and probably represents a silent heat.



Fig. 6. Fecal progestagen (P4) profile for adult cow moose "Luna." Her days of estrus were unknown. Profiles suggest a silent heat on 18 September and first overt estrus around the 29 September - 1 October. Additional nadirs in P4 suggest her second and third estrus occurred on 25 October and 11 November.



Fig. 7. Fecal progestagen (P4) profile for adult cow moose "Deshka." Her day of estrus was unknown. Profiles indicate a silent heat between the 21 and 27 September and first overt estrus between 2 and 6 October. Additional nadirs in P4 indicate her second and third cycle occurred on 27 October and sometime after 13 November.

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Fig. 8. Fecal progestagen (P4) profile for adult cow moose "Satorene." Her day of estrus was unknown. Profiles indicate a silent heat on 28 September and first overt estrus on 7 October. Additional nadirs in P4 indicate her second and third cycle occurred on 28 October and sometime after 13 November.



Fig. 9. Fecal progestagen profile for primiparous cow moose "Sabrina." Her day of estrus was unknown. Unusually high progestagen concentrations in late October and early November make interpretation of her P4 profile difficult. Additional samples are currently being analyzed.

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Fig. 10. Fecal progestagen profile for primiparous cow moose "Sue." Her day of estrus was unknown. Unusually high progestagen concentrations in late October and early November make interpretation of her P4 profile difficult. Additional samples are currently being analyzed.



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Fig. 11. Mean urinary U:C and K:C x 100 ratios (solid lines), and dry matter intake (broken line) for 10-day intervals for moose at the Kenai Moose Research Center. Vertical lines at day 50 and day 90 indicate beginning and end of restricted feeding. Letters in upper left hand corners of graphs indicate animal designations in Table 1, with the exception of moose E, the 10-year-old bull. Error bars represent 1 SE.

Table 1. Mean dry matter intake, percent weight change, and mean (SE) urinary U:C and K:C x 100 ratios prior to Day 50 (Period 1) and thereafter (Period II) for 4 male moose in winter, Kenai Moose Research Center, 1989.

K:C x 100		102.4(17.0)	101.8(13.5)	117.0(11.0)	75.5(8.7)	
		76.8(14.6)	118.4(15.9)	110.4(15.3)	99.5(17.6)	
U:C		5.9(0.7)	5.7(0.2)	6.0(0.3)	5.6(0.2)	
	_	4.6(0.3)	5.0(1.1)	4.7(0.4)	5.8(0.6)	
eight 1 <u>ge</u>		1.	L-	+5	0	
% We		+	+3	9+	-9	
Intake (<u>g/kg BW^{0.75})</u>	II	79.4	77.6	75.9	49.2	
		83.4	98.8	91.3	94.8	
	<u>Age</u>	Calf	Calf	Calf	3+	
	Animal	A	В	C	D	

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Trial	Age	Period 1	Period 2	Period 3
Hay	Adult	2.4(1.6)	2.5(0.8)	2.1(0.2)
	Calf	4.6(0.3)	2.8(1.3)	3.6(0.9)
MRC	Adult	5.7(1.6)	4.7(0.4)	4.6(1.0)
	Calf	7.0(0.5)	5.5(1.2)	6.7(1.5)

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Table 2. Mean (SE) urinary U:C ratios for adult and calf moose fed either hay or a formulated ration (MRC) for 11 weeks in winter, Kenai Moose Research Cneter. Periods 1-3 were 2 weeks, 6 weeks, and 10 weeks into the trial, respectively.

APPENDIX A.

XYLAZINE IMMOBILIZATION OF MOOSE WITH YOHIMBINE OR TOLAZOLINE AS AN ANTAGONIST: A COMPARISON TO CARFENTANIL AND NALTREXONE

Charles C. Schwartz, Thomas R. Stephenson and Kris J. Hundertmark

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ABSTRACT: When moose (Alces alces) are kept in captivity, it is often necessary to immobilize them for research purposes or animal care. Carfentanil, a very potent narcotic, used in combination with xylazine hydrochloride is the preferred drug mixture when immobilizing moose in the wild. However, carfentanil is both expensive and potentially dangerous to the handler. We evaluated the use of xylazine hydrochloride, an analgesic sedative, used alone, or in combination with either carfentanil citrate or ketamine hydrochloride (cyclohexamine) to immobilize moose at the Moose Research Center. Mean induction time for Xylazine alone was not different from xylazine:ketamine and carfentanil:xylazine mixtures. Drugged animals could be approached and handled immediately when given carfentanil:xylazine. Xylazine or xylazine:ketamine drugged animals often laid down 8-12 minutes before completely immobilized. The antagonist yohimbine had no apparent effect on reversal of xylazine immobilized moose, and recovery times averaged 3:38 + 2:01 hours. The antagonist tolazoline hydrochloride reduced recovery times significantly, and animals reversed with this drug were standing within 4 to 31 minutes (x = 21 minutes). Animals immobilized with a mixture of carfentanil:xylazine and reversed with naltrexone were usually standing within 7 minutes with a range from 3 to 21 minutes after administration of the antagonist. Comparisons of individual drugs, mixtures and antagonists are discussed relative to cost, efficiency, effectiveness, safety, and reliability of immobilizing moose.

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APPENDIX B.

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URINE CHEMISTRY PROFILES OF SUPPLEMENTALLY-FED MOOSE Kris J. Hundertmark¹, Glenn D. DelGiudice², and Charles C. Schwartz¹

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ABSTRACT: We describe urine chemistry profiles for 4 healthy male moose (<u>Alces alces</u>) being fed an ad libitum or moderately restricted high protein-high energy diet, and for 1 male being fed the same diet ad libitum but recovering from severe undernutrition. Urinary urea (U) and potassium (K) were expressed as ratios to creatinine (C) to account for differences in urine concentrations and to facilitate analysis of urine deposited in snow. Urea:C ratios did not differ among animals or over time. Mean K:C ratios for 10-day intervals varied over time and were correlated with allometric dry-matter intake for the previous 10-day interval, indicating that urinary K excretion reflects short term variation in intake. These urinary metabolite ratios can be considered as a baseline for moose fed supplementally during winter. Analysis of urine deposited in snow can be a useful indicator of population condition in moose, but a clear understanding of the physiological processes indicated by these profiles and their inherent limitations is essential to proper implementation.

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APPENDIX C.

Duration: From: 1 July 1996 To: 30 June 2001

WILDLIFE RESEARCH STUDY PLAN

Alaska Department of Fish and Game Division of Wildlife Conservation

STUDY TITLE: Evaluation and testing of techniques for moose management: nutritional requirements for reproduction with respect to body condition thresholds.

THE NEED/PROBLEM:

1. Statement

Managers increasingly must manage moose populations more intensively within the state. To understand the factors limiting current moose populations and avoid overexploitation, we require a more detailed understanding of the relationship between moose and their environment. Specifically, we need to understand how nutrition, body condition, and reproduction interact to determine population productivity.

2. Justification

To facilitate intensive management of populations, we must be able to predict survival and reproductive success of individuals within these populations. Although population size is dictated by numerous factors such as weather and predation, ultimately habitat quality defined by the nutritional quality of diets will determine the maximum number of moose that an area can support. Reproductive performance of cow moose is likely related to their body condition. We intend to refine the use of an individual animal's condition as an indicator of the nutritional quality of it's habitat, as well as a predictor of its potential for reproduction and survival. Furthermore, although body condition and reproductive performance are interrelated, gradual changes in body condition may be more detectable and require smaller sample sizes than fecundity and survival data. The Boolean (yes or no) nature of fecundity and survival data typically requires larger sample sizes to detect significant shifts within populations.

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3. Background

Theoretically, as population density increases, a density dependent decline in resource availability will result in a reduction in fecundity and survival. Estimation of carrying capacity, the number of animals that a unit of area can support without habitat degradation, has frequently been pursued but rarely achieved by biologists (Regelin et al. 1987b). Our ability to quantify forage quality and quantity has generally been hampered by the extreme variability inherent in natural systems. Recently, methodology for applying the "animal indicator concept" (Franzmann 1985) was validated. Stephenson (1995) developed equations to predict total body fat in moose from ultrasonographic fat measurements. Hundertmark et al. (1994) also developed equations to predict body composition using bioelectrical impedance analysis. The animal indicator approach assumes that because the animal is a product of its environment, it represents the quality of its environment. Thus, rather than define carrying capacity in numbers of animals, this approach provides a relative indication of the proximity of the population to K. Recently, Grubb (1995) defined nutritional condition as "the state of body components controlled by nutrition and which in turn influence an animal's fitness." Saltz et al. (1995) noted that Grubb's definition clearly identifies the role of nutrition in determining an animal's condition and ultimately its reproductive success.

Because body fat is the primary energy store of the body (Price and White 1985), measurement of lipid reserves has been the focus of much research aimed at estimating nutritional condition (Chan-McLeod et al. 1995, Franzmann and Ballard 1993, Harder and Kirkpatrick 1994, Gerhart 1995). In addition, estimation of muscle mass (i.e., protein) is of interest, particularly with respect to the demands of pregnant and lactating females (Allaye-Chan 1991; Torbit et al. 1985). Torbit et al. (1985) discussed the importance of protein reserves during the latter stage of starvation. Ultrasonography also exhibits potential for estimating protein reserves (lean mass) in ungulates (Johns et al. 1993). Although body mass correlates well with reproductive parameters in specific applications, its application is limited because of the difficulty in accounting for variation related to alimentary fill (Adamczewski et al. 1987) and reproductive status. Albon et al. (1986) demonstrated that body condition, independent of body mass and fat and protein stores, may enable determination of protein reserves.

Assessment of body condition provides insight into the ability of individuals in a population to survive and reproduce. However, in order to evaluate the role of body condition in determining an animal's reproductive fitness, we must be able to assess reproductive performance including ovulation, conception, fetal numbers and survival, and natal survival. Ultrasonography, particularly when used with animals conditioned to permit handling, offers the potential to directly observe ovulation (Pierson and Ginther 1988).

Although summer twinning rates have been used to indicate the quality of moose habitats (Franzmann and Schwartz 1985), undetected predation may lead to biased postpartum estimates (Stephenson et al. 1995). Ultrasonography has been used to successfully determine in utero pregnancy and twinning in moose during both early (Stephenson et al. 1995) and late gestation (J. W. Testa, unpubl. data). Because ultrasonography requires specialized equipment and expertise, a serum assay that diagnoses twinning is of interest. Willard et al. (1995) recently developed a

quantitative pregnancy-specific protein B assay for domestic sheep that permitted detection of fetal twins with up to 82% accuracy.

Varying emphasis has been placed on the role of summer and winter ungulate ranges in providing for the nutritional requirements of populations. Merrill and Boyce (1991) and Mautz (1978) emphasized the importance of high quality summer habitat in allowing animals to compensate for the nutritional stresses of winter and lactation. Regardless of winter severity, winter ranges in different locations offer different forage species of varying nutritional quality and biomass (Schwartz et al. 1988b; Hubbert 1987; Stephenson 1995). Further complicating matters, Clutton-Brock et al. (1983) proposed the existence of carry-over effects on body condition among years. Debate remains regarding the mechanisms of winter's cumulative effects (Messier 1995, McRoberts et al. 1995). Thus, depending upon the level of stress resulting from reproductive demands and winter severity and habitat quality, the requirements of summer habitats will differ.

Although the existence of threshold "set points" of body condition have been hypothesized for ungulates (Schwartz et al. 1988a; Renecker and Samuel 1991; Gerhart 1995), their existence relative to reproduction in moose remains unquantified. Using logistic regression, Albon et al. (1986) determined fertility probabilities in red deer based on body weight, kidney fat, jaw length, reproductive status, and age. The positive relationship between body condition and pregnancy rate in caribou and deer has been well documented (Thomas 1982, Cameron et al. 1993, Cameron and Ver Hoef 1994, Allaye-Chan 1991, Clutton-Brock et al. 1983). In caribou, pregnancy declines with decreasing autumn body mass (Cameron et al. 1993). Furthermore, ovulation rate in reindeer was related to lipid reserves (Leader-Williams and Rosser 1983). In addition, Cameron et al. (1993) predicted perinatal calf survival from the body condition of the female during late gestation. Gerhart (1995) determined that body fat content was the body condition parameter that best explained differences in pregnancy rate in Porcupine Herd caribou. An understanding of thresholds required for ovulation, gestation, and neonatal calf survival will enhance our insight into the importance of different seasonal habitats and the management of these habitats.

Sinclair and Arcese (1995) provided evidence that vulnerability to predation is related to body condition. Furthermore, Mech et al. (1991) suggested that vulnerability of white-tailed deer to wolf predation was related to maternal and grandmaternal nutrition and body mass. Recently, Sams et al. (1996) identified a relationship between serum immune parameters and neonatal mortality of white-tailed deer fawns. Poor maternal nutrition may lead to failure in the passive immunity process between mother and offspring and increase susceptibility to diarrhea, septicemia, and other diseases in neonates. Low neonate serum levels of colostral antibodies may occur from inability to efficiently nurse, poor colostral absorption, or depressed colostrum production (Sams et al. 1996). Indices of fawn viability such as immunocompetency or maternal condition may provide insight relative to the additive or compensatory nature of predation.

Thompson and Peterson (1988) suggested that multifactorial hypotheses should be considered more frequently if we are to enhance our understanding of predator-prey interactions. Captive studies permit us to individually isolate and test components of complicated models. Verme (1963) and Sams et al. (1995) noted the necessity of duplicating the extremes of natural environments when duplicating diets under captive conditions. Although we may expect moose reproductive responses to altered nutrition that are similar to those recorded for deer (Verme 1963, 1965, 1967, 1969; Robinette et al. 1973), our ability to measure lipid reserves allows us to collect new data that may be more readily used to assess natural habitats. In particular, we may now more accurately predict the reproductive success of a population based on the condition of a representative sample of individuals from that population. Ultimately, we wish to isolate the importance of habitat quality, predation, weather, and other factors that affect population dynamics.

To validate the animal condition approach, we must conduct experiments with animals foraging on natural browse in addition to animals on trials using pelleted rations. Although Schwartz et al. (1988a) documented that moose fed a poorer quality ration compensated by increasing intake, this may not accurately represent conditions of wild moose as density increases, forage availability declines as a result of snow, and the very poor nutritional quality of some natural winter forages. With increasing snow depth, the cost of foraging increases in addition to a decline in forage availability. In addition, changes in moose diets relative to vegetation succession must be manifested in terms of nutritional quality and ultimately body condition and reproductive performance. As plant communities mature, the biomass of important moose forages typically declines (Oldemeyer and Regelin 1987, Regelin et al. 1987a, Wolff and Zasada 1979).

As a population approaches carrying capacity, increased competition for forage resources should reduce average body condition. Hobbs and Swift (1985) hypothesized that as population density increases, the upper limit on nutritional quality of diets obtainable will decline progressively. A deterioration in the nutritional status of individuals would be expected as population density increases and the condition of individuals could be monitored to assess diet quality. Determining the ability of moose to compensate as density changes will enable us to understand the limitations of using the animal condition approach to assess habitat quality and the mechanisms of density dependence. Perhaps the concern of Saether et al. (1996) that densityindependent variation in demographic variables because of a stochastic environment will overshadow density-dependent response in fecundity and reproduction will encourage application of the animal condition approach. If thresholds for reproduction and survival exist, variation above the thresholds could only be monitored by examining body condition. The ability to precisely monitor condition within a population over an extended time scale may offer the greatest potential to refine monitoring habitat quality.

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22 **MIN**C

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OBJECTIVES

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- 1. Determine overwinter nutritional requirements for reproductive success in female moose.
- H_a: There is a difference in neonatal and in utero calf survival and indices of calf condition with respect to the dam's nutrition during pregnancy.
- 2. Determine thresholds in body condition at which reproductive performance declines.
- H_a: There is a difference in body condition among female moose relative to reproductive performance (ovulation, pregnancy and twinning, and perinatal and neonatal calf health).

3. Evaluate the existence of yearly carry-over effects in female moose relative to body condition, reproductive performance, and nutrition.

- Ha: Given suboptimal nutrition, reproductive performance of female moose is related to body condition.
- 4. Refine estimation of moose body composition.
- Ha: There is a relationship between ultrasonic fat and muscle measurements and total body fat and muscle mass.

5. Develop and refine methodology for diagnosing twinning in moose using ultrasonography and a quantitative serum assay.

Ha: There is a relationship between serum PSPB concentrations in moose and the number of fetuses in utero.

6. Evaluate body condition and reproductive success of habituated moose relative to dietary intake of natural browse.

Ha: There is a decline in moose body condition and reproductive performance as moose per unit area increases.

EXPECTED RESULTS AND BENEFITS

The results of this research will refine our ability to use body condition and reproductive performance of an animal as an indicator of habitat quality. In addition, we may be able to use body condition of adult moose as an indicator of adult and calf vulnerability to predation. Assessing the additive or compensatory nature of predation will aid in determining appropriate management alternatives.

STUDY APPROACH

Integration of captive experiments, field studies, and modeling will most fully elucidate the relationships between nutrition, reproduction, predation, and weather. Captive experiments will refine the relationship between nutrition, body condition, and reproduction. Subsequently, field studies will further incorporate predation and weather into complex equations.

<u>Job 1.</u> Conduct feeding trials to evaluate the relationship between moose nutrition, body condition, and reproductive performance.

Captive moose at the Kenai Moose Research Center will be used in this project. Ten adult female moose will be randomly assigned to 1 of 2 treatment groups (5 per group). Individuals will receive the same diet treatment during 2-3 years to assess diet x year interactions and carry-over effects. After the initial 2-3 year period, moose will be switched to the alternate treatment for an additional 2-3 years utilizing a crossover design. Treatment groups will consist of a high quality pelleted moose feed (Schwartz et al. 1985) and a poorer quality submaintenance ration (Schwartz et al. 1988a). Both rations will be offered in predetermined quantities calculated to approach above maintenance and submaintenance levels. In particular, feed will be offered to encourage reasonable gut fill on both diets; digestibility will be reduced for the submaintenance ration to achieve this. Animals will be confined together and will access feed using individual-specific feed gates (Calan) operated by electronic keys attached to neck collars. Known amounts of feed will be offered and orts collected daily to permit calculation of daily energy and protein intake for each animal. Trials will be conducted during 1 December - 30 April. During the remainder of the year animals will be maintained on natural browse and have access to the high quality pelleted ration as well. Moose will be immobilized during September (end of lactation and shift from green browse to greater dependence on pelleted feed), late November (beginning of feeding trial), March (typical capture period for field projects), and April (end of feeding trial). The rump region will be scanned to measure lipid reserves using a portable real-time ultrasound device. Subcutaneous fat thickness will be used to predict total body fat (Stephenson 1995). In addition, moose will be weighed in September and weekly during feeding trials. Serum will be collected during all immobilizations for determination of PSPB and serum urea nitrogen levels. Transrectal ultrasonography will be used to detect the presence and number of fetuses.

Repeated measures analysis-of-variance will be used to test for differences in body condition relative to diet quality. Logistic regression will be used to evaluate the relationship between body condition and pregnancy and twinning probabilities.

These data will be compared with data from wild populations that will be collected opportunistically in cooperation with other investigators.

Job 2. Evaluate relationship between calf health and the dam's nutrition and body condition.

Newborn calves, born to cows in feeding trials, will be located by ground surveillance of cows, then capture by hand. Calves will be handled after >12 have elapsed since birth to avoid abandonment by the dam. Captured calves will be equipped with expandable break-away radio collars and numbered eartags. Sex, body mass, total body length, and hind foot length will be recorded at capture. Serum will be collected and evaluated for determination of immunocompetence (Sams et al. 1996), specifically concentrations of gamma-globulins (colostral antibodies) and gamma-glutamyl transferase (GGTP; used to indirectly measure the efficiency of colostral absorption). Logistic regression will be used to determine significant correlates of neonatal mortality.

Job 3. Validate approaches for determining body fat and body protein in live moose.

Captive moose on various nutritional planes and during different seasons will be further evaluated for body composition. To estimate fat reserves, the rump region of immobilized moose will be scanned using an Aloka model 210 portable ultrasound device (Corometrics Medical Systems, Inc., Wallingford, Conn.) with a 5 MHz 8 cm linear-array transducer (Stephenson 1995). Ultrasonic fat thickness will be measured at 2 sites along a line between the spine, at its closest point to the tuber coxae (hip bone), and the tuber ischii (pin bone). Subcutaneous fat thickness will be measured with electronic calipers to the nearest 0.1 mm at the midpoint and point of maximum thickness detected along the line (MAXFAT), and 2) the sum of the maximum thickness at the midpoint (SUMFAT). To estimate protein reserves, ultrasonic muscle thickness of the biceps femoris and gluteus medius will be recorded directly under the hip and pin bones, respectively. In addition, longissimus dorsi muscle thickness will be measured at 1993).

Further evaluation of bioelectrical impedance analysis to determine body composition (particularly protein reserves) will be conducted in conjunction with ultrasonography. Electrodes

from a plethysmograph (Model BIA-101, RJL Systems, Inc. Detroit, MI) will be placed in the hindleg and foreleg of sternally recumbent moose. Resistance and conductance will be recorded.

Animals will be euthanized immediately following ultrasonic and BIA measurements while still chemically immobilized. Whole body mass will be determined and then each animal will be eviscerated and skinned (subcutaneous fat will remain on the carcass). The carcass will be bisected longitudinally along the vertebral column, with one half frozen for chemical analysis. The gastrointestinal tract will be emptied of ingesta (Hundertmark et al. 1994). The fetus(es) and amniotic fluid of pregnant females will be removed and their mass determined to permit fetus-free calculations. Kidney fat mass will be recorded as the mass, to the nearest 1 g, of trimmed fat attached to the kidney. Marrow samples will be collected and frozen for determination of percent marrow fat. The entire viscera and samples of shaved hide will be frozen for analysis. The frozen carcass half and visceral mass will be sliced at 51 and 25 mm intervals, respectively, on a commercial band saw. The homogenate at the base of the blade will be collected for each component, mixed and refrozen. Hide samples will be freeze-dried and ground in a Wiley mill to create a homogenate. Chemical analysis of frozen samples will be conducted at Washington State University's Wildlife Habitat Laboratory. Crude fat will be determined by ether extraction (AOAC 1975). Samples will be analyzed in triplicate.

Regression analysis will be used to develop additional predictive equations for body composition. Furthermore, additional samples will be used to validate existing predictive equations.

Job 4. Develop serum assay to detect twinning.

This will be a cooperative project with the University of Idaho, Department of Animal and Veterinary Sciences. Placentas will be collected from road-killed pregnant female moose during winter (middle to late gestation) and stored frozen. Pregnancy-specific protein B (PSPB) will be isolated from moose cotyledonary tissue by radioimmunoassay at the University of Idaho and used to develop a standard curve that will permit quantitative assessment of PSPB in moose sera (Willard et al. 1995). Sera collection and ultrasonography from cows immobilized as part of Job 1 and routine immobilizations will be used to evaluate the accuracy of the PSPB test. Repeated collections of sera from individual moose will enable evaluation of the pattern of increased PSPB concentrations with increasing placental mass as gestation proceeds. Calving observations will provide further evaluation of the tests accuracy. The assay will be evaluated for accuracy in diagnosing twin and singleton litters, as well as predicting breeding date.

Job 5. Monitor body condition and reproductive performance of cow moose stocked at different densities feeding on natural browse.

Beginning in October 1998, adult female moose will be stocked initially at optimal densities (minimal competition for food) in Pens 3 and 4 (each 1 mi²) at the Kenai Moose Research Center. Pen 3 contains vegetation that was partially crushed during 1987 and now represents early successional birch/aspen communities, in addition to uncrushed mature stands. Pen 4 contains mature spruce/birch/aspen vegetation of a later successional stage. Densities will be maintained for 2 consecutive years to isolate density x year interactions. Subsequently,

densities will be increased every 2 years to increase competition among moose for forage. Although Pens 3 and 4 were stocked with extremely high densities $(10 - 44 / mi^2)$ of moose during the 1970's (Franzmann et al. 1977), moose have been excluded from these pens during the past 5–10 years.

Upon completion of the rut in October in Pen 2A, bred cows will be moved to Pens 3 and 4. Cows will subsist entirely on natural browse during mid-October through late April. In late April, cows will be returned to Pen 2A for calving season. Calves will be processed as in Job 2. Cows and their calves will spend the summer in Pen 2 on natural browse and will be returned to Pen 2A in September for the rut. Following the rut, cows will be separated from their calves and returned to Pens 3 and 4 for another winter trial.

Moose will be immobilized during September, December, and April. The rump region will be scanned to measure lipid reserves using a portable real-time ultrasound device. In addition, moose will be weighed. Serum will be collected for determination of PSPB and serum urea nitrogen. Transrectal ultrasonography will be used to detect the presence and number of fetuses.

To determine diet quality and browse intake, we will record foraging behavior of habituated moose. Moose will be located at random intervals and observed for 2-6 hour periods. Species composition, as well as species-specific bite sizes, and bite and intake rates will be recorded (Stephenson 1995). Diameter-at-point-of-browsing (DPB) regressions will be used to convert recorded DPB's to bite sizes. In addition, forage samples will be collected periodically to determine digestible energy and protein of forages. Calculation of protein and energy intake will permit modelling energy balance of each animal.

Job 6. Preparation of reports and publications.

PERSONNEL

Thomas R. Stephenson, PCN 11-2197; 6 months PFT

Kris J. Hundertmark, PCN 11-2104; 2 months PFT

Charles C. Schwartz, PCN 11-2030; 1 month PFT

Sandra L. Kennedy, PCN 11-2160; 6 months P/S

COOPERATORS

Related research, currently being conducted by the Alaska Department of Fish and Game, includes moose projects in Unit 20A (Rodney Boertje, principal investigator), Unit 13A (Ward Testa, principal investigator), and Unit 26A (Geoff Carroll, principal investigator). In addition, moose research is being conducted in Denali National Park (Victor Van Ballenberghe, principal investigator).

SCHEDULE

		EST. (OPERA'	TING C	OSTS x	1000	
JOB NO.	ACTIVITY	FY97	FY98	FY99	FY00	FY 01	
1	Feeding trials and moose handling ^a	22.5	22.5	17.5	22.5	22.5	1
2	Capture neonatal calves ^b	0.5	0.5	0.5	0.5	0.5	
3	Body composition analysis ^C	1	1	1	1	1	
4	Develop twinning assay ^d	5	5	5	-	-	
5	Diet analysis and moose handling ^e	-	-	5	5	5	

^aFY97=purchase and construct Calan feeding gates, purchase and install continuous power supply for Calan gates, immobilize moose to measure fat reserves and determine reproductive condition, buy moose feed, and conduct nutrient analysis of feed.

FY98=purchase an ultrasound unit by paying annual installments, purchase and construct a squeeze chute, immobilize moose to measure fat reserves and determine reproductive condition, buy moose feed, and conduct nutrient analysis of feed.

FY99-01=purchase an ultrasound unit by paying annual installments, immobilize moose to measure fat reserves and determine reproductive condition, buy moose feed, and conduct nutrient analysis of feed.

^bhematological analysis of calf blood

^cconduct proximate analysis

^dlab analysis at University of Idaho

^echemical analysis of forage quality and immobilize moose to measure fat reserves and determine reproductive condition

GEOGRAPHIC LOCATION

Kenai Moose Research Center

REPORTING SCHEDULE

Annual progress report will be in headquarters by <u>1 October</u> each year of the study.

Final report will be in headquarters by <u>1 October 2001</u>.

APPENDIX D.

N L N

CARBOHYDRASE ACTIVITY IN THE PANCREAS AND SMALL INTESTINE OF MOOSE AND CATTLE

Charles C. Schwartz¹, David L. Harmon², Kris J. Hundertmark¹, Charles T. Robbins³, and Barbara A. Lintzeñich²

¹Alaska Department of Fish and Game, Moose Research Center, 34828 Kalifornsky Beach Road, Soldotna, AK 99669; ²Department of Animal Science 805 W.P. Garrigus Building, University of Kentucky, Lexington, KY 40546-0215; ³Natural Resource Sciences, Washington State University, Pullman, WA 99164-6410.

<u>ABSTRACT</u>: Moose (*Alces alces*) are difficult to keep in captivity and often die of apparent digestive problems. It has been hypothesized that some of the problem may stem from an inability to produce adequate quantities of the enzymes necessary to digest the starch contained in cereal grains formulated into synthetic diets. We tested this hypothesis by quantifying concentrations of the important enzymes found in the pancreas and small intestine of 2 moose. We compared these values to a grain fed steer. Pancreatic a-amylase concentrations were higher in moose (4,228 µmol•g tissue⁻¹•min⁻¹) than the steer (1,104 µmol•g tissue⁻¹•min⁻¹), and intestinal maltase concentrations were similar between moose (0.33 µmol•g tissue⁻¹•min⁻¹) and the steer (0.47 µmol•g tissue⁻¹•min⁻¹). Additionally, moose produced concentrations of isomaltase (0.6 µmol•g tissue⁻¹•min⁻¹) at a rate similar to values published for cattle (0.4-0.6 µmol•g tissue⁻¹•min⁻¹). Only lactase values were lower in moose than cattle. Although our sample size was small, these data suggest that moose are quite capable of producing the enzymes necessary for the breakdown of both starch and disaccharides.

ALCES VOL. 33(1996) pp. 000-000

EVALUATION AND TESTING OF TECHNIQUES FOR UNGULATE MANAGEMENT

By

Thomas R. Stephenson Kris J. Hundertmark Charles C. Schwartz Sandra L. Kennedy

Research Progress Report Federal Aid in Restoration Grant W-24-4 Study 1.45

December 1997

RESEARCH PROGRESS REPORT

STATE:	Alaska	Study No.: 1.45
COOPERATORS:	Kenai National Wildlife Refuge, Soldotna, Alaska	
GRANT NO:	W-24-4	
STUDY TITLE:	Evaluation and Testing of Techniques for Ungulate	Management
Period:	1 July 1995–30 June 1996	

SUMMARY

We continued to collect baseline information on parameters of calving in nutritionally unrestricted caribou (*Rangifer tarandus*) for later comparison with nutritionally stressed animals. We also improved facilities and developed new methods for collecting such information. By allowing bulls access to cows only during daylight hours of the rut, we were able to observe all 6 planned breedings. Four calves were born and processed (weighed, measured, sexed, ear tagged, and blood sampled) without loss. During 1994–1996, male calf weights were related to postpartum dam weights. Using a digital electronic scale, we weighed all caribou biweekly, with minimal stress. Seven adult females were immobilized during February–March; 4 of 7 were pregnant, based on serum assay. Mean ultrasonic rump fat thickness was 1.08 cm and 1.03 cm for pregnant and nonpregnant caribou, respectively. Mean gestation length was $222 (\pm 3.9 \text{ SD})$ days. We successfully trained 6 adult female caribou to use a restrictive feed gate system. No caribou mortalities or transfers occurred this year. The MRC caribou herd now consists of 19 animals.

Key words: Body weight, caribou, gestation, nutrition, Rangifer tarandus, reproduction

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BACKGROUND

Recent data from the Southern Alaska Peninsula caribou herd (SAP) indicate a reduced population, small adult body size, low birth weights, late calving dates, and low calf survival. Undernutrition is the suspected agent affecting the population dynamics of that herd (Pitcher et al. 1991). It is unclear what the appropriate management strategy for the herd should be because of our uncertainty as to the role of density-dependent food limitation in the decline.

Body condition of adult female caribou and reindeer affects reproductive performance and calf survival. Lenvik (1988) found that conception date in reindeer was related to weight (and possibly energy reserves) of females during the breeding season. Pregnancy rate was closely associated with fat reserves and body weights of Peary caribou in Arctic Canada (Thomas 1982). Calves of undernourished female reindeer had reduced birth weights and reduced survival (Espmark 1980, Skogland 1984). Gerhart (1995) determined that body fat content was the body condition parameter that best explained differences in pregnancy rate in Porcupine Herd caribou.

Undernutrition of Rangifer females during gestation and possibly before breeding resulted in late calving (Espmark 1980, Reimers et al. 1983, Skogland 1984). Late calving reduces the summer growth season during the first year (Klein et al. 1987) and likely reduces survival of calves into the following winter (Haukioja and Salovaara 1978). For caribou, there are strong indications that nutrition, growth, condition, productivity, and survival are linked; however, our knowledge of these relationships is incomplete and additional information is needed to guide management.

OBJECTIVES

To determine the effects of nutrition on breeding chronology, calving chronology, birth size, neonatal survival, and maternal body condition.

METHODS

During May/June 1995–July 1996, captive caribou at the MRC were provided an ad libitum pelleted reindeer ration (13% crude protein, 15% crude fiber). Before this, animals were fed a 1:1 ratio of pelleted moose ration (10% crude protein, 5% crude fiber; Schwartz et al. 1985) and a different pelleted reindeer ration (16% crude protein), periodically supplemented with alfalfa hay. Caribou were confined to a 4 ha enclosure with access to additional grasses (*Calamagrostis* spp.) and forbs.

We weighed caribou older than neonates biweekly, using a 12-volt electronic platform scale (Tru-Test Limited Model 700, Auckland, New Zealand). During 24 February–19 March 1996, 7 adult females and 1 male calf were immobilized with either xylazine hydrochloride or a carfentanil citrate/ xylazine hydrochloride mixture and reversed with Yohimbine or Naltrexone, respectively. Tolazoline was tested for reversal of 1 male calf immobilized with xylazine hydrochloride. Immobilized adults were maintained in sternal recumbency, and we measured total length, chest girth, and mandible length. We attached individual-specific "keys" for the self-feeding gates, using standard domestic dog collars. Serum was collected by jugular venipunture and frozen (-20 C) for eventual pregnancy-specific protein B assay (Stephenson et al. 1995) to diagnose pregnancy. Portable real-time ultrasound was evaluated for measuring lipid reserves (Stephenson 1995). The rump region was scanned using an Aloka model 210 portable ultrasound device (Corometrics Medical Systems, Inc., Wallingford, Conn.) with a 5-Mhz 8 cm linear-array transducer. Ultrasonic fat thickness was measured along a line between the spine, at its closest point to the tuber coxae (hip bone) and the tuber ischii (pin bone). Subcutaneous fat thickness, to the nearest 0.1 mm, was measured with electronic calipers at the point of maximum thickness along the line.

We weighed newborn calves, using a spring scale (Salter No.235, London, England), ear tagged, and determined sex, total length, jaw length, hind foot length 1 (metatarsus), and hind foot length 2 (heel to toe). In addition, blood (5 cc) was collected by cephalic venipuncture using a syringe. We processed calves within 12-24 hours after birth.

During the 1995 breeding season, we confined 7 adult females in a 50×50 m enclosure. To insure known breeding dates, we observed a 3-year-old male that was allowed access to the females during a 1-hour period in the morning and evening.

We tested a controlled-access feeding system (American Calan, Inc., Northwood, NH) for suitability with caribou. The system utilizes a feed container, accessible only through a neck slot controlled by a 24-volt electronically locking gate that is unlocked by an individual-specific sensing "key" collar worn by the animal (Mazaika et al. 1988). Animals were trained to use the gate feeders in pairs by initially unlocking the 2 gates specific to their keys and propping the gates open for 1 day. The following day gates were closed but remained unlocked. During subsequent days, gates were locked for both individuals, and they were required to use their "keys" to access feed.

In preparation for feeding trials this winter, we are remodeling our handling facility to better accommodate caribou. In particular, we are constructing a more efficient weighing and immobilization facility. In conjunction with Calan feed gates, an additional enclosure is being constructed for confinement of animals in feeding trials.

RESULTS AND DISCUSSION

We observed copulations for 6 confined adult females during the presumptive first estrus. The seventh adult cow, B.R., was not confined with the bull due to her history of poor fertility and no apparent indication of estrus. One yearling female was not observed during the rut and did not produce a calf. Only 4 of the 7 adult cows were determined to be pregnant by pregnancy-specific protein B assay in February/March (Table 1). The 4 pregnant females calved successfully in May (Table 2). Two of the 3 females not pregnant are older animals (≥ 10 years) but apparently ovulated since they permitted copulation during the rut. We may only speculate as to whether they aborted or failed to conceive before the February/March assay. B.Y., the third nonpregnant

adult cow, delivered late calves during 2 of the last 4 years and may have chronic low fertility. All 7 of the adult cows had calves at heel and were assumed to be lactating just before the rut. Although sample sizes were too small to permit statistical analysis, rump fat thickness seemed similar between pregnant (mean = 1.08) and nonpregnant (mean = 1.03) cows. Gerhart (1995) suggested that lactational infertility may occur regardless of maternal fat reserves, particularly for cows that lactate well into autumn. B.R. undoubtedly lactated late into autumn since her calf was born on 4 July 1995. Cameron (1994) suggested that periodic reproductive pauses were a response by caribou to nutritional stress. Two additional cows (Snow and Violet) with rump fat thickness <1 cm were 2-year-olds and perhaps less likely to exhibit a reproductive pause (Cameron 1994); however, both animals exhibited relatively late breeding dates.

Upon initial arrival of caribou at the MRC, we began feeding an experimental pelleted 16% crude protein reindeer ration developed by the University of Alaska, Fairbanks. However, because our animals developed scours, we blended this ration 1:1 with a 10% crude protein pelleted moose ration. We fed this diet during 1992–1995. During May–June 1995, we began using a 13% crude protein pelleted reindeer ration. However, although MRC captive caribou were intended to be on a high nutritional plane, the poor reproductive performance observed this year indicates we still may inadvertently be maintaining our caribou on a less-than-optimal nutritional plane. Diet analysis of free-ranging caribou in Interior Alaska indicates a summer diet high in crude protein and digestible energy (Boertje 1985, 1990).

Two males and 2 females were born during 1996. Mean gestation length in 1996 was 219 days (range 215-223). Mean gestation length for all years (n = 15) was 222.5 (\pm 3.9 SD) days (range 215-231); the median and mode were 224 days (Fig. 1).

During 1994–1996, calf weights on or about 1 September were related to birth weights (P = 0.00007) indicating that accelerated growth (Schwartz et al. 1994) does not occur by that date (Fig. 2). Mean weight gain between birth and approximately 1 September was 45.5 kg (range 30.6–54.0). Furthermore, among male calves, birth weights were related to postpartum (2–4 weeks) dam weights (Fig. 3; P = 0.006). However, among female calves, birth weights were not related to postpartum dam weights (Fig. 4; P = 0.24), although sample size was small (n = 6). Possible controls for this relationship include genetics, nutrition, and age and will be evaluated further.

Five of the 6 cows rapidly determined how to access the locked Calan gates by correctly positioning their heads over the gate (to correctly position their "key") and opening the unlocked gate with their nose or neck. The sixth cow (B.R.) only occasionally managed to open her gate, and we were temporarily forced to discontinue training due to the upcoming calving season. Additional work with her in the fall should insure successful access to her feed gate.

RECOMMENDATIONS

For diets simulating an optimal nutritional plane, we plan to switch to a higher quality 16% crude protein textured ration (W. Hauer, UAF LARS), particularly during summer months. Chemical analysis of future diets will be conducted to determine actual levels of digestible energy and protein provided by diets for comparison to nutritional requirements for maintenance and reproduction. Feeding trials this winter will focus on determining the nutritional quality of optimal nutritional plane diets and ad libitum intake rates. Before placing animals on restricted rations, we need to bring all trial animals to equivalent levels of body condition and reproductive status, particularly if we are to evaluate the existence of cumulative effects.

We also plan to collect first incisor teeth for aging from all wild-caught caribou of unknown ages. This may prove useful in evaluating past reproductive pauses.

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ARLIS







ARLIS.







Animal	Approx A	ge Sex	Recent	Total Length	Chest Girth	Mandible	Rump Fat	Pregnant ²
	(yrs)		weigni (kg)	(cm)	(cm)	(cm)	t nickness (cm) ¹	
Blue	<u>≥</u> 10	F	188	208	124	30	1.6	Yes
BR	<u>></u> 10	ĹĿ,	115	202	122	30	1.5	No
ВΥ	>11	Ľ	103	195	124	29	0.8	No
Orange	Ŝ	Ц	114	206	118	30	1.0	Yes
Snow	ę	Ц	104	197	116	29.5	0.8	Yes
Violet	ŧ	ц	107	193	116	30	0.9	Yes
White	≥10	Ц	111	ł	8	ı	0.8	No
V95	calf	M	65	178	. 86	26	0.5	NA

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STAR C

²Pregnancy status determined by radio-immuno assay for Pregnancy-specific Protein B.

	- - - -			-						0	•
		D	ate								
Animal	Sex	Conception	l Birth	Gestation length (days)	Birth weight (kg)	Total length (cm)	Mandible length (cm)	Metatarsus length ¹ (cm)	Hind foot length ² (cm)	Dam	Sire
BB96	M	11 Oct 95	21May 96	223	9.1	89.0	14.0	27.0	36.0	Blue	Hebou
96V	X	19 Oct 95	21May 96	215	7.2	86.0	13.5	25.0	33.5	Violet	Hebou
9600	Ц	14 Oct 95	22May 96	221	7.2	89.0	13.5	24.0	36.0	Orange	Hebou
S 96	Ĺ	25 Oct 95	29May 96	217	5.9	83.0	13.0	24.0	32.0	Snow	Hebou
¹ Length c	of the hind fo	ot minus the	hoof.							NAME OF A DESCRIPTION OF A	

Table 2. Descriptive data for caribou calves captured within 24 hours of birth at the Moose Research Center during 21-29 May 1996.

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²Length of the hind foot to tip of hoof.

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INFLUENCE OF SELECTIVE HARVEST SYSTEMS ON POPULATION GENETICS OF ALASKAN MOOSE

By

Kris J. Hundertmark Charles C. Schwartz Thomas R. Stephenson Sandra L. Kennedy

Research Progress Report Federal Aid in Wildlife Restoration Project W-24-4 Study 1.48

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RESEARCH PROGRESS REPORT

STATE: Alaska

STUDY. No.: 1.48

COOPERATORS: Kenai National Wildlife Refuge, Soldotna, Alaska; Boone and Crockett Club; Dr. Gerald Shields, University of Alaska Fairbanks; Dr. Michael H. Smith, Savannah River Ecology Lab, University of Georgia, Aiken, SC; Doug Larson, Charlie Land, Matt Robus, Roy Nowlin, Ted Spraker, Herman Griese, Bob Tobey, Tim Osborne, Robin Eagan, Alaska Department of Fish and Game

GRANT NO.: W-24-4

A R C M

STUDY TITLE: Influence of Selective Harvest Systems on Population Genetics of Alaskan Moose

PERIOD: 1 July 1995-30 June 1996

SUMMARY

Tissue samples from 457 moose were analyzed for allozyme variation using horizontal starch gel electrophoresis. All data are not yet entered into a computer database, but preliminary analysis of data from 3 populations (Matanuska-Susitna [Mat-Su] Valleys, Alaska, Southeast Alaska, and Colorado) indicate that genetic differences exist among populations. Four polymorphic loci (Mdh-1, Mpi, Pep-2, and Ck-1) of 19 examined to date were identified for the Alaska populations, whereas 2 loci (Mdh-1 and Mpi) were polymorphic in Colorado moose. Allele frequencies differed significantly among the populations for Mdh-1 ($X^2 = 45.0$, P < 0.00001), Mpi ($X^2 = 12.9$, P = 0.002), and Ck-1 ($X^2 = 6.5$, P = 0.038). Direct count estimates of heterozygosity of polymorphic loci varied from 9.1-40.0% for Mat-Su moose, 0.0-40.0% for Southeast Alaska moose, and 23.1-57.7% for Colorado moose. These preliminary data support the conclusions that genetic differentiation of moose populations can be measured with allozymes and that moose exhibit levels of genetic diversity similar to those of other mammals and are not monomorphic.

Key words: Alces alces, antlers, genetics, genetic diversity, mitochondrial DNA, moose, selective harvest system.

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BACKGROUND

In 1987, the Alaska Board of Game approved a selective harvest system (SHS) for bull moose (*Alces alces*) on the Kenai Peninsula. This system limited bull harvest to those with either a spike or forked antler, or animals with at least a 50-inch (127 cm) antler spread or at least 3 brow tines on one antler. One of the many reasons cited for instituting this system was that focusing harvest on spike/fork yearlings would serve to eliminate "inferior" bulls from the gene pool. This statement was predicated on the assumptions that antler characteristics are inherited, that age-specific variation in antler size is related to genetics, and that antler characteristics are indicative of overall individual fitness.

The SHS implemented on the Kenai Peninsula has proven to be an effective method for managing moose harvest (Schwartz et al. 1992). Consequently, the Alaska Board of Game has adopted this system to many Game Management Units (GMUs) connected by the state road system between Anchorage and Glenallen, as well as most areas of Southeast Alaska. Implementation of this SHS will affect a large proportion of the state's moose populations. In light of this proposal, we need to gain a better understanding of the genetic aspects of harvest systems based upon antler configuration. Specifically, the assumptions driving this system, as well as the changes in genetic structure brought about by this system, need to be quantified before we can understand the effect of SHS on moose genetics.

As public demand for consumptive and nonconsumptive use of moose increases, it is contingent upon the state to manage populations more intensively, which in turn requires a more complete knowledge of population processes. In attempting to understand temporal and spatial differences in the attributes of moose at the population (e.g., natality, mortality) and individual animal (e.g., antler size, body condition) levels, biologists focus primarily on nutrition, predation, and harvest

rates. The possibility that genetic factors are responsible for many intra- and interpopulation differences in these parameters is distinct; however, information concerning population genetics of moose is scarce. In order to manage moose populations more effectively, we must understand the degree to which genetics contributes to antler development and the extent antler development reflects fitness. Additionally, the potential effects which antler-based management strategies may have on genetics must be described.

The genetic component of phenotypic expression, although universally recognized by biologists, has not been considered in a management context perhaps due to the lack of simple techniques for data collection and analysis or the perception that cause-effect relationships could not be ascertained. However, during the last 2 decades techniques have been developed to assess population genetics in wild animals (Hedrick and Miller 1992) and subsequent investigations have demonstrated that information gained from such analysis can be useful to managers (Dratch and Pemberton 1992).

The initial efforts to describe genetic variation in wild populations focused on electrophoretic variation of loci coding for enzymes. These studies focused on the relationships between overall genetic variability (most often expressed as heterozygosity) and physiological or morphological characteristics of individuals or populations. Mitton and Grant (1984:489-90) summarized the prevailing theories explaining these relationships as: "... a) the enzymes mark blocks of chromosomes and are fortuitously linked to genes directly affecting growth and development; b) protein polymorphisms constitute a sample of genes whose heterozygosity reflects a continuum between highly inbred (low heterozygosity) and randomly outbred (high heterozygosity) individuals; and c) the genotypes of enzyme polymorphisms typically exhibit different kinetic characteristics; these differences affect the flow of energy through metabolic pathways and thereby influence growth, development, and oxygen consumption." In essence, this means that (a) the dynamics of enzyme polymorphisms mirror those of closely linked loci and therefore act as markers, (b) the genotypes observed in a population are indicative of the breeding history of that population, and (c) individuals exhibiting heterozygosity are thought to be able to take advantage of multiple metabolic pathways for energy processing, making them better able to adapt to a variable environment.

The most widely studied game species in this context is the white-tailed deer (*Odocoileus virginianus*), which exhibits a great amount of genetic variability (Smith et al. 1984). Studies at the Savannah River Ecology Lab have demonstrated relationships between heterozygosity and body condition of overwintering females (Cothran et al. 1983), conception timing (Chesser and Smith 1987), male body size and antler characteristics (Scribner and Smith 1991), number of fetuses (Johns et al. 1977), and rate of fetal development (Cothran et al. 1983).

Although genetic diversity is thought to be maintained in natural populations by means of stabilizing selection (Pemberton et al. 1991), populations subject to hunting can exhibit unexpected trends in genetic composition due to different mortality rates. Improperly designed hunting seasons can cause dramatic changes in the genetics of populations without causing a decline in population size. Thelen (1991) demonstrated that certain SHS for elk (*Cervus elaphus*) based on antler characteristics actually resulted in a decrease in desirable genetic traits, while others had the opposite effect. Ryman et al. (1981) demonstrated that certain harvest regimes for

moose can cause rapid declines in effective population size (N_e) , an index of the rate of genetic drift (random loss of genetic material), and that populations in which only males are harvested are more susceptible to these changes because they have an inherently lower N_e because of their characteristic skewed sex ratios. Scribner et al. (1985) demonstrated that 2 different hunting methods (still vs. dog hunting) had different effects on genetic diversity of white-tailed deer populations without changing population composition. Hartl et al. (1991) detected differences in allele frequencies in populations of red deer (*Cervus elaphus*) that differed in the amount of hunting pressure on spike-antlered yearlings. Therefore, the type of SHS imposed on a population consequently influence population processes of interest to managers.

Electrophoretic variation has also been used to determine population subdivisions, or breeding units. Species in which population subdivision has been detected include white-tailed deer (Manlove et al. 1976), elk (Dratch and Gyllensten 1985), caribou (*Rangifer tarandus*, RØed and Whitten 1986), mule deer (*Odocoileus hemionus*, Scribner et al. 1991), and moose (Ryman et al. 1980, Chesser et al. 1982). Describing this variation is useful in quantifying such concepts as dispersal and population identity as well as understanding interpopulation differences in population parameters. As populations should be managed at the level of the breeding unit (Smith et al. 1976, Ryman et al. 1981), this information can be of extreme importance to management agencies.

Recently, genetic analyses have identified relationships between alleles at specific loci and selective pressures. Pemberton et al. (1988, 1991) detected a relationship between gene frequencies at a particular locus and juvenile survival and adult fecundity in red deer. Hartl et al. (1991) demonstrated that selective harvesting of spike-antlered red deer caused a decline in frequency over time of a specific allele. This latter study is supported by Templeton et al. (1983), who demonstrated that the number of antler points in white-tailed deer is probably controlled by a single gene. In a subsequent study, Hartl et al. (*in press*) concluded that red deer that were homozygous for a particular allele at the Idh-2 locus developed significantly more antler points than did individuals with alternative genotypes. Animals homozygous for a particular allele at the Acp-2 locus exhibited larger antlers overall, compared with animals with other genotypes.

The degree to which genetics contributes to antler expression (heritability) in moose is unknown. Arguments for either nutrition or genetics as the primary force behind antler growth are common (Goss 1983). The limited data available indicate the form of the antler and its potential size are genetically controlled. Harmel (1983) reported that of the offspring produced by a male white-tailed deer with superior antlers, only 5% exhibited spikes as yearlings, whereas 44% of the offspring of a male with inferior antlers had spikes. As all of the deer in this study were maintained on high-quality feed, it is apparent the size of antlers is heritable. The heritability of brow tines is unknown.

OBJECTIVES

1. Determine genetic structure of moose populations across the state.

 H_{10} : Estimates of genetic diversity will not differ among moose populations across the state.

H_{1A}: Estimates of genetic diversity will differ among moose populations across the state.

2. Determine if differences in antler characteristics noted for different regions of Alaska are related to genetic factors.

 H_{20} : Populations characterized by superior antlers (larger age-specific antler spreads and palmated brows) will not exhibit more genetic diversity than those characterized by inferior antlers.

 H_{2A} : Populations characterized by superior antlers (larger age-specific antler spreads and palmated brows) will exhibit more genetic diversity than those characterized by inferior antlers.

3. Determine the degree to which antler characteristics are heritable.

H₃₀: Antler morphology of offspring has no relation to antler morphology of parents.

H_{3A}: Antler morphology of offspring is related to antler morphology of parents.

4. Determine if antler characteristics are related to other phenetic correlates such as body size and growth rate.

 H_{40} : Antler morphology (size) is not related to body size or growth rate.

H_{4A}: Antler morphology (size) is directly related to body size or growth rate.

5. Determine if N_e of moose populations subjected to SHS changes over time in comparison with control populations.

H₅₀: Temporal changes in N_e will not differ between populations subject to SHS and general hunts.

H_{5A}: Temporal changes in N_e will differ between populations subject to SHS and general hunts.

6. Determine if SHS causes a decline in the number of animals with inferior antlers.

 H_{60} : The percentage of spike-fork yearlings in populations subject to SHS will not decrease over time.

 H_{6A} : The percentage of spike-fork yearlings in populations subject to SHS will decrease over time.

7. Determine if genetic diversity of populations is related to historical population trends.

H₇₀: Populations characterized by historically low bull:cow ratios and/or low population densities will exhibit no differences in genetic diversity compared with populations that are close to management objectives.

H_{7A}: Populations characterized by historically low bull:cow ratios and/or low population densities will exhibit lower genetic diversity compared with populations that are close to management objectives.

METHODS

JOB 1. COLLECT TISSUE SAMPLES FROM MOOSE POPULATIONS ACROSS THE STATE

A sample of skeletal muscle, and kidney, liver, and heart tissue if possible, were collected from animals shot by hunters. These samples were frozen at -20°C as soon as possible after collection. Additionally, a sample of ear tissue was collected from animals collared as part of other research projects and were stored frozen. These tissues were transferred to an ultracold freezer (-80°C) in 1995 to facilitate long-term preservation. A listing of sample sizes and collection locations was presented in Hundertmark et al. (1996).

JOB 3. CONDUCT A CAPTIVE BREEDING PROGRAM TO ASSESS HERITABILITY OF ANTLER AND BODY SIZE

In May 1993 5 newborn calves (2 male, 3 female) were captured in the Three Day Slough area of the Koyukuk River, an area known for producing moose with large brow formations. These animals, along with a female calf born at the MRC, were hand-reared at the MRC to allow them to become accustomed to human presence and handling. The calves were allowed to forage on natural vegetation during the summer and were provided a formulated ration (Schwartz et al. 1985) ad libitum during the winter to maximize nutritional effects on antler and body growth.

Selective breeding followed the methodology of Harmel (1983). The cows were divided randomly into 2 groups, and a bull was placed with each group based upon random selection and allowed to breed. All offspring were ear-tagged and weighed at birth. Male offspring were placed in a large pen and fed a formulated ration ad libitum. Females were retained to be bred to their fathers as yearlings and 2-year-olds. We will weigh male offspring weekly in September and remove, weigh, and measure their antlers. We will analyze weights and antler measurements by partitioning the variance among sires and sibs (Wright 1969). Pedigrees of all MRC moose will be constructed to determine if these data can be used in this analysis.

JOB 5. LABORATORY ANALYSIS OF TISSUE SAMPLES

Electrophoretic analyses of tissue samples were conducted at the Savannah River Ecology Lab at Aiken South Carolina in March 1996. The loci examined are listed in Table 1. Horizontal starch gel electrophoresis was performed according to the procedures of Selander et al. (1971), Manlove et al. (1975), and Harris and Hopkinson (1977). Each gel was loaded with tissue extract from up to 25 individuals and was exposed to electric current overnight. Gels were sliced and stained, and allozyme genotypes for each individual were scored. Gels which contained more than 1 genotype were photographed to provide a permanent record.

Tissue samples for 457 individual moose were analyzed for genetic diversity via electrophoresis. These data are being entered into a computer database to assist with analysis. Presently, genotypes at 19 loci for moose from 3 areas (Mat-Su Valley, Southeast Alaska, and Colorado) are entered into the database, available for analysis. These loci are Mdh-1,-2, Mpi, Pep-2, 6Pgd, aPgd, Ldh-1,-2, Ada, Icd-1,-2, Pgi, Pgm-2, Ck-1,-2, Adk-1,-2, Fh, and Sod-1 and are described in Table 1.

Genetic variability will be analyzed with the computer program BIOSYS-1 (Swofford and Selender 1981) and will be expressed as heterozygosity (h), alleles per locus (A), and percent polymorphic loci (P). Genetic differentiation among populations will be determined by use of F statistics (Wright 1965) and Nei's genetic distance (Nei 1978).

RESULTS AND DISCUSSION

JOB 3. CONDUCT A CAPTIVE BREEDING PROGRAM TO ASSESS HERITABILITY OF ANTLER AND BODY SIZE

Data from the most recent breeding season were summarized in Hundertmark et al. (1996).

JOB 5. LABORATORY ANALYSIS OF TISSUE SAMPLES

The 2 Alaskan populations (Mat-Su and Southeast) were polymorphic at 4 (Mdh-1, Mpi, Pep-2, and Ck-1) of 19 loci, whereas the Colorado population was polymorphic for 2 (Mdh-1, Mpi) of these (Table 2). No variation at the Pgm-2 locus was observed, contrary to findings from moose from the Kenai Peninsula (Hundertmark et al. 1992). These data support the conclusion of Hundertmark et al. (1992) that moose exhibit levels of genetic variation similar to other mammals and are not monomorphic as previously reported (Ryman et al. 1980). Other variable loci described by Hundertmark et al. (1992) have not been analyzed at this time.

Estimates of genetic variability for the 3 populations were less than those reported for Kenai Peninsula moose by Hundertmark et al. (1992) (Table 3). This was to be expected for the Colorado population because it originated as a transplant of 23 animals. Moreover, the Southeast population (comprised mainly of animals from the Haines area) was established by migration from Canada, and it would not be unusual if genetic diversity in the founded population was less than that in the parent population. It is surprising, however, to note less diversity in the Mat-Su population, compared with animals from the Kenai Peninsula. Mat-Su is a large, open population that would not experience significant amounts of genetic drift, at least in recent times. We should note that measures of genetic variability are influenced by the choice of loci included in the analysis, and the loci included in this analysis were not identical to those of Hundertmark et al (1992). These results may change somewhat in the final analysis.

Allele frequencies differed significantly among the 3 populations for Mdh-1 ($X^2 = 45.0$, P < 0.00001), Mpi ($X^2 = 12.9$, P = 0.002), and Ck-1 ($X^2 = 6.52$, P = 0.038). Genotype frequencies at each locus within populations did not differ from Hardy-Weinberg expectations with the exceptions of Mdh-1 (Mat-Su) and Ck-1 (Southeast). In both of these instances the rare allele was found in the homozygous condition in one animal and no instances of heterozygotes were

recorded. These data might be attributable to small sample sizes or improperly scored gels and should not be considered as definitive evidence of a heterozygote deficiency.

Allele frequencies for Mdh-1 and Mpi show a geographic trend. The common allele in Mdh-1 in Mat-Su is the rare allele in Colorado, and these alleles are intermediate in frequency in Southeast Alaska. For Mpi, the rare allele in Mat-Su becomes more common in moose from Southeast Alaska and is more common still in Colorado (Table 2). These trends support the hypothesis of Hundertmark et al. (1992) in which moose colonized the lands south and east of Interior Alaska through a stepping stone model of migration from the Beringian glacial refugium. Allele frequencies for certain loci were altered through genetic drift and founder effects and became fixed for some loci such as Ck-1. Although the moose from Colorado were introduced, the founding individuals were from Utah and Wyoming, which does not bias this analysis.

RECOMMENDATIONS

Electrophoretic and mtDNA analyses will be completed during the next reporting period, and a final report will be prepared. Microsatellite markers known to work on white-tailed deer (*Odocoileus virginianus*) will be tested on moose in cooperation with Dr. Rodney Honeycutt, Texas A&M University. These markers will be useful in population identification and in examining genetic processes at the population level.

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Table 1. Enzymes studied via gel electrophoresis.

		Gel	Stain
Enzyme name	<u>E.C.1</u>	Buffer ²	Reference ³
a-Glycerophosphate dehydrogenase (a-Gpd)	1.1.1.8	LiOH, TC8.0	3
Aconitase (Aco)	4.2.1.3	ТМ	3 '
Adenosine deaminase (Ada)	3.5.4.4	LiOH	2
Adenylate kinase (Adk-1,-2)	2.7.4.3	TM, TC8. 0	2
Aspartate aminotransferase (Aat-1,-2)	2.6.1.1	TC8.0, LiOH	3
Creatine kinase (Ck-1,-2)	2.7.3.2	ТМ	2
Esterase (Est) (bnp substrate)	3.1.1.1	TM	3
Fumarate hydratase (Fh)	4.2.1.2	ТМ	3
Glucose-6-phosphate isomerase (Pgi-1,-2)	5.3.1.9	РК	3
Isocitrate dehydrogenase (Icd-1,-2)	1.1.1.42	LiOH	3
Lactate dehydrogenase (Ldh-1,-2)	1.1.1.27	LiOH, TC8.0	3
Malate dehydrogenase (Mdh-1,-2)	1.1.1.37	AC,TC8.0	3
Malic enzyme (Mod-1,-2)	1.1.1.40	ТМ	2
Mannose-6-phosphate isomerase (Mpi)	5.3.1.8	LiOH, TC8.0	3
Menadione reductase (Mnr)	1.6.99.2	LiOH	3
Peptidase (Pep-2) (lgg substrate)	3.4.11	LiOH	3
Phosphoglucomutase (Pgm-1,-2)	5.4.2.2	TC8. 0	3
6-Phosphogluconate dehydrogenase (6Pgd)	1.1.1.44	LiOH	3
Superoxide dismutase (Sod-1,-2)	1.15.1.1	TM	3

¹Enzyme Commission number

² PK (Poulik) = discontinuous tris-citrate (Manlove et al. 1975); LiOH = lithium hydroxide (Manlove et al. 1975);

TM = tris-maleate (Manlove et al. 1975); TC8.0 = continuous tris-citrate (Manlove et al. 1975)

³ 1 = Manlove et al (1975); 2 = Selander et al. (1971); 3 = Harris and Hopkinson (1977)

			Locus		
Population	Allele	MDH-1	MPI	PEP-2	CK-1
Mat-Su	Α	0.240	0.120	0.045	0.045
(N = 25)	В	0.720	0.880	0.955	0.955
	С	0.040	0.000	0.000	0.000
	h	0.400	0.160	0.091	0.091
Southeast	Α	0.560	0.320	0.083	0.200
(N = 26)	В	0.440	0.680	0.917	0.800
	С	0.000	0.000	0.000	0.000
	h	0.400	0.400	0.167	0.000
Colorado	Α	0.885	0.442	0.000	0.000
(N = 26)	В	0.115	0.558	1.000	1.000
	С	0.000	0.000	0.000	0.000
	h	0.231	0.577	0.000	0.000

Table 2. Allele frequencies and direct-count estimates of heterozygosity (h) for polymorphic loci detected in 3 moose populations.

Population	h	Α	Р
Mat-Su	0.035	1.24	19.05
Southeast	0.046	1.19	19.05
Colorado	0.038	1.10	9.52
Kenai	0.077	1.35	30.00

Table 3. Estimates of heterozygosity (h), alleles per locus (A), and percent polymorphic loci (P, 0.99 criterion) in 3 moose populations analyzed in this study and compared with a fourth population ("Kenai," Hundertmark et al. 1992).
Alaska's Game Management Units



ARLIS

Alaska Resources Library & Information Services Anchorage, Alaska The Federal Aid in Wildlife Restoration Program consists of funds from a 10% to 11% manufacturer's excise tax collected from the sales of handguns, sporting rifles, shotguns, ammunition, and archery equipment. The Federal Aid program allots funds back to states through a formula based on each state's geographic area and number of paid hunting license holders. Alaska receives a maximum 5% of revenues collected each year. The Alaska Department of Fish and Game uses federal aid funds to help restore, conserve, and manage wild birds and mammals to benefit the

public. These funds are also used to educate hunters to develop the skills, knowledge, and attitudes for responsible hunting. Seventy-five percent of the funds for this report are from Federal Aid.



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