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# Evaluation and Testing of Techniques for Ungulate Management

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Research Final Performance Report 1 July 1992–30 June 2000 Federal Aid in Wildlife Restoration Grants W-24-1 through W-27-3 Study 1.45

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# **RESEARCH FINAL REPORT**

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#### SUMMARY

This project addressed a diverse array of activities during the reporting period. We requested and received from the Alaska Legislature a capital improvement project for deferred maintenance. That funding was used to upgrade animal handling facilities, upgrade power generation capabilities, and upgrade the access road. We found that carfentanil and xylazine were effective means for immobilizing moose, but that the traditional antagonist of xylazine, yohimbine, was ineffective in moose. Tolazoline hydrochloride proved to be an effective antagonist to xylazine in moose. We documented that fat depots located in viscera and carcass of moose each decline linearly with total body fatness, and at maximum adiposity the viscera contained 50% of its mass as fat, whereas the carcass contained 15% of its mass as fat yet the carcass held more fat overall due to its greater mass. We determined that measurement of rump fat thickness by ultrasound is a viable and accurate index of adiposity in moose. We also determined that ultrasound can be used to measure rump fat, and therefore adiposity, in mule deer and that the predictive relationships between moose and mule deer have similar characteristics. Moose calves that are conceived after the first estrus do not differ in birth mass from their first-estrus counterparts but they are born later. Late-born calves do not exhibit catch-up growth during their first year and have lower mass entering their first winter, which may lead to greater overwinter mortality in that cohort. We determined that pregnancy status and in utero twinning rates could be measured with ultrasound and a serum assay for pregnancy-specific protein B. We also determined that ovarian activity and pregnancy status in moose also can be monitored by concentration of fecal progestagen, which provides us with a noninvasive technique for monitoring reproductive status. We investigated the composition of rut urine in bulls to determine the identity of a putative estrus-priming pheromone. We identified components of urine that elicited positive responses from estrus females by broad chemical class, but specific chemical compounds have not yet been identified. The identity of pheromones in moose urine will allow us to determine which segment of the bull population should be conserved to facilitate rut synchrony. We investigated the response of

moose to an artificial stressor (ACTH administration) and documented a rapid rise in serum levels of cortisol within 60 minutes that indicated an unusually well-developed adrenocortical response in moose. Although anecdotal accounts suggested that moose could not digest complex carbohydrates, we determined that carbohydrase activity in the small intestine of moose is great enough to digest carbohydrates effectively, which is an important consideration in formulating moose feeds. Moose exhibit sexual segregation in seasonal habitat use, and we need to understand the extent and cause of this behavior if we are to manage moose and their habitats effectively. We documented that jaw morphology differs between the sexes, even when corrected for sexual dimorphism in mass. We hypothesize that a differential ability to forage, combined with different nutritional needs of the sexes, leads to sexual segregation. During this reporting period Moose Research Center (MRC) personnel wrote and edited chapters for a new book on moose ecology and management. MRC personnel also participated in a workshop at the Fourth International Moose Symposium concerning the application of new technologies to moose management. We focused on assessment of body condition and pregnancy by ultrasound and application of molecular genetics techniques for population dynamics and forensic applications. We reviewed concepts underpinning maximum sustained yield and discussed the dangers inherent in applying this concept as a management edict in wildlife populations. We documented that elk introduced onto Afognak Island show similar genetic heterogeneity to the source population on the Olympic Peninsula, Washington, indicating that a severe genetic bottleneck probably is not a cause of abnormally small antlers on Afognak elk. Although moose were thought to show little genetic diversity, we documented moderate amounts of heterogeneity in moose from the Arctic National Wildlife Refuge. Allelic diversity was great enough over 11 microsatellite loci to yield a probability of identity of 1 in 267,600,000. Leptin is a protein produced by adipose cells and is directly related to total body fatness in humans and a potentially powerful tool for measuring condition in moose. We assayed serum samples with five different commercial leptin assays, but none of those assays was successful in detecting leptin.

**Key words:** Alaska, *Alces alces*, body composition, capital improvements, deferred maintenance, genetics, leptin, microsatellites, techniques.

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## BACKGROUND

The Moose Research Center (MRC), with approximately 30 confined moose and 15 confined caribou, and facilities to handle them, provides unique conditions for developing and testing techniques applicable to ungulate management. This study has been continuously active since 1969 when the MRC became functional. Four Federal Aid final

reports covering the period from 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 35 journal publications. Most findings from these studies were published as articles in scientific journals, book chapters, proceedings, and popular articles. Recently, the focus of this study was extended to cover other ungulates, particularly caribou.

The work conducted under this study was divided into 7 jobs. When we completed a segment of a job, it usually was submitted for publication in a refereed journal. In appendices of this report, we provide abstracts from manuscripts that are published or in the process of publication. The studies are described briefly in the body of this report, but we encourage interested readers to refer to the original publications for further information. The body of this report describes work in progress.

# JOB 1. MAINTENANCE AND OPERATIONS

This job encompassed all activities associated with the physical plant of the MRC. Our facility comprises five log cabins, approximately 13 miles of 8-ft high woven wire fence, animal handling areas and equipment, feeding and watering equipment for captive animals, and 4.5 miles of single-laned, unimproved roadway. The roadway was established as a single-laned tractor trail that connected a series of existing seismic exploration trails. The US Fish and Wildlife Service, Kenai National Wildlife Refuge plows and grades the roadway and has provided us with a John Deere 300 backhoe/loader to assist with maintenance. Propane is used to operate lights, heaters, ovens and refrigerators. A 13-kW generator on site also is powered by propane, and all cabins are wired for 210/240 volt AC power. Due to the high cost of propane, the generator is run only when AC power is necessary; otherwise, propane- or gasoline-powered equipment is used. Due to the age of the facility, upgrades to power production, animal handling areas, sections of fence, and the road surface became necessary. Those deferred maintenance items were too expensive to be included in the normal budget request, so we prepared a capital improvement project (CIP) request.

## JOB 2. DRUG TESTING

The primary focus of this job is the evaluation of immobilizing drugs and their antagonists for moose and caribou. A secondary focus is the evaluation of other pharmaceuticals that may be useful for moose management or research but that have not been licensed or tested in moose or other cervids. During this report period we evaluated the efficacy and cost of immobilizing moose with either a) xylazine hydrochloride antagonized by either tolazoline or yohimbine, or b) carfentanil citrate antagonized by naltrexone. The abstract of the publication resulting from that study is in Appendix A.

## **JOB 3. GENETICS STUDIES**

This job was developed to investigate the application of new molecular genetics tools to management and research questions and to bring a population genetics perspective to resource issues. Activities under this job included the analysis of microsatellite variation in moose. Microsatellite variation was also assessed in elk (*Cervus elaphus*) from Afognak and Raspberry Islands in Game Management Unit 8 and compared with variation in elk from the Olympic Peninsula, Washington. Eight elk (5 females and 3 males) were introduced onto Afognak and Raspberry Islands in March 1929. All of those animals had been born in 1928 on the Olympic Peninsula. We sought to determine if the small size of the transplant caused a genetic bottleneck in the Alaskan population that may be responsible for certain morphological anomalies.

## Social Application for DNA Profiling

An Assistant Attorney General for Alaska contacted us for assistance with their prosecution of a case concerning wanton waste of two moose from the Brooks Range. The State wished to use DNA profiling to match meat seized at a suspect's residence with unsalvaged meat collected from the carcasses in the field. Before probabilities of identity could be applied to that case, we needed to determine microsatellite allele frequencies from a random sample of moose from the surrounding area. Our frozen tissue archive contained samples from 57 moose collected in the Arctic National Wildlife Refuge (ANWR) and represented the nearest samples to the crime scene. We analyzed allele frequencies for moose from ANWR to determine the probability that two unrelated moose would have the same genotype. That information was necessary before the value of a match between evidence samples could be evaluated.

## JOB 4. PHYSIOLOGICAL INDICES OF BODY CONDITION

We investigated the relationship between total body fat and fat depots in the carcass and viscera (Appendix B). We also investigated ultrasound measurements of subcutaneous rump fat as an index of total body fatness in moose (Appendix C and D) and mule deer (*Odocoileus hemionus*, Appendix E). We also compared predictive equations among cervids (Appendix F).

Circulating levels of leptin in blood serum or plasma are related to percent body fat in humans and certain laboratory animals (Campfield et al. 1996). The gene responsible for producing this protein is expressed only in fat cells and leptin is secreted in proportion to the size and number of such cells. Thus, it would seem that this protein would be an excellent indicator of body fatness in mammals, but this assumption needs to be investigated in moose. Moreover, there is a large amount of variation in leptin levels that has been documented at given levels of fatness (Considine et al. 1996). To be considered as a reliable tool for assessing animal condition, this variance must be reduced by determining the physiological variable(s) associated with it. For instance, it is known that there is a circadian rhythm in circulating leptin levels (increase at night but are stable during the day). Also, fasting for 24 hours has depressed leptin levels in humans but levels return to normal after refeeding (Campfeld et al. 1996). Therefore, assessment of circadian variation in leptin levels as well as effects of intake on these levels may add predictive power to the leptin-fat relationship.

## **JOB 5. MOOSE REPRODUCTION**

One of the reasons for maintaining a bull:cow ratio near 20:100 in moose populations is to achieve rut synchrony and to foster the breeding of all females during their first estrous cycle. It had been assumed that calves born to females bred during the second estrus were born later than their first-estrus counterparts and would enter their first winter at a smaller body size, which would decrease their ability to survive the winter. Schwartz and Hundertmark (1993) demonstrated that calves born to females bred after the first estrus were indeed born significantly later than those born to females bred during the first estrus. During this report period, we investigated the potential for late-born calves to exhibit compensatory, or catch-up, growth during their first months of life as a way of making up for their late birth and therefore minimizing any survival rate differential associated with body size. The abstract of the resulting publication is in Appendix G.

We investigated the potential for diagnosing pregnancy and twinning rates by 2 different techniques: ultrasonography of the uterus and a serum assay for pregnancy-specific protein B (PSPB, Appendix H). Finally, we determined the efficacy of using fecal progestagen concentration as an indicator of the estrous cycle and pregnancy (Appendix I).

Moose are spontaneous ovulators, a process that is governed primarily by environmental cues (Sadlier 1982). The role of the male in moose reproduction traditionally has been viewed as that of a sperm donor but having nothing to do with timing of ovulation in females. Nonetheless, the behavior of female moose in rutting pits, where females attempt to coat themselves with male urine-soaked mud before mating, indicates some degree of physiological function associated with reproduction (Miquelle 1991). Schwartz et al. (1990) documented the presence, albeit at extremely low levels, of pheromones in the saliva of bull moose during rut. Those pheromones are involved in priming ovulation in female swine (*Sus scrofa*) and are hypothesized to facilitate breeding synchrony in swine populations (Perry et al. 1980).

We were interested in continuing investigations about the function of pheromones in moose reproductive biology because the potential role of the male to induce ovulation is an important factor in determining proper sex ratios in managed moose populations as well as the age structure of the male segment of populations. We supported data collection by a graduate student concerning the response of female moose to rut and non-rut urine collected from different bulls at the MRC. The abstract of her MS thesis is presented in Appendix J.

## **JOB 6. CARIBOU REPRODUCTION**

The original objective of this study was to examine the extent to which nutritional factors influenced reproduction in caribou, and how that relationship might have contributed to the decline of the Southern Alaska Peninsula caribou herd. Problems related to personnel changes, inexperience with caribou husbandry, and poor caribou survival in captivity resulted in little progress toward that objective. Data collected thus far have been presented elsewhere (Stephenson et al. 1999).

#### JOB 7. MISCELLANEOUS INVESTIGATIONS

This job gives us the flexibility to evaluate new techniques and address specific management issues in a timely manner. During this report period we evaluated the physiological response of male moose to stressful stimuli. We simulated a stress event by administration of adrenocorticotropic hormone and measured the response by assessing the levels of cortisol in blood serum. The abstract from that publication is in Appendix K.

The development of a formulated ration for moose at the MRC (Schwartz et al. 1985) allowed moose to be kept in captivity successfully for the first time. Although the MRC ration was used successfully at our facility for years, nutritionists at Ralston Purina Corporation (St. Louis, MO) suggested that moose were not able to digest the complex carbohydrates contained in the MRC ration and implied that this was a cause of a lack of vigor often seen in moose kept on a formulated ration for extended periods (Schwartz 1992). Purina developed the Mazuri moose ration as an alternative to the MRC ration. The Purina ration differed mainly in the carbohydrate source: simple sugars versus complex carbohydrates. We tested this claim by measuring the activity of carbohydrase, an enzyme necessary for the breakdown of complex carbohydrates, in three sections of the small intestine from 2 moose fed an MRC diet and compared those levels to those observed in cattle. The abstract from that publication is in Appendix L. Further research into this wasting syndrome in captive moose indicated similarities with inflammatory bowel disease, perhaps caused by outbreaks of certain bacteria in the lower gut (Appendix M).

Moose exhibit resource partitioning between the sexes by segregating in different habitats seasonally (LeResche 1974, Miquelle et al. 1992). The degree to which moose segregate sexually must be considered if the habitat requirements of the sexes are to be met. For instance, white-tailed deer (Odocoileus virginianus) in California exhibit such extreme sexual segregation that Kie and Bowyer (1999) recommended that the sexes be managed as if they were distinct species, each with specific habitat needs. An understanding of the mechanisms causing moose to segregate sexually is necessary before we can determine the specific needs of either sex. Many hypotheses have been formulated to explain sexual segregation, but a recent hypothesis attributed that behavior to differences in nutritional requirements and limitations between males and females (Barboza and Bowyer 2000). To gain a better understanding of the mechanisms behind sexual segregation in moose, we supported research in which a graduate student examined aspects of diet selection by male and female moose. One finding of his research concerned differences in jaw morphology that may cause differences between the sexes in the ability to process food, and therefore may have an effect on sex-specific diet selection and habitat choice. The abstract of the manuscript describing that research is in Appendix N.

Although much research has been conducted on moose over the last 4 decades, no exhaustive compilation of the knowledge acquired over this period existed, leaving Peterson (1955) as the ultimate, yet dated, comprehensive source for moose. In 1991 the Wildlife Management Institute agreed to sponsor a new book concerning the ecology and management of North America moose to be edited by A. W. Franzmann and C. C. Schwartz. The book was published in 1998 (Franzmann and Schwartz 1998), and former

and current MRC personnel contributed some of the chapters. The citations for those chapters are listed in Appendix O.

During the Fourth International Moose Symposium, held in Fairbanks, Alaska in 1997, new technologies and their applications to moose research and management were presented. Certain technologies investigated under this study were included, and the abstract of a paper summarizing that symposium is in Appendix P.

One new aspect of wildlife management in Alaska is a legislative mandate for a management goal of maximum sustained yield from certain intensively managed ungulate populations. Maximum sustained yield is an important theoretical construct in wildlife biology, but management of populations at that level is risky. We recommend maintaining intensively managed populations at densities above the actual point of maximum sustained yield to avoid potential overharvests caused by stochastic variation in recruitment. A review of the concepts involved in this issue was prepared under this study, and the abstract of that publication is in Appendix Q.

## **OBJECTIVES**

The objective of this study is to test and evaluate techniques that are potentially useful for management of ungulates.

## **METHODS**

With exceptions listed below, methods for individual projects are described in the report or article referenced in each appendix. The activities listed below are still active or have not reached a stage of final analysis and publication.

## JOB 1. MAINTENANCE AND OPERATIONS

We submitted a request for capital improvement funding for deferred maintenance, which was granted for FY1998–2002. Primary items identified in the request were 1) road upgrade, 2) power plant upgrade, 3) roof repair for animal handling facility, 4) upgrade of caribou handling facilities, and 5) repair of fence corner assemblies. Funding for deferred maintenance was granted by the Alaska Legislature in the amount of \$100,000. This project took effect on 1 July 1998 and will last 5 years.

#### **JOB 3. GENETICS STUDIES**

#### Elk Genetics

Tissue samples, (hair, hide, or muscle) were solicited from hunters in Alaska and from biologists in Washington. DNA was extracted using the Qiagen system. Ten microsatellite loci were amplified via the polymerase chain reaction according to the protocols of Talbot et al. (1996). Microsatellite alleles were documented by electrophoresis in an automated sequencer (ABI Model 373) using dye-terminated primers. Genotypes were scored with

Genotyper software (ABI) and locus-specific variation between populations was analyzed with the program Cervus ver. 1.0 (Marshall et al. 1998).

## Moose Genetics

Tissue samples from 30 moose from ANWR were removed from our tissue storage archive and were shipped to the University of Alberta, Department of Biology for DNA profiling. DNA was extracted from samples with Qiagen tubes. PCR was conducted using a reaction buffer of 50 mM KCl, 10 mM Tris HCl, pH 8.8, 0.1% Triton X-100, 0.16 mg/ml BSA, and sterile water. Concentration of MgCl<sub>2</sub> varied among the protocols for different primer sets, but a typical reaction mix contained 2 mM MgCl<sub>2</sub>, 120 µm dNTPs and 0.16 µm each primer. Template DNA was added to that mixture and was amplified on a thermal cycler using a standard time and temperature regime. Primers selected were BL42, BM203, BM4513, BM848, BM888, BM1225, FCB193, RT24, RT30, RT9, and RT5 (Talbot et al. 1996, J. Coffin, personal communication). Microsatellite alleles were documented by electrophoresis in an automated sequencer (ABI Model 373) using dye-terminated primers. Genotypes were scored with Genotyper software (ABI) and locus-specific variation.

## JOB 4. PHYSIOLOGICAL INDICES OF BODY CONDITION

We collected serum samples from 15 moose for which body fat levels were determined by proximate analysis (Hundertmark et al. 1997). These samples were submitted to Linco, Inc., a biotechnology firm that specializes in creating and manufacturing assays to detect leptin in serum. Personnel at Linco analyzed the samples by means of 5 different radioimmuno assays (RIAs), 4 of which were specific to a certain species or group (human, mouse, rat, and primate) and a fifth assay that consisted of a polyclonal antibody that detects leptin in numerous species (multispecies assay). We compared the variation in circulating leptin levels with our body fat estimates from those moose.

# **RESULTS AND DISCUSSION**

# JOB 1. MAINTENANCE AND OPERATIONS

Our progress to date on deferred maintenance has included the purchase and installation of a restraining device for caribou. This device traps the caribou in a V-shaped slot so that they can be handled without chemical immobilization. Our efforts to upgrade our power generation capabilities consisted of the purchase and installation of an alternative energy system comprised of a wind-powered generator, two banks of solar panels, a 24-V bank of batteries for power storage and a 24-VDC–120-VAC inverter. The wind and solar generators provide power streams to the battery bank that can be used at any time. The inverter converts the 24-VDC power to 120-VAC power to operate standard electric appliances. We also purchased a new propane-powered main generator rated at 10-kW standby power. This generator will be installed in the fall of 2000 and will become the main source of power, with the old generator becoming a backup. Finally, the access road to the MRC was upgraded by the application of approximately 4000 yards of gravel. Unocal Corporation donated most of that gravel from its Swanson River oil field. The

gravel was hauled and spread by personnel from the Kenai National Wildlife Refuge with assistance from private contractors.

# **JOB 3. GENETIC STUDIES**

# Elk Genetics

Eight of the 10 loci were amplified reliably and were included in this analysis. Elk from the Olympic Peninsula (OP) were polymorphic at all 8 loci, whereas elk from Afognak Island (AI) were polymorphic at 7 loci (Table 1). Not all individuals were scored reliably at all loci; a mean of 27.1 individuals per locus were scored for AI, whereas the mean for OP was 13.1 individuals per locus. Mean number of alleles per locus was 3.25 for OP and 3.63 for AI. Elk from AI exhibited the greater number of alleles at 2 loci, whereas elk from OP never exhibited a greater number of alleles. Mean expected heterozygosity for AI was 0.511 and was 0.585 for OP.

The heterogeneity observed in AI and OP elk indicates that no significant bottleneck occurred during the founding of the AI population. Mean number of alleles per locus is similar as are expected heterozygosity levels. It would be unusual for a founded population to have more alleles per locus than the source population, but we attribute the differences observed in these data to the sample size differential between the populations. Had more elk from AI been available, we expect that all alleles found in AI elk would have been found in OP elk as well.

When compared with heterogeneity of Canadian elk (J. Coffin, personal communication), we detected fewer alleles at 6 of the 8 loci (Table 2). At each of the remaining 2 loci, we detected 1 more allele than was found in the Canadian sample. The lower overall allelic diversity shown by the combined OP and AI samples may indicate that reduced heterogeneity may have existed in the original founder population (OP). That hypothesis can be tested with more samples from OP.

# Moose Genetics

Of 30 moose submitted for DNA profiling, 1 sample provided no usable DNA and another sample yielded a genotype for only 1 locus. Of the 12 loci examined, 11 were amplified successfully. All loci were polymorphic, exhibiting a range of 3–10 alleles each (Table 3). Mean number of alleles per locus was 5.09 and mean heterozygosity was 64.6%. Probability of identity for specific loci ranged from 0.047–0.413, and overall probability of identity was  $3.74 \times 10^{-9}$ . Thus, the probability of 2 random unrelated individuals having identical genotypes at all 11 loci is approximately 1 in 267,600,000. Despite the relatively low levels of heterogeneity seen in the moose genome, that level of probability indicates that tissue samples taken at different times (e.g., mark-recapture sampling) or places (e.g., forensic purposes) can be matched with virtual certainty.

#### JOB 4. PHYSIOLOGICAL INDICES OF BODY CONDITION

Values for the 5 leptin assays showed little variation. In fact, the rat and primate assays did not detect leptin in any moose serum samples, and the human assay failed to detect leptin in 13 of 15 moose (Table 4). The mouse and multispecies assays showed some variation, but it was not related to variation in body fat content. It was obvious that those assays were not effective in detecting leptin in moose serum, so we chose not to apply statistical analyses.

## **CONCLUSIONS AND RECOMMENDATIONS**

This study has produced numerous findings and publications that are helpful to moose researchers and managers. We recommend continuation of this study so that new drugs and research and management techniques can be evaluated in a timely and efficient manner. The deferred maintenance CIP will allow the MRC to continue to operate efficiently, but long-range plans are necessary for future modifications if we are to continue to effectively serve the wildlife management community.

The genetics data collected under this study indicate the power of new molecular genetics technologies for addressing wildlife management problems. We addressed a question of founder effect in a population of introduced elk and contributed forensic data to a criminal prosecution. We believe that further investigations in the area of molecular genetics will enhance our research capabilities and will be a benefit to wildlife management.

We were not successful in finding an existing assay that was effective in measuring leptin levels in moose blood serum. Nonetheless, we believe that this area of research is very promising, and we will continue to investigate assays as they become available or will work to develop a moose- or cervid-specific assay for leptin. Once that is accomplished, the relationship between leptin and body fatness in moose and other cervids can be explored.

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Table 1 Numbers of alleles detected at each of 8 loci in elk from Afognak Island, Alaska (AI) and the Olympic Peninsula, Washington (OP). The first row of values under the column heads is the number of individuals typed for that locus. Thereafter, columns contain a listing of alleles, listed by their length in nucleotides. These data are preliminary.

BI	_42	BMC	C1009	BM	[203	BM	[848	BM	4107	BM	888	BM	4208	BM	5004
AI	OP	AI	OP	AI	OP	AI	OP	AI	OP	AI	OP	AI	OP	AI	OP
25	15	25	15	25	15	11	9	35	13	32	13	31	12	33	13
252	252	283		224	224		356	161	161	180	182	152	152	133	133
258	258	285	285	226	226	360	360	163	163	182	190	156	156	135	135
260	260	287	287	229	229			171	171	188	192			137	
262	262		289	232	232			173	173	190				139	139
264					234			182		192					

Table 2 Comparison of allelic diversity among elk from Afognak Island, Alaska (AI), and Olympic Peninsula, Washington (OP) determined from this study, and elk from western Canada (J. Coffin, Univ. Alberta, personal communication). These data are preliminary.

	BL42	BMC1009	BM203	BM848	BM4107	BM888	BM4208	BM5004
AI	5	3	4	1	5	5	3	4
OP	4	3	5	2	4	3	2	3
Total	5	3	5	2	5	5	3	4
Canada	4	7	8	5	7	5	5	3

Locus	Number individuals scored	Number alleles	Heterozygosity	Probability of identity
RT5	28	3	0.39	0.413
RT9	28	4	0.59	0.239
RT24	28	5	0.68	0.157
BL42	28	10	0.85	0.047
RT30	28	6	0.72	0.136
BM203	29	5	0.73	0.131
BM888	28	4	0.67	0.182
BM848	28	4	0.60	0.241
BM1225	28	6	0.61	0.194
BM4513	28	5	0.75	0.116
FCB193	28	4	0.53	0.290

Table 3 Allelic diversity among moose from the Arctic National Wildlife Refuge scored for 11 microsatellite loci.

	% Body		Leptin assay reactivity, ng/ml							
Animal	fat	Human	Mouse	Rat	Primate	Multi-species				
Soccer	7.8	< 0.5	0.5	<0.5	<0.5	<1				
Enau	12.1	< 0.5	0.6	< 0.5	<0.5	<1				
Terra	19.3	< 0.5	0.2	< 0.5	<0.5	3				
Vicki	8.9	< 0.5	0.4	< 0.5	<0.5	1				
Betsy	8.5	< 0.5	0.5	< 0.5	<0.5	1				
Angel	0.3	0.5	0.6	< 0.5	<0.5	2				
Butch	1.4	< 0.5	0.4	< 0.5	<0.5	1				
Hydro	7.8	< 0.5	< 0.2	< 0.5	<0.5	3				
Mario	5.8	< 0.5	0.3	< 0.5	<0.5	2				
Sinuk	18.4	< 0.5	0.4	< 0.5	<0.5	2				
Luke	8.9	< 0.5	0.4	< 0.5	<0.5	1				
Oly	1.1	<0.5	0.5	<0.5	<0.5	2				
Wild Bill	4.1	<0.5	0.4	< 0.5	<0.5	2				
Brooks	15.6	0.9	0.4	<0.5	<0.5	1				
Yogi	6.8	<0.5	0.4	<0.5	<0.5	<1				

Table 4 Percentage body fat for 15 moose from the Moose Research Center and associated values for circulating serum leptin concentrations detected by five different assays from Linco Research, Inc.

#### APPENDIX A

Schwarz, C. C., T. R. Stephenson, and K. J. Hundertmark. 1997. Xylazine immobilization of moose with yohimbine or tolazoline as an antagonist: a comparison to carfentanil and naltrexone. Alces 33:33–42.

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 alpha<sub>2</sub> adrenergic sedative and analgesic, used alone, and in combination with either carfentanil citrate or ketamine hydrochloride to immobilize moose at the Moose Research Center. Mean down 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 lay down 8-12 minutes before they became 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 (P < 0.0001), and animals reversed with this drug were standing within 4-31 minutes (mean = 21 minutes). Animals immobilized with a mixture of carfentanil:xylazine and reversed with naltrexone were usually standing within 7 minutes with a range of 3-21 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.

#### **APPENDIX B**

Hundertmark, K. J., and C. C. Schwartz. 2000. Dynamics of lipid reserves in moose. Abstract from poster presentation at the 36<sup>th</sup> North American Moose Conference and Workshop.

We assessed body composition by proximate analysis in 24 moose (11 females, 13 males) representing the range of condition encountered in wild populations. Percent body fat (wet weight) ranged from 0.3–19.3% of ingesta-free body (IFB) mass. The carcass (including hide and subcutaneous fat) held a greater absolute mass of fat (57% of total fat mass) than the viscera (43%) across the entire range of body fatness, but the viscera held a higher percentage of its mass as fat. At maximum adiposity, fat accounted for nearly 50% of the viscera mass, whereas fat accounted for 15% of carcass mass. Fat deposits in the carcass and viscera each declined linearly with total body fat, indicating these pools are utilized simultaneously during periods of energy deficit. We contend that indices of body composition that measure fat levels at this scale, (e.g., half carcass) would accurately predict total body composition.

#### **APPENDIX C**

Stephenson, T. R., K. J. Hundertmark, C. C. Schwartz, and V. Van Ballenberghe. 1993. Ultrasonic fat measurement of captive yearling bull moose. Alces 29:115–123.

The ability to measure fat thickness in live moose offers potential as an index of population condition. Consequently, we evaluated the feasibility of using portable, realtime ultrasound to measure body fat in 5 captive yearling bull moose (*Alces alces*). The rump region of the bulls was scanned, twice weekly for 3 weeks during the rut, using a 5-MHz transducer; an additional set of measurements was obtained in April 1993. Ultrasonic fat thickness was measured at multiple sites along a line between the spine, at its closest point to the tuber coxae (hip bone), and the tuber ischii (pin bone), as well as along a second line perpendicular to the first line at its midpoint. The range of maximum subcutaneous fat thickness at the beginning of the study was 0.3–2.4 cm. Fat thickness declined significantly during the rut. The range of fat loss during the rut at the intersection of the 2 measurement lines was 0.2–1.2 cm. This in vivo technique exhibits potential to monitor body composition.

#### **APPENDIX D**

Stephenson, T. R., K. J. Hundertmark, C. C. Schwartz, and V. Van Ballenberghe. 1998. Prediction of body fat and body mass in moose with ultrasonography. Can. J. Zool. 76:717–722.

Lipids are the primary energy store of the body, and estimation of these reserves provides an indication of nutritional status in moose (*Alces alces*). Estimates of total body fat enhance our understanding of reproductive potential, survival rate, energy balance, and nutritional carrying capacity. We developed predictive equations of total body fat and body mass from ultrasonographic fat measurements for application in live animals. We detected a strong linear relationship ( $r^2 = 0.96$ ) between ingesta-free body fat and rump fat thickness measured by ultrasonography. Rump fat thickness was measurable over a range of body fat levels (5.8–19.1%). Rump fat mass ( $r^2 = 0.92$ ) and kidney fat mass ( $r^2 = 0.95$ ) were curvilinearly related to ingesta-free body fat levels. For adult females, total length and chest girth were not related to body mass. Ingesta-free body fat, however, explained 81% of the variability in body mass, and ultrasonically measured rump fat thickness predicted body mass ( $r^2 = 0.80$ ).

#### APPENDIX E

Stephenson, T. R., V. C. Bleich, B. M. Pierce, and G. P. Mulcahy. Validation of mule deer body composition using in vivo and post-mortem indices of nutritional condition. Manuscript prepared for submission to *Wildlife Society Bulletin*.

Understanding body fat dynamics in Cervidae is of considerable mechanistic importance relative to their nutritional ecology. Estimates of body composition provide insight into an animal's energetic state, potential for reproduction and survival, and the quality of their habitats. We validated the accuracy of both in vivo and postmortem indices for predicting total body fat in mule deer (<u>Odocoileus hemionus</u>). Rump fat thickness measured using ultrasonography was linearly related to ingesta-free body fat but disappeared at ~5.6% body fat. Loin muscle thickness by ultrasonography exhibits potential for quantifying the remaining nutritional reserves below where rump fat is measurable. Whole body mass was not a significant predictor of body fat in adult females but is a good predictor of ingesta-free lean body mass. Kidney fat mass and kidney fat index were moderate predictors of total body fat, but there is concern regarding their curvilinear fit. Of the postmortem indices we tested, the Kistner Score exhibited the strongest linear relationship to ingesta-free body fat. We suggest that using an accurate in vivo method of assessing nutritional reserves reveals much about past nutritional history and future productivity of individuals within a population, especially when determined repeatedly in radiocollared animals.

## **APPENDIX F**

Stephenson, T. R., V. C. Bleich, B. M. Pierce, G. Mulcahy, K. J. Hundertmark, and J. A. Crouse. Predicting body fat using ultrasonography: are the cervids similar? Abstract of poster presented at the 36<sup>th</sup> North American Moose Conference and Workshop, Whitehorse, Yukon, during 5–10 June 2000

<u>Abstract:</u> Understanding body fat dynamics in cervids is of considerable mechanistic importance relative to their nutritional ecology, productivity, and survival. The ability to repeatedly assess total body fat in live animals has extensive research and management application. We continued to validate the application of ultrasonography for predicting body fat in a number of North American cervids. The relationship between ingesta-free body fat and the thickness of subcutaneous rump fat in mule deer was similar to that previously published for moose when adjusted for body size. In contrast, the relationship between caribou and moose differed in both slope and intercept. Differences in fat deposition patterns and mobilization among species may be related to diet composition and/or climatic adaptations.

#### APPENDIX G

Schwartz, C. C., K. J. Hundertmark, and E. F. Becker. 1994. Growth of moose calves conceived during the first versus second estrus. Alces 30:91–100.

It has been hypothesized that a low bull:cow ratio can result in delayed or late breeding in some female moose (Alces alces). A consequence of late breeding is late born calves. It also has been speculated that late born calves grow faster and eventually attain a size similar to early born calves. We tested this accelerated growth hypothesis by breeding cow moose during their first or second estrus and tracking the growth rates of their calves. We conducted the experiment over a 4-year period using 10 mature cow moose that produced 33 calves in 22 litters. Birth mass of calves conceived during the first and second estrus did not differ (P = 0.613) but mass of single calves was greater (P = 0.006) than twins, regardless of date conceived. Body mass gained from birth through autumn (Oct) of calves born to cows bred during their first estrus was significantly (P = 0.0019) greater than calves conceived during the second estrus. However, by spring (May), mass gain was not significantly different (P = 0.1368) between the two groups. We reject the hypothesis that second estrus calves exhibit accelerated growth during their first summer of life. Body mass of second estrous calves, however, increased at a faster rate than that of first estrus calves during winter (P = 0.0094), indicating the potential for accelerated growth at least while on a high nutritional plane. By autumn as yearlings, mass of second estrous born calves was not significantly different (P = 0.125) than mass of first estrous calves, indicating compensatory growth for second estrous calves during their second summer. There was no relationship (P = 0.01424) between April body mass of short yearlings and their gain in body mass over summer. We concluded that second estrous calves do not gain more mass by fall and, consequently, enter winter at a lower body mass. As a result, they are more likely to be susceptible to winter mortality, especially in deep snow years. We discuss management implications are discussed.

## APPENDIX H

Stephenson, TR. R., J. W. Testa, G. P. Adams, R. G. Sasser, C. C. Schwartz, and K. J. Hundertmark. 1995. Diagnosis of pregnancy and twinning in moose by ultrasonography and serum assay. Alces 31:167–172.

We evaluated transrectal ultrasonography and serum assay for detecting pregnancy in captive and wild moose (*Alces alces*). Ultrasonographic determination of twinning appeared most feasible during days 30–80 of gestation. During December, January, and March, pregnancy, but not twinning, was reliably detected ultrasonographically; diagnosis was confirmed by the presence of a fetus or placentomes. In addition, serum was assayed for pregnancy-specific protein B (PSPB). During December, January, and March, both techniques were 100% accurate in diagnosing pregnancy. However, accuracy of diagnosis during November was 95% and 90% by ultrasound and PSPB assay, respectively, based on our assumption that false positives did not occur with ultrasonography. Detection of the presence of a conceptus in utero eliminates calf detection biases associated with postpartum assessment of moose population productivity.

#### **APPENDIX I**

Schwartz, C. C., S. L. Monfort, P. H. Dennis, and K. J. Hundertmark. 1995. Fecal progestagen concentration as an indicator of the estrous cycle and pregnancy in moose. J. Wildl. Manage. 59:580–583.

We established a noninvasive technique for monitoring the estrous cycle and pregnancy in moose (*Alces alces*). We collected fecal samples daily throughout gestation from 3 pregnant adult cows. We analyzed samples by radioimmunoassay (RIA) to quantify the concentration of progestagen metabolites. Peak luteal phase concentrations of progestagens were  $4.5 \pm 0.9$  (SD)  $\mu$ g/g compared with  $0.5 \pm 0.1 \mu$ g/g during the follicular phase. Observed (n = 7) and suspected (n = 2) matings all occurred within  $\pm 2$ days of the nadir in progestagen excretion. Fecal progestagens were above peak luteal phase concentrations by the eighth week of gestation (>7  $\mu$ g/g feces, P < 0.001), permitting accurate pregnancy detection by this time. Relative concentrations of fecal progestagens during the follicular phase, luteal phase, and pregnancy were 1:10:35, respectively. Monitoring fecal progestagen is a useful noninvasive technique for tracking ovarian activity and pregnancy in moose.

## APPENDIX J

Whittle, C. L. 1999. Putative pheromones in the urine of male moose: evolution of honest advertisement? Unpublished MS Thesis. University of Alaska Fairbanks.

I tested hypotheses of how olfactory communication is related to mating behavior in Alaska moose (*Alces alces gigas*). Males dig rutting pits where urine is deposited to which females strongly respond. Consequently, male urine may contain primer pheromones that synchronize estrus of females. Urine samples were collected from captive moose on the Kenai Peninsula, Alaska. Samples included those from the mating season and from the nonrutting period for 2 adult males, 1 yearling male, and 1 male and female calf. After pH adjustment, samples were extracted with methylene chloride to yield 3 fractions (acidic, neutral, and basic), which were analyzed by gas chromatography-mass spectrometry. Putative pheromones include unsaturated alcohols and homologs of tetrahydro-6-methyl pyranone, and 2-nonen-4-one. I hypothesize that these compounds are related to hypophagia and catabolism of body reserves by rutting males, and thereby provide an honest advertisement of body condition in moose.

## APPENDIX K

Bubenik, G. A., C. C. Schwartz, and J. Carnes. 1994. Cortisol concentrations in male Alaskan moose (Alces a. gigas) after exogenous ACTH administration. Alces 30:65–69.

Blood levels of cortisol were determined in 5 yearling Alaska moose after an exogenous administration of 40 I.U. of ACTH. A rapid elevation of cortisol concentration (over 15  $\mu$ g/100ml) within 60 min. of ACTH injection demonstrated an unexpectedly high level of adrenocortical response to a simulated stress. The results in moose are compared to several other deer species.

#### APPENDIX L

Schwartz, C. C., D. L. Harmon, K. J. Hundertmark, C. T. Robbins, and B. A. Lintzeñich. 1996. Carbohydrase activity in the pancreas and small intestine of moose and cattle. Alces 32:25–30.

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  $\alpha$ -amylase concentrations were higher in moose (4228 µmol/g tissue/min) than the steer (1104 µmol/g tissue/min), and intestinal maltase concentrations were similar between moose (0.33 µmol/g tissue/min) and the steer (0.47 µmol/g tissue/min). Additionally, moose produced concentrations of isomaltase (0.6 µmol/g tissue/min) at a rate similar to values published for cattle (0.4–0.6 µmol/g tissue/min). 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.

#### APPENDIX M

Shochat, E., C. T. Robbins, S. M. Parrish, P. B. Young, T. R. Stephenson, and A. Tamayo. 1997. Nutritional investigations and management of captive moose. Zoo Biology 16:479–494.

Historically, moose have been difficult to maintain in captivity when on diets of grass or legume hays and grain due to enteritis that frequently leads to chronic diarrhea/wasting disease. The development of wood-fiber diets has increased the lifespan of moose in captivity, but these diets do not completely prevent chronic wasting. Purina Mills (St. Louis, MO) hypothesized that captive moose are unable to digest starch that escapes the rumen, and therefore abnormal bacterial fermentation in the hindgut causes chronic diarrhea. An earlier study found no evidence of a digestive problem, so we tested the hypothesis that moose have difficulty metabolizing excess proprionate produced from the fermentation of starch found in traditional cervid rations and high-grain wood-fiber diets. When challenged with an i.v. proprionate load, moose metabolized proprionate similar to healthy mule deer and domestic livestock. We then tested the hypothesis that grass forage is an initiating factor to chronic diarrhea/wasting and further hypothesized that grass, alfalfa, and other agriculture-based forages in association with an aierobic bacteria produce inflammatory bowel disease (IBD) in moose. Captive moose that had ad libitum access to a wood-fiber pelleted moose diet and grazed in grass pastures developed chronic wasting symptoms at 2-4 years of age and died at 4.7 + 0.3 years unless restricted from grass before the development of advanced symptoms. We isolated Bacteroides vulgatus in the feces and successfully treated a moose with chronic diarrhea/wasting disease with long-term metronidazole therapy, indicating that the chronic enteritis causing wasting disease arises from a bacteria-associated defective immunosuppressive response similar to IBD in other species. Further support for the IBD cause of wasting in moose is that this animal will relapse within hours if the metronidazole treatment is discontinued even after many months. We developed a highly palatable high-fiber, low-starch moose ration that can be fed as the sole source of nourishment, although additional research and dietary improvements are required.

#### APPENDIX N

Spaeth, D. F., K. J. Hundertmark, R. T. Bowyer, P. S. Barboza, T. R. Stephenson, and R. O. Perterson. 2000. Incisor arcades of Alaskan moose: are the sexes dimorphic? Manuscript submitted for publication in *Alces*.

We tested whether size of incisor arcades of Alaskan moose (Alces alces gigas) differed between males and females of known age. Lower jaws were collected from moose killed by hunters and those dying of natural causes or from collisions with vehicles. We measured the breadth (width) and the depth (protrusion) of the incisor arcades of moose. Age of those jaws was determined by counting cementum annuli of incisors. Arcade breadths of moose followed von Bertalanffy models of growth with an asymptote at about 4 years of age for both sexes. Regression models differed between the genders of moose; size of arcade breadth for males was significantly larger than for females. Body masses of females also reached an asymptote at 4 years old; however, males do not attain an asymptote until 8-10 years of age. When incisor breadth was considered relative to maximum body mass, incisor breadth of males was less than that of females. Coefficients of incisor breadth relative to body mass scaled similarly for male (0.249) and female (0.260) Alaska moose. Incisor depth, however, did not differ between the sexes when depth was corrected for age. Our data indicate that incisor breadth, but not depth, scaled with body mass. Thus, muzzle morphology may exhibit more plasticity than previously thought. We hypothesize that muzzle architecture of moose, as reflected in incisor breadth and depth, relates to the diets of the sexes when they are spatially segregated. Whether incisor dimensions are a cause or consequence of sexual segregation, however, is uncertain.

#### **APPENDIX O**

Chapters contributed to the book *Ecology and management of the North American moose*, edited by A. W. Franzmann and C. C. Schwartz, by past and present personnel from the Kenai Moose Research Center.

- Crichton, F. J., W. E. Regelin, A. W. Franzmann, and C. C. Schwartz. 1998. The future of moose management and research. Pages 655–663 *in* A. W. Franzmann and C. C. Schwartz, eds. Ecology and management of the North American moose. Smithsonian Institution Press. Washington, D.C.
- Franzmann, A. W. 1998. Restraint, translocation and husbandry. Pages 519–557 *in* A.W. Franzmann and C. C. Schwartz, eds. Ecology and management of the North American moose. Smithsonian Institution Press. Washington, D.C.
- Hundertmark, K. J. 1998. Home range, dispersal and migration. Pages 303–335 in A.W. Franzmann and C. C. Schwartz, eds. Ecology and management of the North American moose. Smithsonian Institution Press. Washington, D.C.
- Renecker, L. A., and C. C. Schwartz. Food habits and feeding behavior. Pages 403–439 *in* A. W. Franzmann and C. C. Schwartz, eds. Ecology and management of the North American moose. Smithsonian Institution Press. Washington, D.C.
- Schwartz, C. C. 1998. Reproduction, natality and growth. Pages 141–171 *in* A. W. Franzmann and C. C. Schwartz, eds. Ecology and management of the North American moose. Smithsonian Institution Press. Washington, D.C.
- ——, and L. A. Renecker. 1998. Nutrition and Energetics. Pages 441–478 *in* A. W. Franzmann and C. C. Schwartz, eds. Ecology and management of the North American moose. Smithsonian Institution Press. Washington, D.C.

#### **APPENDIX P**

## Rodgers, A. R., S. M. Tomkiewicz, E. J. Lawson, T. R. Stephenson, K. J. Hundertmark, P. J. Wilson, B. N. White, and R. S. Rempel. 1998. New Technology for moose management: a workshop. Alces 239–244.

This paper provides outlines of presentations made during a special session devoted to new technology for moose management at the 4<sup>th</sup> International Moose Symposium and 33<sup>rd</sup> North American Moose Conference and Workshop, Fairbanks, Alaska, May 17–23, 1997. The intent of this session was to provide an overview of emergent technology that may benefit moose management and to suggest future directions for research. Advancements in the use of Global Positioning Systems technology for tracking moose and other wildlife were outlined. The performance of these new systems under both controlled and field situations were discussed. Recent progress in the application of ultrasonography to the assessment of moose nutritional and reproductive condition was presented. Prospects for the application of new genetic techniques, particularly molecular genetic markers, to the understanding and management of moose populations was outlined and demonstrated. This special session highlighted a wide variety of new technologies that may have significant impacts on moose management in the near future and into the 21<sup>st</sup> century.

### APPENDIX Q

Hundertmark, K. J., and C. C. Schwartz. 1996. Considerations for intensive management of moose in Alaska. Alces 32:15–24.

The Alaska Legislature recently passed a law directing the Alaska Board of Game to identify certain game populations that will be managed intensively. This mandate implies management for maximum sustained yield (MSY), yet managing populations for MSY is problematic. Overharvest at MSY may cause populations to decrease to low levels, and in the presence of predation low-density equilibria can be established. We recommend maintaining intensively managed populations at densities above the actual point of MSY to avoid potential overharvests caused by stochastic variation in recruitment. Managing intensively will require better information on factors that influence recruitment and corresponding rates of increase in moose populations, including age at first reproduction; rates of pregnancy, twinning, age-specific survival, dispersal, predation, and population sex ratios. Population modeling indicates that rate of increase is most sensitive to changes in adult survival, but under most circumstances in real moose populations, calf survival is very important. Factors affecting calf survival include habitat quality, weather, and predation; maintaining moose densities slightly above those that maximize recruitment can minimize effects of these factors. An intensive management strategy for moose populations in Alaska must include the ability to implement cow harvests, predator management, and habitat management. Aggressive monitoring of population parameters, cause-specific mortality rates, trends in habitat quality, and a knowledge of carrying capacity will be essential to selecting appropriate management strategies. Gaining this information will be expensive, but the alternatives are potential mismanagement and risk of population declines.