Alaska Department of Fish and Game
Division of Wildlife Conservation

Federal Aid in Wildlife Restoration
Research Progress Reports
1 July 1994- 30 June 1995

MOOSE RESEARCH CENTER REPORTS

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PAT COSTELLO

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Study 1.45 Evaluation and Testing of Techniques for Moose Management
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Studies 1.42, 1.45 (2), and 1.48
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STATE OF ALASKA
Tony Knowles, Governor

DEPARTMENT OF FISH AND GAME
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DIVISION OF WILDLIFE CONSERVATION
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ESTIMATION OF BODY COMPOSITION IN MOOSE

STUDY 1.42

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RESEARCH PROGRESS REPORT

STATE: ALASKA **STUDY. NO.:** 1.42

COOPERATOR: KENAI NATIONAL WILDLIFE REFUGE, SOLDOTNA, ALASKA

GRANT NO.: W-24-3

STUDY TITLE: ESTIMATION OF BODY COMPOSITION IN MOOSE

PERIOD: 1 JULY 1994-30 JUNE 1995

SUMMARY

We evaluated techniques for estimation of body composition in moose under field conditions. Body water was estimated via bioelectrical impedance analysis (BIA) for 3 moose. These animals were euthanized and tissue samples were analyzed for protein, water, fat, and ash content. Additionally, the peroneus muscle group was dissected from these individuals and submitted to the same analyses. Percentage fat in each of 3 depots (hide, carcass, and viscera) declined linearly with declines in percentage body fat (ingesta-free body), indicating that fat from all depots were mobilized simultaneously during periods of energy deficit. Fat in carcass and viscera was expressed as percentage of total fat in the body (rather than percent of total depot mass) and was plotted against percentage body fat. At body fat levels approaching 20%, these 2 depots each represented approximately 50% of the total body fat. As total body fat declined, the visceral fat was almost completely catabolized, leaving the small amount of carcass fat as the predominant energy source. Our data indicated 2 divergent relationships between percentage body fat and percentage fat in the peroneus muscle. We cannot provide an explanation of this observation at this time. Predicting volume and percentage of water and fat in the ingesta-free body via BIA was improved dramatically by analyzing each sex separately. Using stepwise regression with BIA parameters and body measurements as predictors, we found all models with the exception of water volume in females contained a BIA parameter.

Key words: *Alces alces*, BIA, bioelectrical impedance analysis, body composition, body fat, body water, moose, urea dilution, weight.

CONTENTS

BACKGROUND

Body condition was identified as a critical variable within the moose carrying capacity model (Hubbert 1987, Schwartz et al. 1988*a*, 1988*b*), and body fat is a major driver of the moose submodel. Body fat must be accurately measured in moose. A proposal was prepared to test methods for estimating body composition of moose (Schwartz et al. 1988c), focusing primarily on measurement of urea space (Preston and Kock 1973), as an in vivo technique, and measurement of composition of the peroneus muscle group (peroneus tertius, extensor digitorum longus, and extensor digiti III proprius, Huot and Goodreault 1984), as a technique for use on dead animals.

Over the last decade a body composition estimation technique known as bioelectrical impedance analysis (BIA) has been demonstrated to be a precise and unbiased predictor of human body composition (Lukaski 1987) and is being investigated for potential use in animal applications (Hall et al. 1989, Jenkins et al. 1988, Swantek et al. 1991). This technique works on the principle of measuring the impedance (resistance to alternating current) of hydrated body tissues to an alternating current of known frequency. Nyboer et al. (1943) demonstrated that

$$V = rL^2/I$$

where V = body water volume, r = volume resistivity and is constant for a given conductor, L = conductor length, and I = impedance. Impedance is computed by $(R_s^2 + Xc^2)^{0.5}$ where R_s =

resistance and Xc = reactance. This technique was tested as a potential indicator of moose body composition.

Implicit in the estimation of body composition by the aforementioned techniques is an accurate measurement of body weight. Obtaining weights of free-ranging moose is difficult because they are often too heavy to be lifted and weighed by a helicopter. Franzmann et al. (1978) and Haigh et al. (1980) reported high correlations between certain body measurements and body weight of moose; however, body measurements are insensitive to changes in the fat depots of moose. Franzmann et al. (1976) developed a subjective 11-point scale for assessing condition class of moose based on physical appearance that may, when combined with body length, more precisely predict body weight.

OBJECTIVES

To determine the relationship between urea space measurements, impedance measurements, chemically determined composition of the peroneus muscle group, and chemically determined body composition in moose.

To determine if moose body weight can be predicted accurately from measurement of body length and heart girth, and appraisal of physical appearance.

To determine if these techniques have potential to estimate moose body composition in field applications.

METHODS

JOB 1. ACQUIRE AND MAINTAIN EXPERIMENTAL ANIMALS

Experimental animals were obtained from the pool of surplus animals of the Kenai Moose Research Center (MRC) herd and included 4 adult females and 1 male calf. These animals were kept in captivity at the MRC and were fed a controlled ration (Schwartz et al. 1985). All animals were sampled and euthanized between 11-25 January 1994.

JOB 2. DETERMINE THE BODY WATER CONTENT OF EXPERIMENTAL ANIMALS VIA UREA DILUTION

Work on this aspect of the project was halted in 1993.

JOB 3. DETERMINE THE BODY WATER CONTENT OF EXPERIMENTAL ANIMALS VIA BIA

A plethysmograph (Model BIA-101, RJL Systems, Inc. Detroit, MI) was used to estimate electrical impedance of moose. The animals were allowed to assume a sternally-recumbent position after immobilization. Any variation in positioning of animals was corrected so that all animals were tested in similar positions. Electrodes were constructed from trocars removed from 18ga spinal needles and were bent to an angle of 90°, 13mm from the tip. A "source" electrode was inserted subdermally at the carpal joint on the foreleg and at the joint between the metatarsus and the hoof on the hind leg on the side of the body most exposed while the moose was sternally-recumbent. A "detector" electrode was placed 7.5 cm proximal to each source electrode. The tips of the electrodes were oriented distally under the skin. Electrodes were connected to the plethysmograph via alligator clips on the end of 10-ft cables. Resistance and reactance were recorded as well as total body length and heart girth. Electrodes were removed and re-inserted, and resistance and reactance measured a minimum of 3 times per animal to ascertain variation associated with electrode placement. Variation associated with animal position also was estimated by taking readings with the hind leg in various positions. The straight-line distance between the knee and the tarsal joint was measured to the nearest cm. This distance increased as the leg was straightened, and changes in BIA were noted at various distances.

JOB 4. DETERMINE BODY COMPOSITION OF EXPERIMENTAL ANIMALS

The animals were killed immediately after we obtained BIA measurements. The animals were eviscerated and skinned (with as much fat as possible being left on the carcass). The empty carcasses were bisected along the spinal column, with one half of the carcass frozen for analysis. The peroneus muscle groups were dissected from the carcass halves not used in the analysis and were frozen. The digestive tract was emptied, and the ingesta and viscera were weighed separately. Entire viscera and samples of ingesta and shaved hide were frozen. The frozen side of the carcass and the visceral mass were cut into 51- and 25-mm slices, respectively, with a commercial band saw. The sawdust that accumulated at the base of the blade was collected for each component, thoroughly mixed and refrozen, and shipped along with ingesta, hide and peroneus samples to the Wildlife Habitat Laboratory of Washington State University for determination of crude fat, crude protein, ash, and water content (Huot and Picard 1988). We determined crude fat by methanol-chloroform extraction, crude protein content by the Kjeldahl procedure (AOAC 1975), ash content by burning in a muffle furnace at 550°C for 2h, and percent organic dry matter (1.00 - moisture content) by drying samples in a 100°C oven for 12-16h and subtracting ash content. Three replicates of each sample were analyzed. Peroneus and hide samples were freeze-dried and ground in a Wiley mill before being subject to chemical analysis.

JOB 5 DETERMINE IF BODY WEIGHT OF MOOSE CAN BE PREDICTED ACCURATELY FROM TOTAL BODY LENGTH AND VISUAL APPRAISAL

Moose at the MRC that were immobilized and weighed during this reporting period were subject to visual appraisal of condition class (Franzmann 1977). Total body length, heart girth and condition class were used in a multiple regression analysis to predict live weight.

JOB 6 EXAMINE THE RELATIONSHIP BETWEEN UREA SPACE, IMPEDANCE VALUES, PERONEUS FAT, AND BODY COMPOSITION ESTIMATES

Peroneus composition and BIA values ([total length]^2/I and I) were compared to body composition estimates by simple linear and/or stepwise multiple regression with live weight and total length as additional predictors. In this report references to "body" composition refer to the ingesta-free body, which is the entire body less hair and the contents of the gastrointestinal tract. Swantek et al. (1991) demonstrated that resistance and/or reactance (and by extension their product I) were occasionally better predictors of body fat and water than the traditional parameter [total length]^2/I (TL^2/I). Nyboer (pers. commun.) suggested that conductance and susceptance, which are the reciprocals of resistance and reactance, respectively, were truly the parameters of interest. Packed cell volume was also used as a predictor because it served as an index of dehydration, which accompanies malnutrition and affects BIA measurements (Brodie et al. 1991). We constrained the stepwise regression procedure to minimize multicollinearity by instructing the software not to enter predictors that were highly correlated with predictors already in the model (Wilkinson 1990). Adjusted coefficients of multiple determination (R_a^2) were reported for multiple regressions rather than raw R^2 values because unlike R^2, R_a^2 is not influenced by the number of independent variables in the model (Neter and Wasserman 1974:229).

RESULTS AND DISCUSSION

JOB 2. DETERMINE THE BODY WATER CONTENT OF EXPERIMENTAL ANIMALS VIA UREA DILUTION

We discontinued this part of the research program when it became apparent that urea dilution was not a practical technique for monitoring condition of animals in captivity or in the field (Hundertmark et al. 1993).

JOB 3. DETERMINE THE BODY WATER CONTENT OF EXPERIMENTAL ANIMALS VIA BIA

Resistance and reactance measurements of all moose are listed in Table 1.

Variation in BIA measurements was related to position of the hind leg. As distance between the knee and tarsal joint increased, TL^2/R decreased (Fig.1). Analysis of covariance indicated that animal ($F = 37.3$, $P < 0.0001$) and distance ($F = 12.4$, $P = 0.0038$) effects both were significant ($P < 0.0001$) predictors of variation in TL^2/R.

JOB 4. DETERMINE BODY COMPOSITION OF EXPERIMENTAL ANIMALS

Percent body fat of all animals ranged between 0.3% and 19.4% (Table 2), and based upon weight and condition class of these animals, we believe this represents the entire range of fatness likely to be encountered in wild moose as well as extremely lean animals. We observed linear relationships between body fat, visceral fat, skin fat, and empty carcass (skinned and eviscerated) fat, expressed as percentages of weight (Fig. 2). This illustrated that mobile fat depots (with the possible exception of marrow fat, which was not measured in this study) were used simultaneously and that the sequence of fat mobilization described by Harris (1945) referred only to the sequence of disappearance of these depots based upon visual appraisal, which was dependent upon their original size.

Water content of the ingesta-free body was related inversely to fat content. A Model II (major axis) regression yielded the following relationship: Y(% water) = 73.3 - 0.92*X(% fat), indicating the fat-free body consisted of 73.3% water which is in agreement with the interspecies mean of 73.2% (Pace and Rathbun 1945).

When weight of depot fat was expressed as a percentage of the weight of body fat (g depot fat/100 g total fat), we found carcass fat represented the largest fat depot available to the animal, followed by visceral fat. Previously (Hundertmark et al. 1992, 1993) we reported the percentage of body fat comprised of each depot was constant over the observed range of body fat. With the addition of new data, we detected curvilinear relationships (Fig. 3). Carcass fat contributes a greater percentage to body fat at low body fat concentrations and declines as the animal fattens; the opposite relationship exists for visceral fat. At extremely low levels of body fatness, fat in the carcass decreases at a greater rate than visceral fat, which may be attributable to the catabolism of marrow fat.

JOB 5. DETERMINE IF BODY WEIGHT OF MOOSE CAN BE PREDICTED ACCURATELY FROM TOTAL BODY LENGTH AND VISUAL APPRAISAL

The animals we sampled this year were added to this analysis and the regression parameters (SEE and R^2) indicated that variation among animals increased (Table 3). The best predictive equation for live weight, based upon these parameters, included total length and condition class, and accounted for 88% of the variation in live weight. It is not surprising that heart girth was not included in the best model as this measurement has a high level of variability because it is difficult to measure on an immobilized moose.

There were some notable differences between our results and those reported elsewhere for weight-length relationships (Franzmann et al. 1978, Haigh et al. 1980). The correlation (r) between TL and LW for our data was 0.83, which was not as high as 0.94, reported by Franzmann et al. (1978) but was higher than 0.71, reported by Haigh et al. (1980). These differences may be attributable to the considerably greater sample size reported by Franzmann et al. (1978) and the fact that Haigh et al. (1980) measured weight by suspending moose from a helicopter and recording weight only to the nearest 5 kg. Haigh et al. (1980) also subtracted a subjective estimate of antler weight from live weight of males, increasing variation of the estimate.

Regression equations presented by Franzmann et al. (1978) and Haigh et al. (1980) for predicting live weight as a function of total length do not describe the variation in our data (Fig. 4). A major difference between our study and theirs is that we have only 1 animal less than 1.5 yr old in our sample. Also, Haigh et al. studied a smaller subspecies (*A. a. andersoni)* than that studied by Franzmann et al. (1978) and this study (*A. a. gigas*). The relative positions of the regression lines (Fig. 4) indicated representatives of *A. a. andersoni* are shorter than representatives of *A. a. gigas* of the same weight. This is an important consideration when choosing a model for predicting weight for different subspecies. Nonetheless, we expected better agreement between our data and the relationship described by Franzmann et al. (1978) because both studies dealt with the same subspecies. We attribute the difference to condition of animals at the time of sampling. Franzmann et al. (1978) sampled wild animals, whether they were free-living or enclosed by fences at the MRC, during winter; therefore, their sample likely contained mostly animals on the lower end of the condition scale, indicating moderate to poor body condition. Our sample included animals held in captivity that represented what was likely a wider array of body condition. The distribution of our data in Fig. 4 with respect to condition class supports this explanation.

During this reporting period we assisted T. Stephenson (Univ. of Idaho) in additional testing of the effectiveness of a portable ultrasound unit in measuring subcutaneous rump fat depths. Data collected this year will supplement those collected previously (Stephenson et al. 1993). This technique measured fat depths accurately and delineated the size of the rump fat depot. This exercise demonstrated to us that rump fat can exist on an animal even though visual appraisal and/or palpation did not detect it. As the presence/absence of rump fat is a criterion in determining condition class, use of this technique may provide a less biased alternative in appraising animal condition.

JOB 6 EXAMINE THE RELATIONSHIP BETWEEN UREA SPACE, IMPEDANCE VALUES, PERONEUS FAT, AND BODY COMPOSITION ESTIMATES

The peroneus muscle group was collected from the 3 moose sampled this year. Adding these observations to those collected in previous years, we determined that 2 distinct trends were apparent in the relationship between peroneus fat and body fat (Fig. 5). Hout and Goodreault (1985) reported a high correlation between percent peroneus fat and percent body fat ($r=0.96$) for caribou (*Rangifer tarandus*). Their model, represented by the line in Fig. 5, describes variation in 1 group of our data. A second group, however, is distinct from this model, and we cannot explain why these data would diverge so dramatically.

Analysis of covariance of a model with sex as a treatment and TL^2/R as a covariate indicated that both variables are significant predictors of IFB water volume (sex: $F = 5.46$, $P = 0.034$; TL^2/R: $F = 25.73$, $P < 0.01$). This model was analyzed without the data for the animal Angel, whose BIA readings are suspect (Hundertmark et al. 1994).

Simple and multiple linear regression models derived were computed via stepwise regression to describe variation in body water and fat (volume and percentage) for each sex separately (Table 4). All models were highly significant and all, with the exception of water volume in females, included a BIA parameter. Live weight was included in water volume models for both sexes and fat volume for males. Fat volume in both males and females can be predicted by models not containing a body weight term, which would be advantageous in field situations. Standard errors decreased dramatically from those reported in previous years, primarily due to a separation of sexes in the analysis.

We realize that our sample sizes are extremely small for this type of analysis, and that regression parameters could change significantly with the addition of more animals. We present these data as preliminary and caution against drawing conclusions from the information presented here. We believe, however, that our data indicate further research is warranted.

RECOMMENDATIONS

This study should continue for 1 year. During the coming year we will complete our analysis with the addition of 4-6 animals to our sample. Historic records of moose measurements from the MRC will be analyzed for relationships to predict weight.

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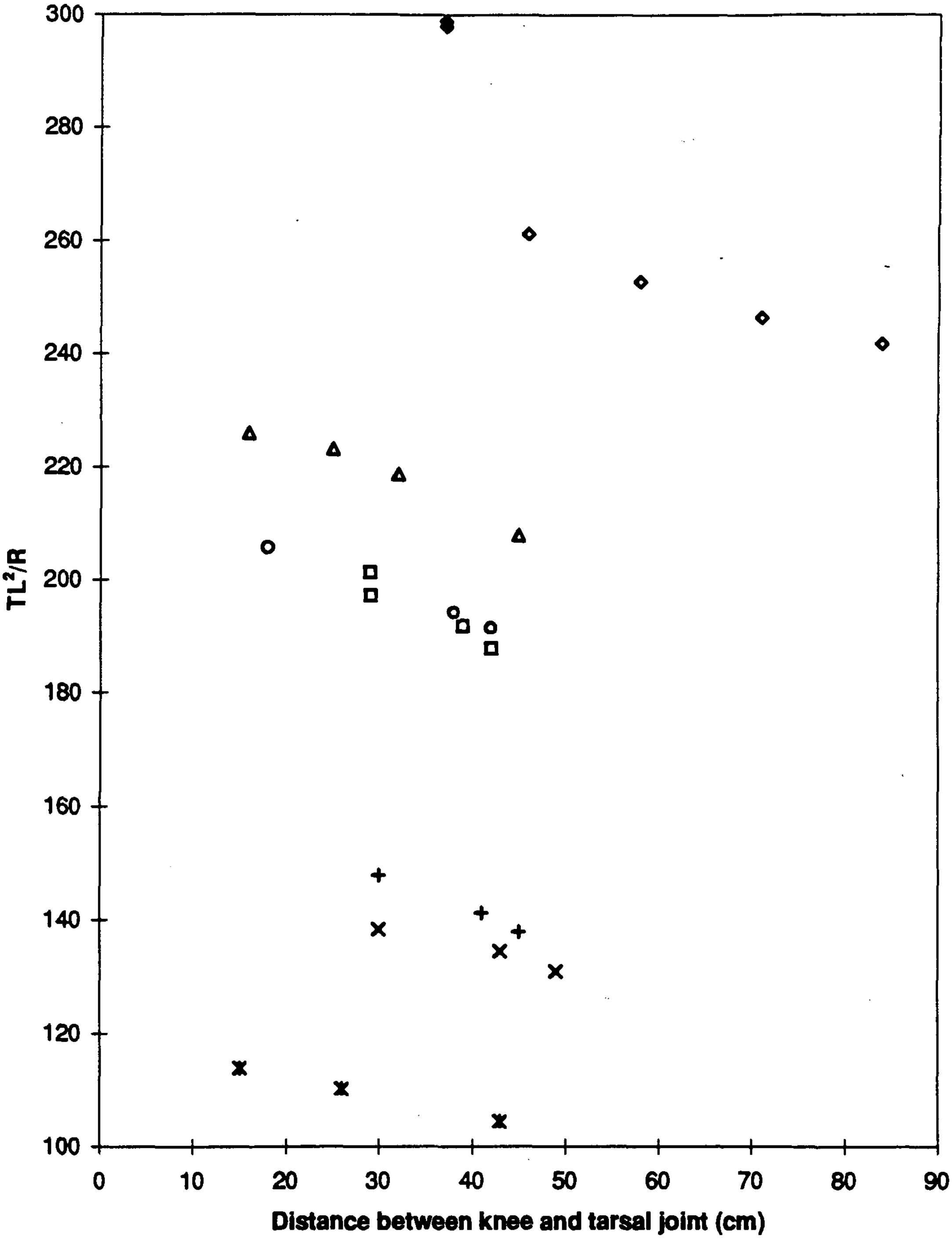

Figure 1 The relationship between the distance between the knee and tarsal joint, representing an index of hind leg straightness and TL^2/R. Individual animals are represented by different symbols.

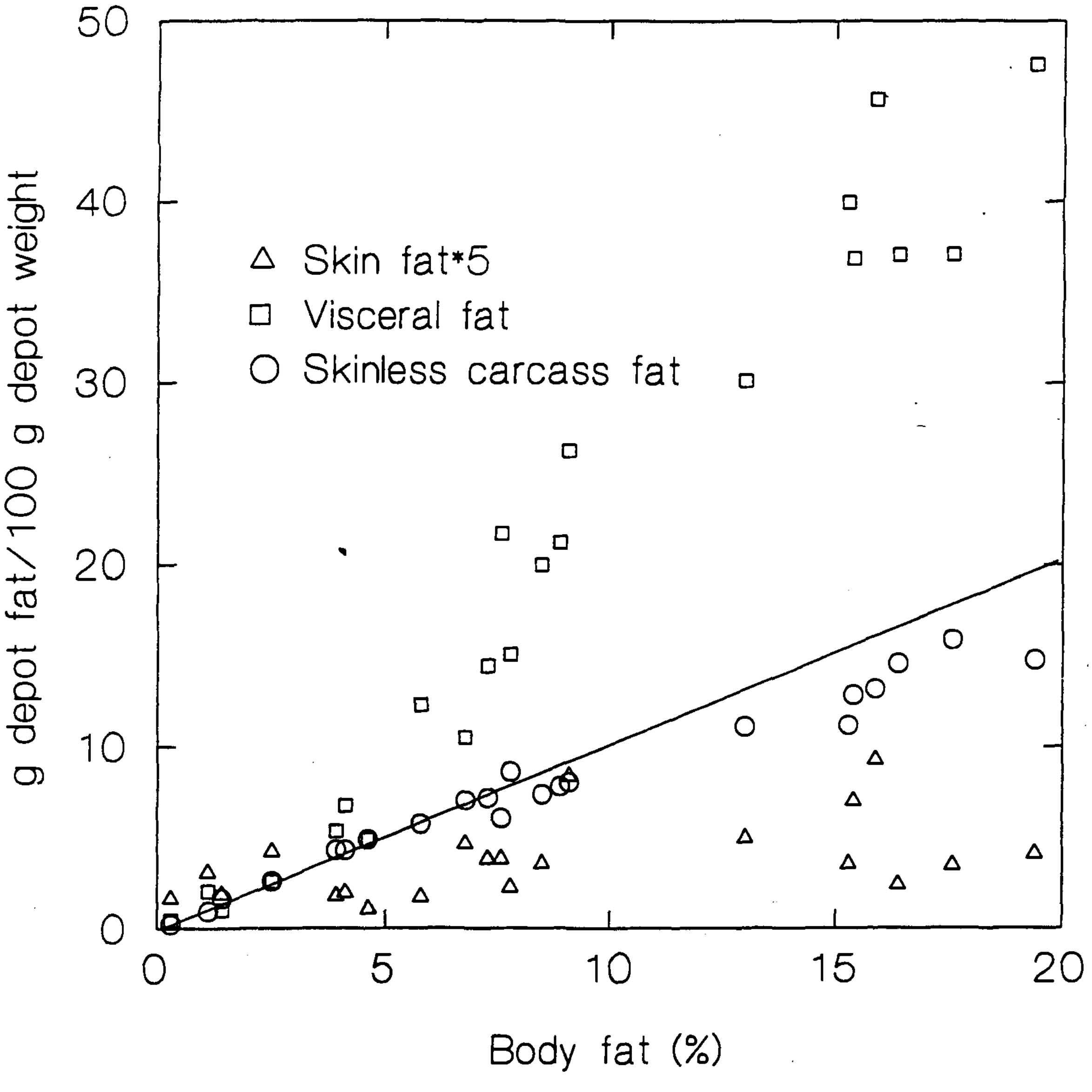

Figure 2 The relationship between percent body fat and percent fat in the shaved skin, skinless carcass and viscera components. The line represents the relationship between carcass fat and IFB fat in caribou (Huot and Goodreault 1984).

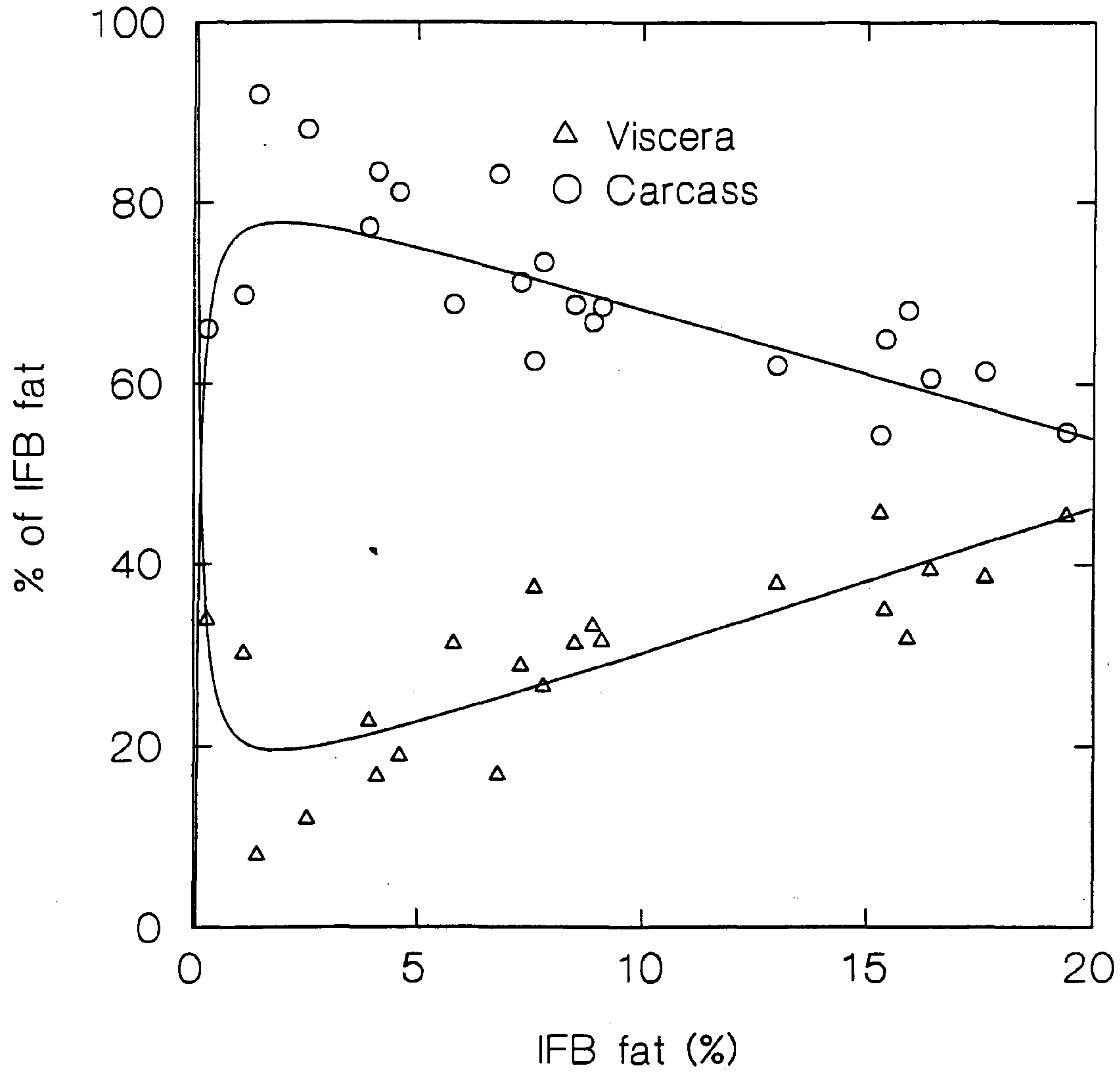

Figure 3 The relationship between percent body fat and the percentage of body fat comprised by the fat in carcass and viscera depots (expressed as g depot fat/100 g body fat). The models are of the form $y=a+bx+cx^{-1}$.

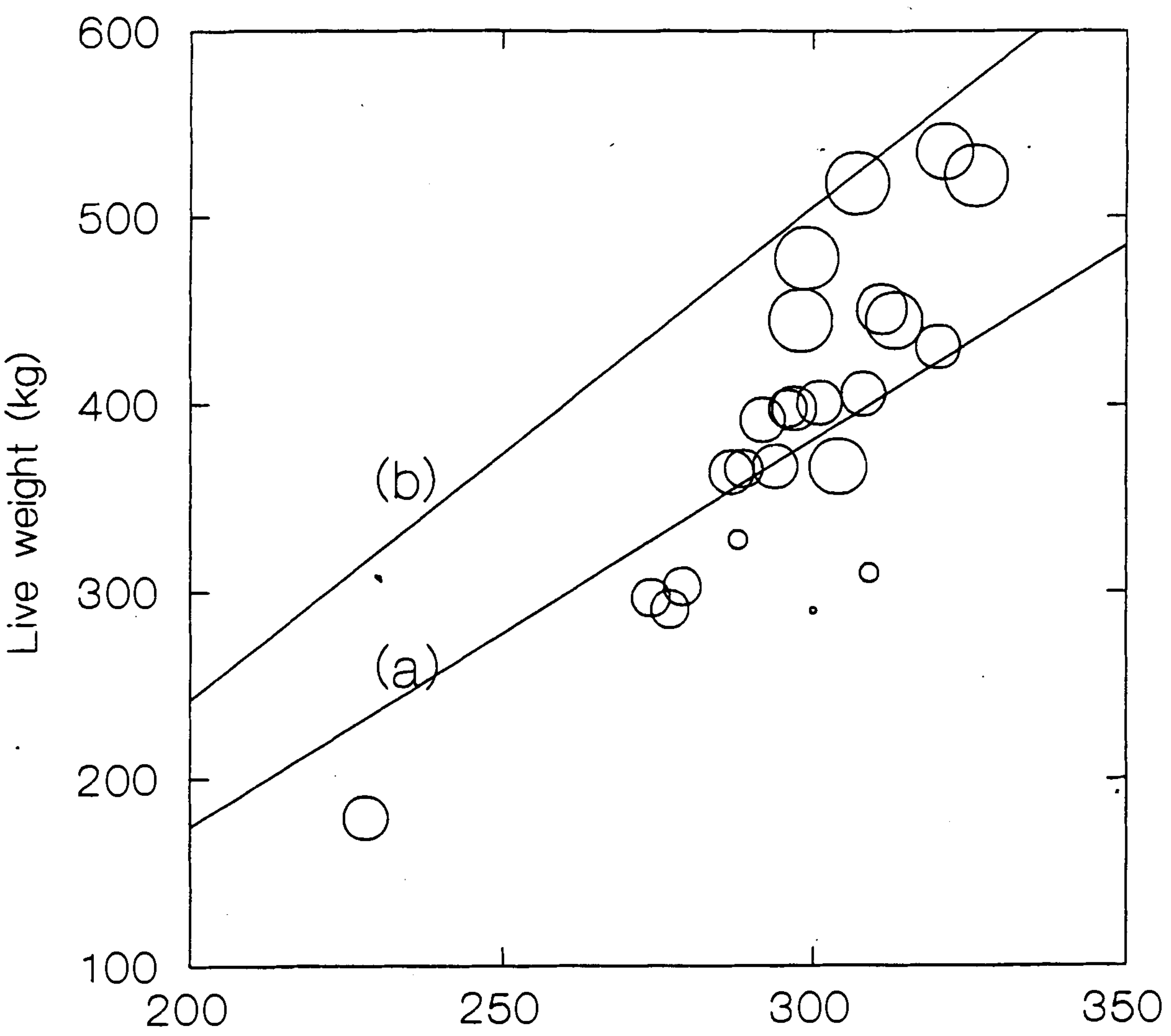

Figure 4 The relationship between LW and TL. Lines were calculated from predictive equations from (a) Franzmann et al (1978) and (b) Haigh et al. (1984). Relative size of data points indicates CC, with the largest circles representing CC = 10 (best) and the smallest circle representing CC = 1 (worst).

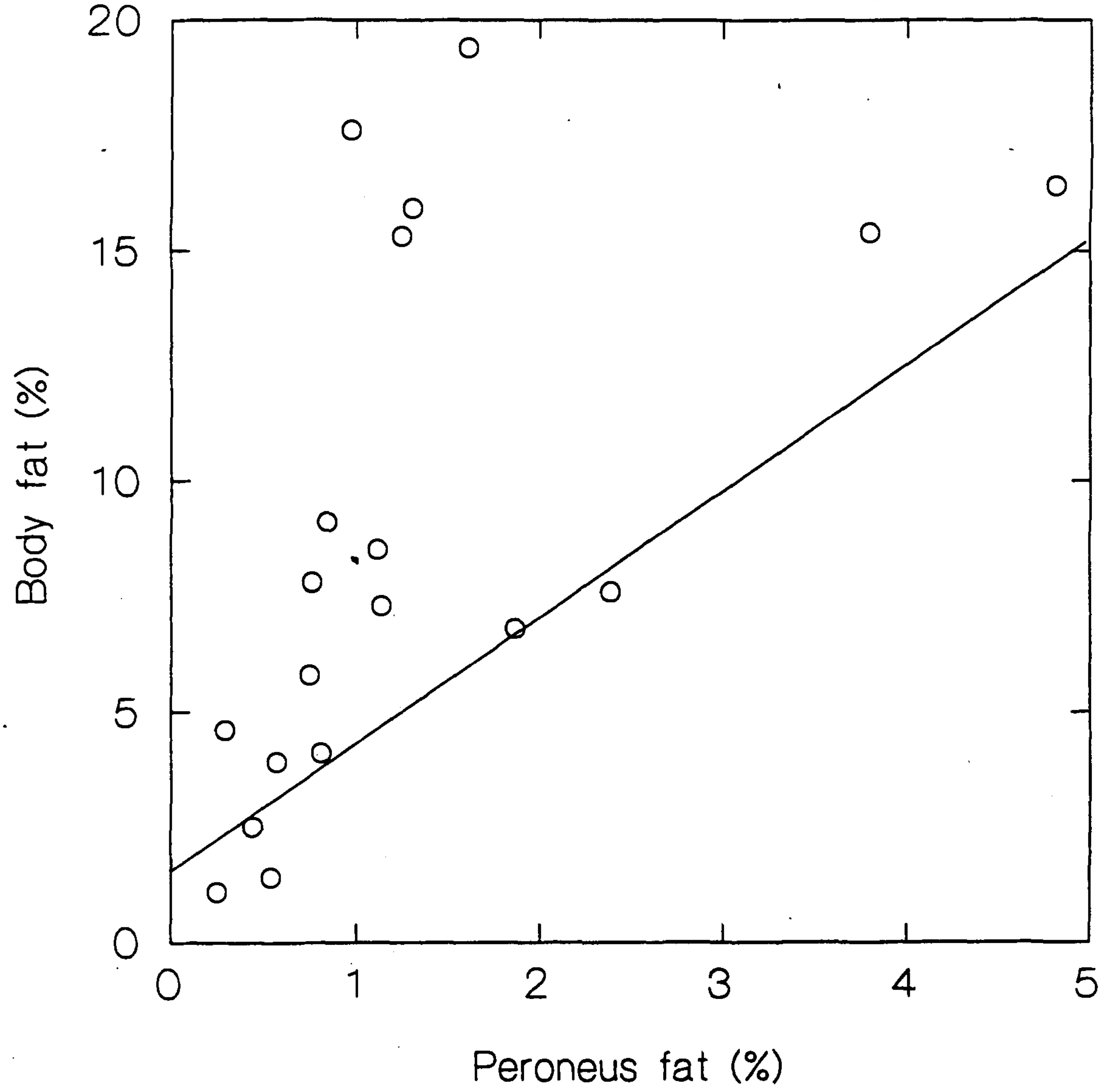

Figure 5 The relationship between peroneus fat and body fat. The line was calculated from the relationship between these 2 parameters in caribou (Huot and Goodreault 1985).

Table 1. Mean values of R and Xc and associated estimates of variation for 18 moose used in the body composition study, Moose Research Center.

			R			Xc	
Animal	N	Mean	SE	CV	Mean	SE	CV
Allye	4	406.0	15.0	3.7	29.3	1.3	4.3
Angel	5	235.6	3.5	3.3	14.0	0.0	0.0
Betsy	3	709.7	11.3	2.8	41.3	1.2	5.0
Brooks	3	302.0	1.2	0.7	21.0	0.0	0.0
Deneki	4	373.8	3.5	1.9	34.0	1.1	6.4
Hydro	3	365.3	6.1	2.9	29.3	0.3	2.0
Kelley	3	473.7	13.6	5.0	31.0	1.2	6.5
Kobuk	3	365.3	7.1	3.3	34.3	0.3	1.7
Luke	3	363.0	0.6	0.3	30.7	0.3	0.3
Mario	7	397.3	13.5	9.1	29.6	1.8	6.1
Oly	5	581.6	8.2	3.2	42.0	2.8	14.7
Sinuk	4	536.8	9.0	3.4	36.5	0.6	3.5
Sol	4	317.0	1.1	0.7	30.0	0.7	4.7
Sony	4	459.8	14.1	3.1	34.8	1.0	2.8
Stripes	3	411.7	2.3	1.0	30.0	0.6	3.3
Terra[1]	3	478.3	10.4	3.8	35.3	1.2	5.9
Terra[2]	3	449.0	2.5	1.0	31.0	0.6	3.2
Vicki	3	653.0	10.1	2.6	39.7	0.7	2.9
Zumu	3	446.7	2.0	0.8	39.3	0.7	2.9

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[1] Measured on a wet substrate

[2] Measured on a dry substrate

Table 2. Live weight (LW), ingesta-free body weight (IFBW) and proximate analysis of composition of the ingesta-free body of moose used in this study.

			Weight (kg)				% of IFBW			
Animal	LW	IFBW	Water	Fat	Protein	Ash	Water	Fat	Protein	Ash
Allye	445	402	235.8	65.9	79.2	20.6	58.7	16.4	19.7	5.1
Angel	290	252	184.5	0.7	53.6	13.2	73.2	0.3	21.3	5.2
Betsy	310	293	193.9	22.8	62.5	13.3	66.3	7.8	21.4	4.6
Bill	397	375	257.6	14.6	83.9	18.4	68.8	3.9	22.4	4.9
Brooks	535	472	278.0	74.8	94.7	24.5	58.9	15.9	20.1	5.2
Butch	350	297	218.1	4.0	59.3	15.0	73.6	1.4	20.3	5.2
Deneki	385	351	206.0	54.2	69.8	21.3	58.6	15.4	19.9	6.1
Hydro	364	312	202.7	26.5	66.3	16.8	64.9	8.5	21.2	5.4
Kelley	180	148	108.6	3.7	32.1	3.4	73.5	2.5	21.7	2.3
Kobuk	344	298	208.7	12.2	60.5	16.5	70.1	4.1	20.3	5.5
Luke	415	397	251.7	36.0	84.9	24.3	63.4	9.1	21.4	6.1
Mario	535	474	312.2	27.4	108.9	25.4	65.9	5.8	23.0	5.4
Oly	340	275	192.9	3.0	59.9	19.4	70.1	1.1	21.8	7.0
Rex	238	217	150.5	16.4	40.8	9.1	69.4	7.6	18.8	4.2
Sinuk	525	448	260.5	78.8	87.8	20.8	58.2	17.6	19.6	4.6
Sol	392	342	224.8	25.1	73.4	18.9	65.7	7.3	21.4	5.5
Sony	478	408	244.5	62.3	81.1	19.6	60.0	15.3	19.9	4.8
Stripes	309	259	177.4	11.8	55.6	14.4	68.4	4.6	21.5	5.6
Terra	510	462	259.6	89.5	93.4	19.4	56.2	19.4	20.2	4.2
Vicki	367	324	203.6	28.9	73.5	17.5	62.9	8.9	22.7	5.4
Yogi	263	212	140.8	14.5	43.9	12.8	66.4	6.8	20.7	6.0
Zumu	445	401	247.8	52.2	81.7	18.9	61.9	13.0	20.4	4.7

Table 3. Regression equations, coefficients of determination (R^2), standard errors of the estimate (SEE), and error degrees of freedom (DF) for prediction of moose live weight (LW) from total length (TL), heart girth (HG), and condition class (CC), Moose Research Center, $N = 23$.

Regression equation	R_a^2	SEE	DF
LW = 3.45(TL)-636	0.64^a	50.3	23
= 2.23(TL)+1.77(HG)-613	0.70^a	45.5	19^b
= 2.88(TL)+18.7(CC)-598	0.88^a	28.9	22
= 3.42(TL)-174.4(1/CC)-596	0.77^a	39.8	22
= 3.49(TL)-133.4(1/CC^2)-639	0.73^a	43.6	22
= 2.50(TL)+0.55(HG)+16.5(CC)-578	0.88^a	29.0	18^b
= 2.35(TL)+1.56(HG)-155.9(1/CC)-580	0.83^a	34.2	18^b
= 2.28(TL)+1.77(HG)-127.9(1/CC^2)-619	0.80	36.8	18^b

[a] $P<0.01$

[b] Heart girth measurements were not available for 4 animals.

Table 4. Estimates of ingesta-free body composition components using TL, LW, PCV and BIA parameters (I, TL^2/I, S, C), fit by stepwise regression, and associated regression parameters.

Regression equation	Error df	R^2	SEE	P^a
Females:				
water (kg) = 75.8 + 0.36(WBW)	7	0.95	6.7	<0.001
water (%) = 154 - 0.21(TL) - 992(S)	6	0.90	1.62	0.001
fat (kg) = -384 + 0.84(TL) + 0.17(WBW) + 3784(S)	5	0.98	5.2	<0.001
fat (kg) = -550 + 1.42(TL) + 6007(S)	6	0.88	11.1	0.002
fat (%) = -116 + 0.31(TL) + 1275(S)	6	0.92	1.9	<0.001
Males:				
water (kg) = 78.5 + 0.59(WBW) - 2482(S)	5	0.99	5.3	<0.001
water (kg) = -543.6 + 2.33(TL) + 0.24(I)	5	0.98	10.4	<0.001
water (%) = 80.18 - 0.060(TL^2/I)	6	0.85	1.85	0.001
fat (kg) = -89 + 0.16(TL^2/I) + 2254(S)	5	0.96	5.2	<0.001
fat (%) = -4.9 + 0.052(TL^2/I)	6	0.73	2.3	0.007

[a] Significance level of *F* statistic from regression ANOVA.

EVALUATION AND TESTING OF TECHNIQUES FOR MOOSE MANAGEMENT

STUDY 1.45

CHARLES C SCHWARTZ

RESEARCH PROGRESS REPORT

STATE: ALASKA **STUDY. NO.:** 1.45

COOPERATORS: KENAI NATIONAL WILDLIFE REFUGE, SOLDOTNA, ALASKA; DR. STEVE MONFORT, NATIONAL ZOOLOGICAL PARK, SMITHSONIAN INSTITUTION, FRONT ROYAL, VIRGINIA

GRANT NO.: W-24-3

STUDY TITLE: EVALUATION AND TESTING OF TECHNIQUES FOR MOOSE MANAGEMENT

PERIOD: 1 JULY 1994-30 JUNE 1995

SUMMARY

Only 1 study was active during this report period. We designed an experiment to further investigate the role of the bull moose in establishing a synchronous estrous period in cows. Seven cow moose were divided into 2 treatment groups. The control group (3 adult females) were put into a 15 ha holding pen with a mature bull in mid-September and allowed to breed normally. The treatment group (3 mature and 2 primiparous females) were isolated from any bulls by a distance of at least 0.8 km. Fecal samples were collected daily from both treatment and control groups, frozen and analyzed later for progestagen (P4) concentrations. Progestagen profiles from control cows were similar to profiles observed during a normal estrous cycle, where a nadir in P4 concentration occurred within 2 days of observed heat. Isolated females appeared to exhibit a silent estrus (nadir in P4) in mid-September followed by another estrus 8-10 days later (second nadir in P4). This second nadir occurred during the normal breeding window observed at the Moose Research Center (28 Sep - 12 Oct). Cows in the treatment group continued to cycle until sample collection terminated in mid-November. The two primiparous females produced exceptionally high (>20ug/g) P4 concentrations in mid to late October. Interpretation of their P4 profiles was more difficult.

Key words: *Alces alces*, estrous cycle, feces, formulated ration, moose, reproduction.

CONTENTS

BACKGROUND

The Moose Research Center (MRC), with 25 confined animals 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 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 more than 35 journal publications (see Schwartz et al. 1993).

Mean (SD) length of the estrous cycle in moose ranges from 22-28 days (Schwartz and Hundertmark 1993). Schwartz et al. (1990) demonstrated that bull moose produce male hormones (androstenone and androstenol) that in swine and red deer (*Cervus elaphus*) 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 evaluate the role of the bull in stimulating estrus in the cow.

This report contains information collected from 1 July 1994 through 30 June 1995. The only active job was the one on reproduction studies (Job 5).

OBJECTIVES

To test and evaluate techniques potentially useful for management of moose.

To investigate the basic parameters of moose reproduction. (Job 5).

METHODS

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 1994. This group is referred to as the treatment group. In addition, 3 adult females were put into a 15 ha holding pen in mid-September with a mature bull (control group). We collected daily fecal samples from 1 September through 15 November for the treatment group and until 5 days after breeding was observed in the control group. Feces was frozen at -20°C in plastic bags until analysis. Fecal samples were analyzed for progestagens following the protocol described by Wasser et al. (1991) as modified by Monfort et al. (1993). Details of the protocol are provided in Schwartz et al. (1995). 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 postestrus in the control group. Additional samples were analyzed to refine profiles as needed.

RESULTS AND DISCUSSION

JOB 5. REPRODUCTION STUDIES

Effect of the bull on reproductive cycle of cow moose: The recently analyzed fecal progestagen profiles differed markedly from those constructed with data from the previous year. Further investigation indicated errors were made by the technician conducting the assay. Replicate samples are being reanalyzed at this time.

RECOMMENDATIONS

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. This year we will repeat the sampling to evaluate the influence of the bull moose on estrous timing in cows.

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APPENDIX A

Cortisol concentrations in male moose (*Alces a. gigas*) after exogenous ACTH administration.

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Abstract: Blood levels of cortisol were determined in five yearling Alaskan moose after an exogenous administration of 40 I.U. of ACTH. A rapid elevation of cortisol concentration (over 15 *u*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.

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APPENDIX B

Considerations for Intensive Management of Moose in Alaska

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Abstract: 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. Over-harvest 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 over-harvests 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, and predation; as well as 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, and the effects of these factors can be minimized by maintaining moose densities slightly above those which maximize recruitment. 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 alternative is potential mismanagement and the risk of population declines.

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The State of Alaska recently enacted legislation instituting a policy of intensive management of selected ungulate populations. The Alaska Board of Game is charged with identifying populations of moose (*Alces alces*) and caribou (*Rangifer tarandus*) for which human consumptive use is the primary management objective. These populations are to be managed intensively to produce and maintain high levels of human harvest. A proposed amendment to this legislation defines harvestable surplus as the number of animals born less the number dying, excluding harvest and predation. At least 50% of this harvestable surplus is allocated to human harvest. Although not stated specifically in the enabling legislation, this policy implies management for maximum sustained yield (MSY) which, in turn, necessitates maximizing recruitment. In this paper, we review briefly the concept of MSY and the reasons why this is not a viable management objective, and we recommend an alternative for optimizing yields. We also review the factors affecting recruitment in moose populations and discuss strategies for managing these factors.

Although it is impossible to address comprehensive moose management strategies without considering the effect of habitat, we have purposely avoided detailed discussion of habitat evaluation and management. Knowledge of habitat stability (static or seral communities), plant species composition, site characteristics, and the effects of manipulations on non-target wildlife, among other factors is critical to proper habitat management, but these topics have been reviewed elsewhere (e.g., Eastman and Ritcey 1987, Joyal 1987, Oldemeyer and Regelin 1987). Here we only identify the density-dependent relationships between habitat productivity and population parameters.

MSY and Population Regulation

The relationship between recruitment and population density is complex; developing an understanding requires long-term research at varying population densities. Moreover, the definition of recruitment varies depending on the type of hunting allowed. In a system in which calves are harvested, recruitment can be estimated from pre-hunting season calf numbers adjusted for non-hunting mortality. If legally-harvested animals are older than calves, recruitment must be estimated from numbers of yearlings.

Maximum sustained yield occurs when a population is managed at the point of maximum recruitment. Using a logistic growth equation, this point occurs at a population density one-half (K/2) that at nutritional carrying capacity (K). Population growth below this point is density-independent and recruitment increases with population size solely because of the increased number of breeders. Mortality from hunting and predation are largely additive at these low densities. Above MSY, density-dependent processes regulate population growth by reducing fecundity and juvenile survival. Mortality from hunting and predation are increasingly compensatory as densities increase.

Management of ungulate populations at MSY is not a new concept, as any density-dependent model of population growth can be used to estimate the density at which MSY is obtained (Caughley 1976). Modeling exercises of ungulate populations indicate that MSY occurs at densities greater than K/2, ranging from 56-72% of K (Caughley 1976, McCullough 1979, Crete et al. 1981), but we recommend caution in using these estimates. Crete et al. (1981) provided the only estimate of MSY for moose (64-72% of K) but they used a definition of K that is less than nutritional carrying capacity. Their estimate of MSY would decrease if expressed as a percentage of nutritional K. In the real world, however, management of ungulate populations at MSY is rarely achieved. The public ultimately decides the density at which ungulate populations will be managed and this often equates to the maximum number of adults that the habitat can support (McCullough 1979).

Management at MSY is fraught with risk (Larkin 1977, Holt and Talbot 1978, MacNab 1985). Although once a mainstay of fisheries management, MSY was found to be too difficult to achieve over the long term and often led to declining stocks (Larkin 1977). Setting a harvest level to maintain a population at MSY can result in over-harvest given stochastic variation in recruitment and nonharvest mortality. A model of moose population dynamics indicated that overharvests at MSY can result in drastic population declines (Van Ballenberghe and Dart 1982). Such an overharvest results in a smaller base population which produces fewer recruits the following year. An identical harvest accelerates the decline and continues the positive feedback loop. Current survey and inventory procedures are not likely to detect overharvests or decreasing densities unless they are extreme, at which point drastic management actions will be required to correct the problem. Crete et al. (1981) observed dramatically increased hunter effort at low moose densities in Quebec and suggested that this would serve to reduce harvests at low densities and consequently break the feedback loop. Their data indicate, however, that effort is a sensitive indicator of density only below a density of 0.2 moose/km^2, which is approximately equal to the density at which predation limits moose populations in Alaska and Yukon (Gasaway et al. 1992).

Predation confounds the management of ungulate populations in many cases, as dynamics of moose and their predators can vary considerably among populations (Van Ballenberghe and Ballard 1994). Three forms of a general model of wolf-moose dynamics were proposed by Messier (1994) in which the degree of limitation of moose population growth by wolf predation varies. In general, wolf predation rate increases sharply with moose density at low densities (below approximately 0.65 moose/km^2) and becomes inversely density-dependent at moderate to high moose densities. The relative strengths of predation and habitat productivity determine whether predation will limit moose populations at some equilibrium level below K (Messier 1995).

Bear predation differs from wolf predation in that it is largely independent of moose density (Ballard and Larsen 1987, Schwartz and Franzmann 1991). The combined effect of bear and wolf predation therefore can increase the potential for low-density equilibria compared with wolf/moose systems (Gasaway et al. 1992, Messier 1995). In systems with little human intervention and containing both bears and wolves, moose populations limited at low densities are to be expected (Van Ballenberghe and Ballard 1994). These same density relationships, however, can be exploited in certain cases where reduction of predation is desired. As neither form of predation is regulatory at moderate moose densities, reduction of one predator will not trigger an increase in predation rate by the other (Dale et al. 1994), which can simplify predator management strategies.

Gasaway et al. (1992) demonstrated that predation is capable of limiting moose populations at low densities, and Messier and Crete (1985) provided evidence suggesting wolf predation may perhaps regulate moose densities. Therefore, managers attempting to generate harvests from moose populations must strive to maintain high moose densities and low predator:moose ratios. Yet as moose density approaches K, the adverse effects on population stability of density-independent factors such as severe winter weather increase (Skogland 1985), which can cause catastrophic population declines and re-establishment of low-density equilibria.

Managing Population Growth for Optimum Yield

When maximum yields from moose populations are desired, we recommend a conservative approach that maintains population size somewhat higher than at MSY and optimizes, rather than maximizes, yield. McCullough (1979) defined such a population level as the fixed removal yield and defined it as "the maximum fixed number of animals that can be removed from a population with fluctuating recruitment without driving the population to extinction" (McCullough 1979:129). A management objective specifying a range of acceptable population densities is superior to an objective of one fixed density and we concur with Gasaway et al. (1992) that a range of optimum density with MSY as the lower limit of the range is desirable. Determining the upper limit of this range is subjective and represents a trade-off between reduced annual yields and increased population stability in the face of stochastic variation in recruitment.

The intrinsic rate of increase (r) of a population, combined with the number of individuals in the population will determine recruitment. Caughley (1976) identified the factors that influence r in ungulate populations as age at first reproduction, litter size, first year survival, adult survival, sex ratio of young, and sex ratio of adults. We would add pregnancy rate and dispersal (immigration and emigration) to this list, although dispersal affects only recruitment, not r.

Age at first reproduction, pregnancy rate, and litter size (twinning rate) affect the number of young born. Age at first reproduction in moose populations is affected by climate and nutrition (Pimlott 1959, Markgren 1969, Schladweiler and Stevens 1973, Boer 1992), and in populations at or near K the first breeding event usually occurs at 2.5 years of age. In populations in which density is well below K, yearlings often breed as well (Gasaway et al. 1992, Boer 1992). In a declining population on the Kenai Peninsula that was at or above K, 22% of yearling moose were pregnant as opposed to 96% of cows aged 2-15 (Schwartz and Hundertmark 1993). Cows older than 15 exhibited a 14% pregnancy rate, which indicates the importance of age structure of a population on recruitment number. These data indicate that most "prime-age" cows become pregnant even when nutrition is not maximized. Pimlott (1959), however, reported a pregnancy rate of 81% for cows older than yearlings from Newfoundland. When segregated by geographic area, pregnancy rates varied from 74-100%.

The environmental and biological factors that influence pregnancy and litter size in moose need to be addressed. Caribou exhibit reproductive pauses, with probability of pregnancy related directly to body weight (Cameron 1994). The energetic constraints imposed by poor habitat and nursing of the previous year's calf can thus influence the probability of pregnancy in the following year. Is such a phenomenon present in moose to some degree?

Twinning rate also is influenced by nutrition. Franzmann and Schwartz (1985) studied two adjacent populations, one of which inhabited highly productive habitat (1969 burn) whereas the other inhabited mature habitat (1947 burn). At the peak of habitat quality, the population inhabiting the 1969 burn exhibited a twinning rate of 70%, whereas the estimate for the 1947 burn population was 22%. Five years later, the habitat in the 1969 burn had declined in quality, and the twinning rate declined to 38% (Schwartz and Franzmann 1989). These data demonstrate that twinning rate responds to habitat quality and likely population density relative to K. Improving habitat quality or holding populations below K should successfully increase yield. The latter goal can be accomplished by harvesting cows as well as bulls, which has the added benefit of lowering the mean life expectancy of cows and thus removing relatively non-productive older cows from the population.

First year survival is related to predation, nutrition of the dam, habitat productivity, and winter weather. Predation by brown (*Ursus arctos*) and black bears (*U. americanus*) is the primary cause of calf mortality in many moose populations (Ballard et al. 1991, Ballard and Larsen 1987). There have been no rigorous field studies to test the notion that intensive brown bear harvest results in long-term increases in moose density or harvest. Experimental removal of bears, however, resulted in increased moose calf survivorship (Ballard and Miller 1990, Stewart et al. 1985). Additional research is needed to address this issue (Boutin 1992). At a minimum, the position of the population relative to K, the dynamics of the habitat (static, improving, or declining in quality), and the causes and extent of all causes of mortality must all be known before predator control is considered as a viable management option (Gasaway et al. 1983, Theberge and Gauthier 1985, Schwartz and Franzmann 1989).

Poor nutrition of the dam during pregnancy can lead to perinatal mortality due to lack of vigor in the calves (Schwartz and Hundertmark 1993), although little is known about the extent of this process in moose. Poor habitat productivity and severe weather, primarily deep snow, can cause decreased survival of calves during winter. Moose calves allocate most energy to growth and do not carry large or even moderate fat reserves into winter. Severe energy deficits caused either by poor nutrition or the increased energy expenditure of moving through and finding food in deep snow can increase calf mortality. Management options that would influence first year survival of moose are holding populations below K and aggressive bear management, although the efficacy of this latter strategy is problematic.

Survival of adults is related to nutrition and predation. Mytton and Keith (1981) reported adult survival rates of 0.84 in an unhunted and predator-free moose population in central Alberta, whereas Hauge and Keith (1981) reported a rate of 0.75 in a population in northeastern Alberta that experienced mortality from hunting and predation. Similarly, Gasaway et al. (1983) estimated annual survival of moose aged 6-10 at 0.67 in a population in interior Alaska experiencing heavy predation. After wolf control, this rate increased to 0.93. Pre- and post-control rates for moose aged >10 were 0.59 and 0.79, respectively. Gasaway et al. (1983) observed that wolf predation held moose populations at a low-density dynamic equilibrium, and that reduction of predation rates allowed the moose to escape the equilibrium. Conversely, Bangs et al. (1989) estimated a survival rate of 0.92 for adult females on the Kenai Peninsula, an area inhabited by wolves and bears, and noted that predation was no more common than hunting or automobile accidents as a cause of mortality. Larsen et al. (1989) determined that grizzly bears and wolves were the primary and secondary causes of moose mortality, respectively, in southwest Yukon, and that predation was limiting the growth of the population. Thus, reduction of wolf or bear predation in some areas can effectively increase adult survival, but the degree of limitation imposed by predation must be known before predator control can be considered. Even if predator management would be an effective strategy for enhancing certain moose populations, the political and social ramifications of such a policy can preclude its implementation (Franzmann 1993).

Sex ratio variation among calves can affect population growth but is difficult to document. Schwartz and Hundertmark (1993) observed no deviation from a 1:1 sex ratio in moose fetuses in a wild population near K and cautioned that large sample sizes are needed before drawing conclusions regarding sex ratio variation. Reuterwall (1981) documented temporal and spatial variation in sex ratio of calves harvested in Sweden. The proportion of males in the harvest in any population varied from 51% to 69%. Reuterwall (1981) demonstrated the management implications of sex ratio variation via computer simulation. She estimated that a change in the proportion of male calves in a population from 50% to 55% would result in halving of the population size in less than 10 years if the management strategy sought to keep the population size constant based on an assumption of sex ratio parity. Reuterwall (1981) discussed a number of hypotheses attempting to explain variation in secondary sex ratios but was unable to identify the ultimate cause. Unfortunately, without a better understanding of the causes of sex ratio variation, it is difficult to propose a strategy for management.

The sex ratio of adults is important to population growth only to the extent that it is related to the total number of females in the population. McCullough (1979:141) modeled recruitment in the population of white-tailed deer (Odocoileus virginianus) on the George Reserve, Michigan using adult sex ratios varying from 20:80 to 80:20. All models produced the same number of recruits at MSY, albeit at different population densities. MSY was reached at lower densities when the sex ratio was skewed toward females.

Sex ratios of ungulate populations should be managed to achieve breeding synchrony (Schwartz and Hundertmark 1993). Child and Aitken (1989) and Aitken and Child (1992) documented changes in reproduction with changes in the adult sex ratio of a moose population in central British Columbia. Changes in hunting regulations in 1981 through 1985 resulted in an increase in the number of mature bulls in the population. Another regulation change in 1986 resulted in heavy hunting pressure on mature bulls and subsequently their numbers declined. Based on analysis of conception dates, the rut remained synchronous throughout the studies, but the variation about the mean date of conception increased with a decrease in abundance of mature bulls (Child and Aitken 1989). The proportion of cows bred during the second or subsequent estrus decreased from 17.5% to 7.7% as bull abundance increased. Additionally, the incidence of twinning was correlated with the bull:100 cow ratio (Aitken and Child 1992). Although these data were collected from *A. a. andersoni*, which exhibits a tending bond system of mating differing markedly from the harem mating of *A. a. gigas*, the concept applies equally to both subspecies, albeit at different scales. These data support the management strategies of Bubenik (1972) that not only is a high bull:100 cow ratio important for adequate and timely reproduction, but that the male component of the population must contain an adequate number of prime bulls. Breeding synchrony is important from a management perspective because calves born to females bred after their first estrus have less time to grow during the summer and do not exhibit accelerated growth (Schwartz et al. 1994). Thus, these animals are at a greater risk of winter mortality than are other members of their cohort.

Dispersal to or from a population can bias attempts to determine population growth rates (Rolley and Keith 1980), which can confound attempts to determine appropriate densities for management. Additionally, moose exhibit limited dispersal, particularly among females (Gasaway et al. 1989, Ballard et al. 1991, which can affect recovery times of populations that are below MSY. Gasaway et al. (1989) demonstrated that creation of productive habitat will cause an increase in population size by increased reproduction of resident moose, but not always by attracting moose from adjacent areas.

Modeling efforts have identified adult survival as the most important parameter in determining r (Nelson and Peek 1982, Eberhardt et al. 1982). For many moose populations in Alaska, however, first year survival clearly is a very important factor affecting recruitment. If populations are at or near K, however, production of large numbers of offspring will not increase recruitment due to the compensatory nature of mortality at high population densities. Bartmann et al. (1992) demonstrated a density-dependent relationship between fawn mortality and population density in mule deer (Odocoileus hemionus) in Colorado. In studies of enclosed populations, fawn mortality due to starvation was directly related to population density. Moreover, in a high-density wild population in which fawn mortality due to predation was reduced via removal of coyotes (Canis latrans), starvation mortality increased and total fawn mortality did not change. This compensation among mortality factors at high densities demonstrates that recruitment can be increased by reducing total population size, but not by predator removal.

Recommendations

Management strategies designed to optimize harvest (Table 1) must move a population toward a density that maximizes recruitment. For high-density populations this involves primarily the harvest of females, which will reduce the base population size and increase recruitment through density-dependent processes. For low-density populations a number of strategies are possible depending upon local conditions, all of which must enhance survival of adults and calves. Viable strategies include habitat and predator management, and should be determined through a step-down planning process similar to that developed by Theberge and Gauthier (1985). Population density objectives likely will fall within the range of 60-80% of K. Once a population goal is reached, managing harvest to optimize adult sex ratios and age structure is important. All of these strategies depend on the manager's ability to determine the position of the population relative to current carrying capacity and to MSY.

Strategies designed to achieve these goals may run contrary to prevailing public opinion and therefore can be highly controversial, and are not limited to predator control. For instance, in Alaska all management strategies involving the harvest of cow moose must be approved yearly by the Board of Game. Local citizen advisory committees have veto power over cow seasons within their jurisdiction, a power they hold for no other management strategy. Many communities associate cow seasons with precipitous population declines in the 1970s and are reluctant to approve new seasons.

To manage moose populations intensively, we must be permitted to implement cow harvests as well as efficient predator control programs when deemed necessary by the Board of Game. Additionally, our ability to implement habitat management on state-owned lands within areas identified for intensive management must be increased. Currently, our ability to use these techniques is limited. Increasing the degree of human intervention in these systems will not be successful if managers are precluded from using effective tools.

Intensive management of moose populations will require collection of precise population-specific information concerning population dynamics and density, predator-prey relationships, harvest statistics, the carrying capacity of the habitat, and trends in habitat quality. Increased survey and inventory activities will be necessary in areas slated for intensive management.

Research programs addressing the relationship between moose recruitment, predation rates, and habitat productivity, wherein prey and predator population densities are manipulated experimentally, would provide much needed information concerning proper approaches to intensive management. These programs will be labor and cost intensive; however, implementing intensive management programs without reliable information ultimately will lead to mismanagement and the risk of population declines.

ACKNOWLEDGMENTS

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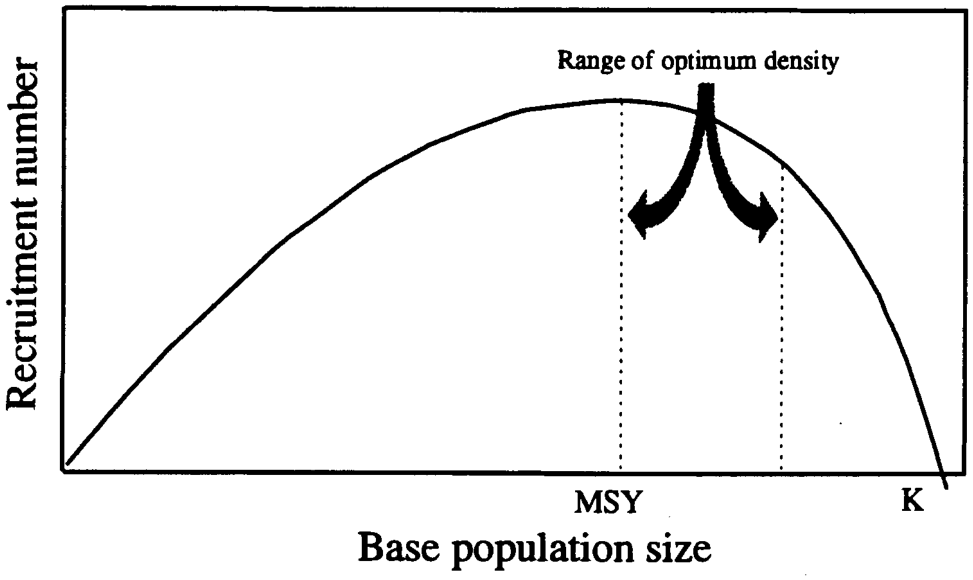

Fig. 1. A theoretical recruitment model illustrating the variability in recruitment number as population size changes. Note that the curve continues beyond nutritional carrying capacity (K), indicating negative recruitment beyond this point. MSY in this model occurs at approximately 0.65K. The range of densities within which moose populations should be managed to optimize yield is indicated (after Gasaway et al. 1992).

Table 1. A listing of factors influencing rate of increase (*r*) and recruitment in moose and potential management strategies to address these factors.

Factor	Strategy
Age at first reproduction	Hold population below K, habitat enhacement
Litter size	Hold population below K, habitat enhancement
Pregnancy rates	Young age structure
First year survival	
⇒ Predation	Bear management?
⇒ Nutrition	Hold population below K, habitat enhancement
⇒ Winter severity	Hold population below K, accept periodic die-offs
⇒ Rut synchrony	Manage for prime bulls
Adult survival	
⇒ Predation	Wolf management
⇒ Nutrition	Hold population below K, habitat enhancement
⇒ Winter severity	Hold population below K, accept periodic die-offs
Sex ratio of adults	Harvest management
Sex ratio of offspring	Unknown

APPENDIX C

Diagnosis of pregnancy and twinning in moose by ultrasonography and serum assay

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Abstract: 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 (1 November - 20 December). 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 post-partum assessment of moose population productivity.

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EVALUATION AND TESTING OF TECHNIQUES FOR UNGULATE MANAGEMENT

STUDY 1.45

KRIS J HUNDERTMARK
CHARLES C SCHWARTZ
CURTIS C SHUEY

RESEARCH PROGRESS REPORT

STATE: ALASKA **STUDY NO.:** 1.45

COOPERATORS: KENAI NATIONAL WILDLIFE REFUGE, SOLDOTNA, ALASKA

GRANT NO: W-24-3

STUDY TITLE: EVALUATION AND TESTING OF TECHNIQUES FOR UNGULATE MANAGEMENT

PERIOD: 1 JULY 1994-30 JUNE 1995

EDITOR'S NOTE: The scope of Study 1.45 was redefined to include ungulate research. This study represents this extension and has the same study number as the previous moose study.

SUMMARY

We continued to collect baseline information on parameters of calving in nutritionally unrestricted caribou (*Rangifer tarandus*) for later comparison with nutritionally stressed animals and to develop improved facilities and methods for obtaining such information. By only allowing the bulls access to the cows during daylight hours of the rut, we were able to observe all 5 planned breedings. Two unexpected breedings of yearlings also took place. Seven calves were born and processed (weighed, measured, sexed, and ear tagged) without loss. Using a digital electronic scale, we weighed all caribou regularly with little stress. We successfully tested a restrictive feed gate system for suitability to caribou. Five male caribou were transferred to a local wild herd. One died in the process, and another was later killed when hit by a car.

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

CONTENTS

BACKGROUND

Recent data from surveys of 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.). 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.

Evidence from studies of domestic and wild reindeer in Norway and caribou in Canada indicates body condition affects reproductive performance of females and survival of their calves. 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).

Several studies found that undernutrition of 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 may reduce 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.

OBJECTIVE

The objective of this study is to determine the effects of nutrition on breeding chronology, calving chronology, birth size, and neonatal survival.

METHODS

Eight adult (>1 year) caribou (6 female, 2 male) were captured in September 1990 from the Nelchina Basin caribou herd with the use of a helicopter-mounted net gun. They were drugged (Xylazine HCL) and transported in a sling to a staging area, where they underwent bioelectrical impedence analysis, were inoculated (Ivermectin), treated with a dual-penicillin type antibiotic, weighed, measured, ear tagged, crated, and loaded on a truck for transport to the MRC. They were maintained in a 10 x 20 meter divided holding pen for several months and soon adapted to a 1:1 ratio of pelleted reindeer ration and pelleted moose ration, supplemented with alfalfa hay. They were then permanently moved to a 4-ha enclosure and provided ad libitum feed and water.

A 12-volt electronic platform scale (Tru-Test Limited Model 700, Auckland, New Zealand) was used to weigh all caribou older than neonates. Newborn calves were weighed using a spring scale (Salter No.235, London, England), ear tagged, sexed, and measured for total length, jaw length, hind foot length 1 (metatarsus), and hind foot length 2 (heel to toe). We attempted to process calves within 12-24 hours after birth.

During the 1994 breeding season, 3 adult (>1 year) male, 7 adult female, and 5 calf caribou were confined in a 4-ha enclosure and fed a 1:1 ratio of pelleted reindeer ration and pelleted moose ration ad libitum, gradually switching to a single formula 13% protein reindeer ration during May and June 1995. We tested a restrictive feed gate system (American Calan, Inc., Northwood, NH) for suitability on caribou. The system consists of a feed container accessible only through a neck slot blocked by a 24 volt electronically locking gate, which can be unlocked by an individual "key" collar worn by an animal. The latch was removed from the gate, allowing all caribou access, and feed was provided only through this gate for an extended time.

RESULTS AND DISCUSSION

Copulations were observed for all 5 breeding-age (>1 year) females during the presumptive first estrus. Four of the 5 calved successfully in May, and the fifth (B.Y.) calved successfully on or about 4 July. This late birth was probably the result of a failed conception from the observed copulation and a successful conception during the third estrus. B.Y. has delivered a late calf previously (15 June 1992) and may have chronic low fertility. Two yearling females were assumed to be sexually inactive and were not observed during the rut. Both calved successfully (26 May [estimated] and 6 June). The 3 later births were unexpected; we estimated 2 of the birth dates based on calf activity and condition of the umbilici. Three males and 4 females were born. Mean gestation length in 1995 was 225.3 days (range 220-228). This estimate excluded the 2 younger females whose breeding was not observed and B.Y. who calved extremely late. Mean gestation length for all years (*n* = 11) was 224.4 days (range 217-231); the median and mode were 224 days (Fig. 1). Historical breeding records, neonate measurements, and animal status are listed in Table 1.

Caribou calves exhibited sexual dimorphism at birth (Table 2). Mean weight, total length, hind foot length, and metatarsus length were significantly ($P < 0.01$) greater in males than females. Jaw length did not differ between sexes at birth. Sexual dimorphism continued throughout the first year of growth (Fig. 2), with calves gaining weight rapidly through September and maintaining weight throughout winter. Weights of adults indicate a typical ungulate weight cycle (Fig. 3). Males exhibited a nadir in September associated with the rut whereas females maintain a relatively constant body weight with a nadir in summer associated with parturition.

A yearling male (Buck) was released near a local caribou herd on 18 October 1994 and passed the winter at the fields of a landowner that provided supplemental hay. The buck joined the lowland herd in the spring but was then struck and killed by a car. The 4 males born in 1994 were released in the same area on 21 May 1995. WW94 died the same day, presumably due to the stress of drugging and transporting. The other three have joined the lowland herd.

The oldest male, Lowland, was found dead on 16 July 1995, mostly consumed by scavengers. Condition of the carcass indicated he had died 2-3 days earlier. No previous signs of poor health had been observed. Cause of death is unknown.

All of the caribou adapted to the unlatched feed gate system gradually over a period of 6-8 weeks. The latch was subsequently installed, and the key collar was placed on an adult female (B.Y.). After a tentative, failed attempt to open the gate, she became more bold and successfully gained access to the feed at will. All other caribou (without the key collar) were unable to open the locked gate and obtain feed. We have purchased 6 additional gate systems for use in our restricted nutrition trial.

RECOMMENDATIONS

We intend to train 6 adult females to use the Calan feed gates so that we can restrict the intake of these caribou. We also will be redesigning the animal handling facility to better control caribou during weighing and immobilization operations.

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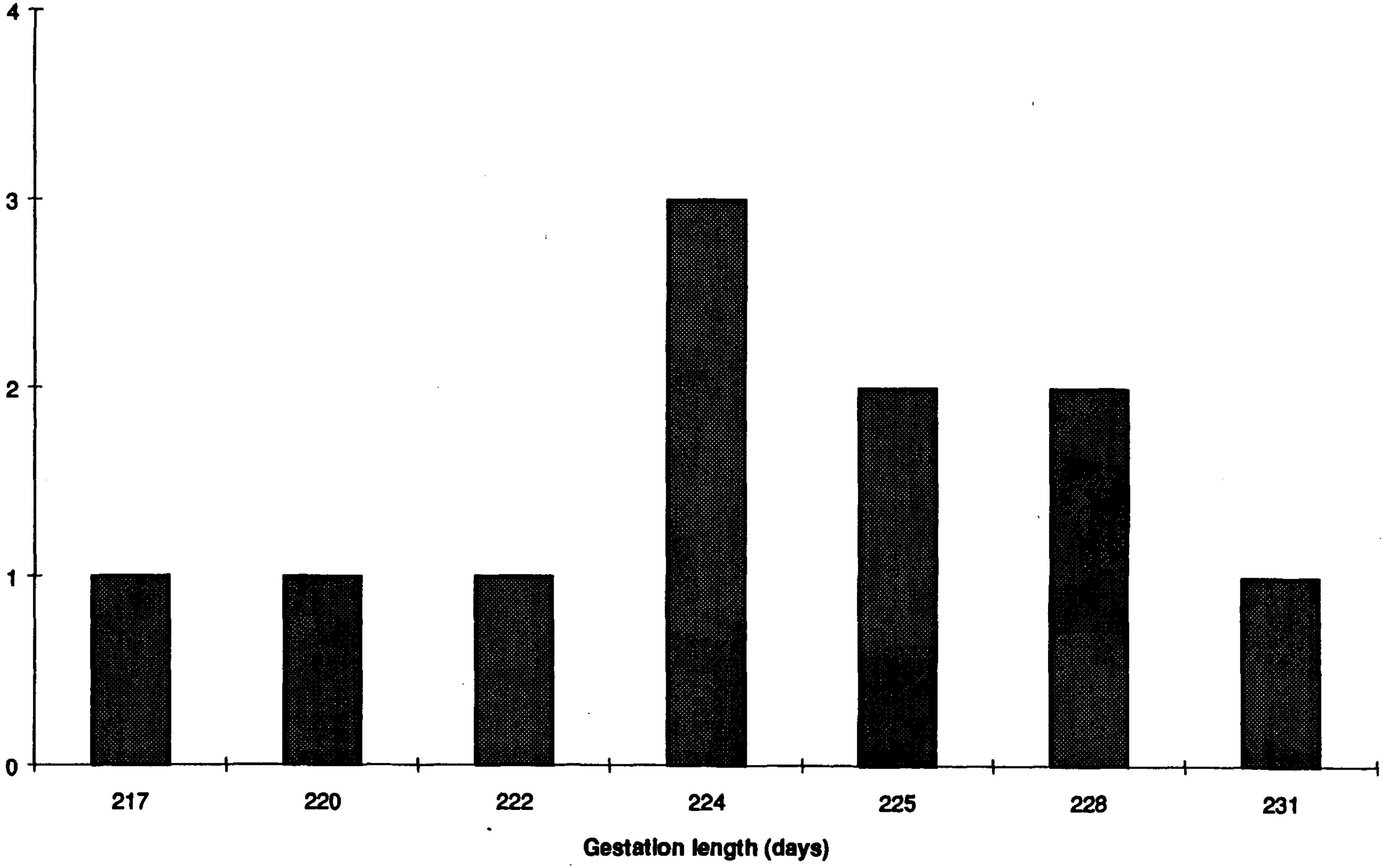

Fig. 1. Distribution of caribou gestation lengths observed at the Moose Reseach Center, 1991- 1995.

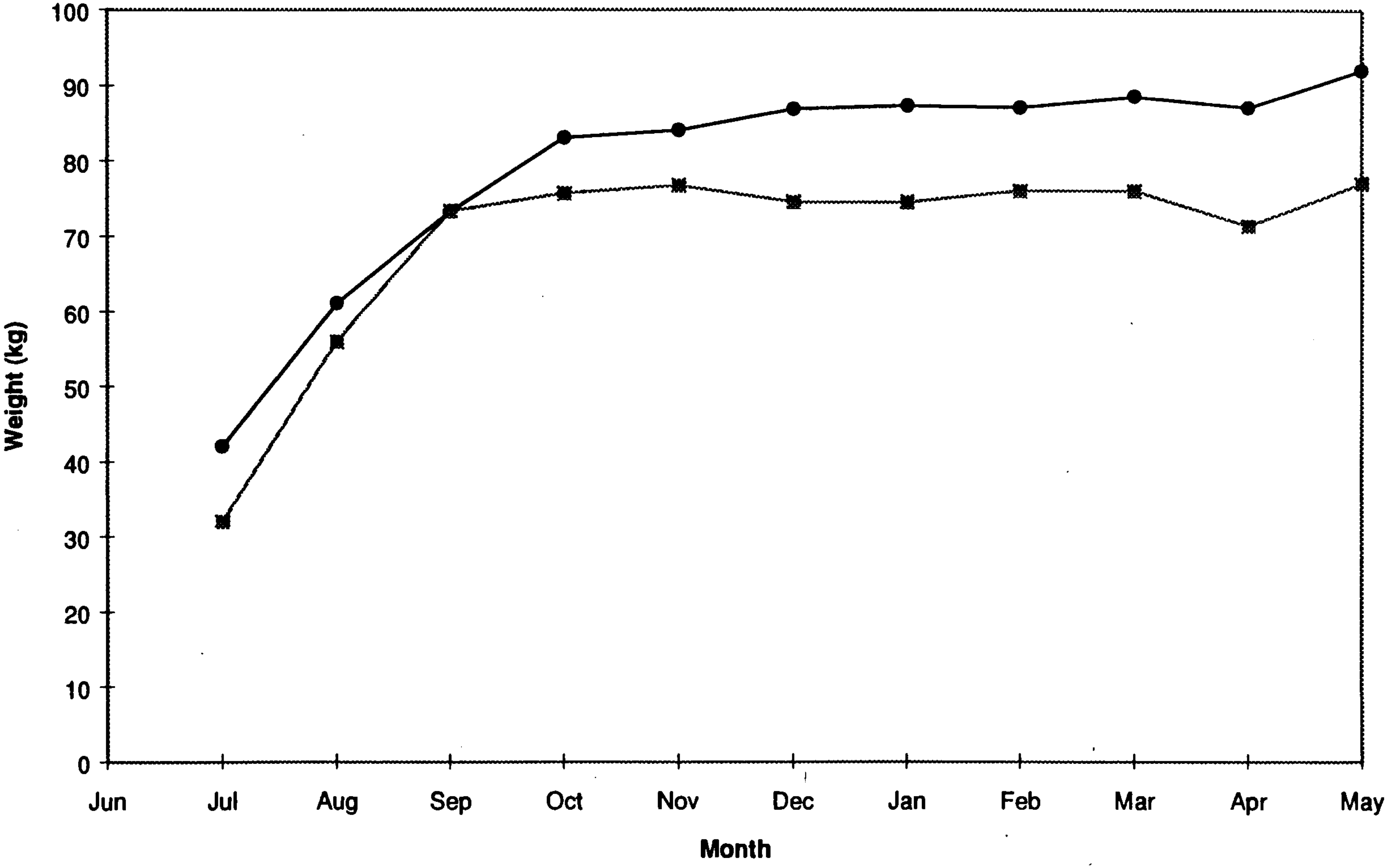

Fig. 2. Mean weights of male (circles) and female (squares) caribou calves, excluding birth weight, during their first year of life, Moose Research Center.

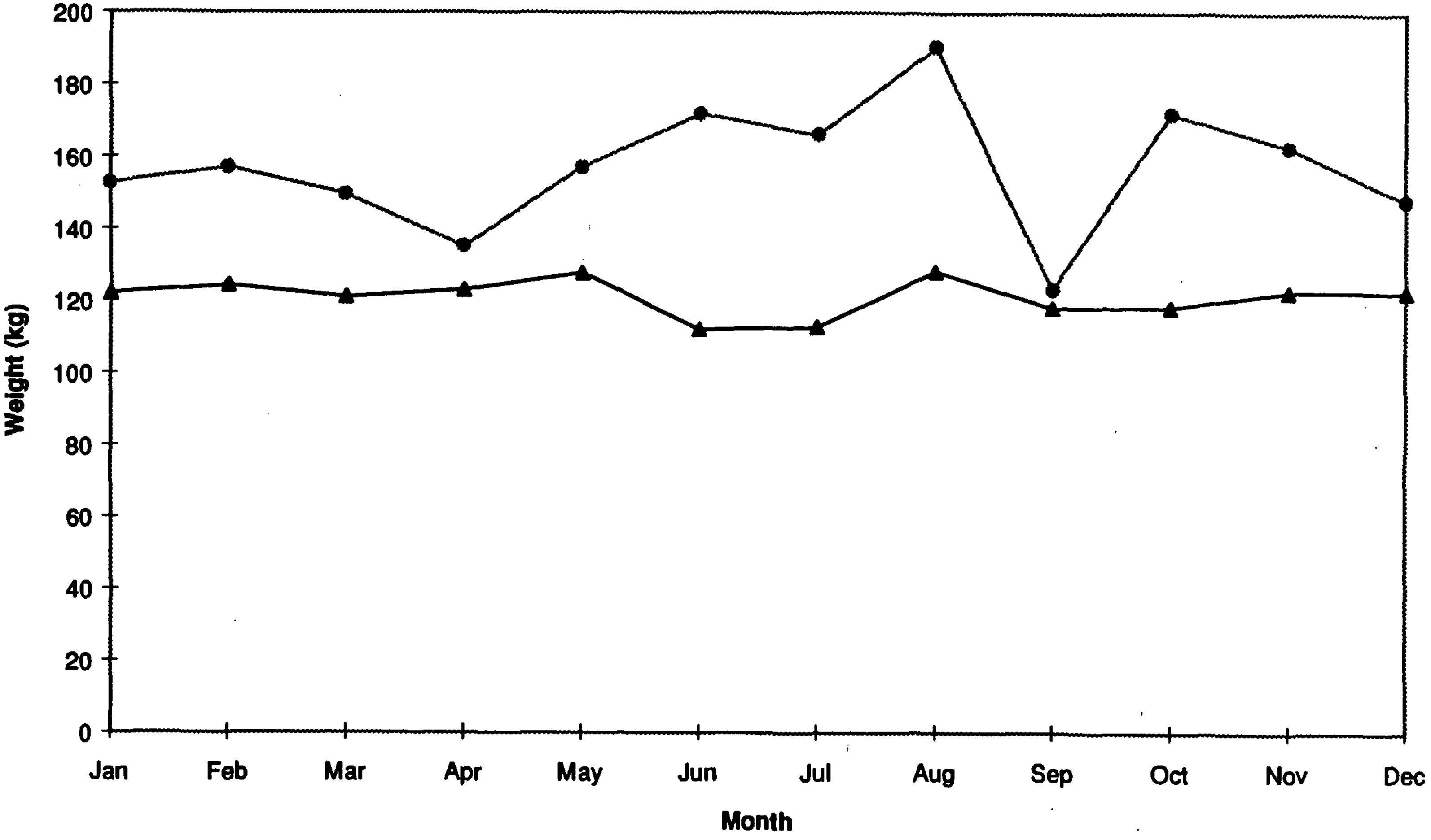

Fig. 3. Mean weights of adult (> 1 year of age) caribou by month, Moose Research Center.

Table 1. Descriptive data for captive caribou held at the Moose Research Center.

Animal	Sex	Date Conception	Date Birth	Gestation length (days)	Birth weight (kg)	Total length (mm)	Mandible length (mm)	Metatarsus length[1] (mm)	HF2[2] (mm)	Source	Dam	Sire	Current status
Yellow	M		1989[3]							NELCHINA, 1990	WILD	WILD	DIED AUG 91
Red	M		1989[3]							NELCHINA, 1990	WILD	WILD	DIED NOV 90
Orange	F		1989[3]							NELCHINA, 1990	WILD	WILD	ALIVE
BR	F		1985-86[3]							NELCHINA, 1990	WILD	WILD	ALIVE
Blue	F		1985-86[3]							NELCHINA, 1990	WILD	WILD	ALIVE
BY	F		PRE-1985[3]							NELCHINA, 1990	WILD	WILD	ALIVE
RW	F		PRE-1989[3]							NELCHINA, 1990	WILD	WILD	DIED MAY 92
White	F		1986[3]							NELCHINA, 1990	WILD	WILD	ALIVE
Unnamed	M		1990[3]							TUSTEMENA, 1991	WILD	WILD	DIED AUG 91
Lowland	M		1990[3]							LOWLAND, 1991	WILD	WILD	DIED 16 JUL 95
Unnamed	M									TUSTEMENA, 1991	WILD	WILD	DIED SEP 91
Killey	M		1990[3]							KILLEY RIVER, 1991	WILD	WILD	DIED MAR 92
BB92	F	14 OCT 91	23 MAY 92	222	7.3	79.0		26.0	34.0	MRC	BLUE	KILLEY	DISAPPEARED JUL 92
Shebou	F	13 OCT 91[3]	24 MAY 92	224[3]						MRC	WHITE	KILLEY	DIED DEC 93
OO92	M		25 MAY 92							MRC	ORANGE	KILLEY	DIED JUN 92
RW92			26 MAY 92							MRC	R.W.	KILLEY	DIED MAY 92
BR92		13 OCT 91	MAY 92							MRC	B.R.	KILLEY	DIED JUN 92
Hebou	M		15 JUN 92		10.0	92.0		28.0	36.5	MRC	B.Y.	KILLEY	ALIVE
Snow	F		16 MAY 93		7.7	92.0	13.5	28.0	35.5	MRC	WHITE	LOWLAND	ALIVE
Tangerine	M		17 MAY 93		7.7	85.0	14.5	27.0	34.0	MRC	ORANGE	LOWLAND	DIED MARCH 94
Buck	M	6 OCT 92	18 MAY 93	224	8.6	91.5	14.5	28.0	36.5	MRC	BLUE	LOWLAND	RELEASED OCT 94
BY93	M		24 MAY 93		10.0	92.0	15.0	27.5	34.5	MRC	B.Y.	LOWLAND	DIED JUN 93
Violet	F		25 MAY 93		7.3	84.5	13.5	26.5	34.0	MRC	B.R.	LOWLAND	ALIVE
Copper	F	7 OCT 93	12 MAY 94[3]	217[3]	7.3	81.5	13.5	27.5	33.0	MRC	ORANGE	LOWLAND	ALIVE
WW94	M		12 MAY 94[3]		9.5	89.0	17.5	28.5	37.0	MRC	WHITE	LOWLAND	RELEASED MAY95
BB94	M	6 OCT 93	18 MAY 94	224	10.0	92.5	14.5	30.0	37.5	MRC	BLUE	LOWLAND	RELEASED MAY95
BR94	M	6 OCT 93	25 MAY 94	231	9.5	94.0	14.5	29.5	37.5	MRC	B.R.	LOWLAND	RELEASED MAY95
BY94	M	13 OCT 93	26 MAY 94	225	10.0	94.0	14.0	29.0	37.0	MRC	B.Y.	LOWLAND	RELEASED MAY95
V95	M		26 MAY 95[3]		8.2	86.0	14.0	29.5	34.5	MRC	VIOLET	LOWLAND	ALIVE
S95	F		6 JUN 95		6.4	78.5	14.0	24.0	31.0	MRC	SNOW	LOWLAND	ALIVE
BR95	F	4 OCT 94	20 MAY 95	228	8.2	91.5	14.5	28.0	35.5	MRC	B.R.	LOWLAND	ALIVE
OO95	M	5 OCT 94	13 MAY 95	220	8.2	88.0	14.0	26.0	33.5	MRC	ORANGE	LOWLAND	ALIVE
BB95	M	2 OCT 94	18 MAY 95	228	10.5	95.0	14.0	29.0	36.0	MRC	BLUE	LOWLAND	ALIVE
WW95	F	30 SEP 94	13 MAY 95	225	8.2	87.0	19.5	27.0	34.0	MRC	WHITE	LOWLAND	ALIVE
BY95	F		4 JUL 95[3]		9.5	83.0	14.0	27.0	36.0	MRC	B.Y.	LOWLAND	ALIVE

[1] Length of the hind foot minus the hoof

[2] Length of hind foot to tip of hoof

[3] Estimated

Table 2. Parameters illustrating sexual dimorphism of newborn caribou, Moose Research Center, 1991-1995. Differences in means were tested assuming unequal variances with the exception of weight.

	Weight (kg)	Length (cm)	Jaw (cm)	Metatarsus (cm)	Hind foot (cm)
Females (n=8)					
Mean	7.75	84.6	14.4	26.75	34.13
Variance	0.89	26.98	5.37	1.71	2.63
Male (n=10)					
Mean	9.40	91.1	14.7	28.4	36.1
Variance	0.85	11.49	1.19	0.93	1.71
t	-3.73	-3.05	-0.31	-2.97	-2.80
P (one-tailed)	0.0009	0.005	0.38	0.005	0.008

INFLUENCE OF SELECTIVE HARVEST SYSTEMS ON POPULATION GENETICS OF ALASKAN MOOSE

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 LARSEN, 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-24-3

STUDYTITLE: INFLUENCE OF SELECTIVE HARVEST SYSTEMS ON POPULATION GENETICS OF ALASKAN MOOSE

PERIOD: 1 JULY 1994-30 JUNE 1995

SUMMARY

Tissue samples have been catalogued from 633 moose from Alaska and other populations from the United States and Canada. Subsets of these samples will be analyzed for electrophoretic variation and mitochondrial sequence divergence. Two bulls obtained from Galena were placed in enclosures with 3 cows each and allowed to breed. Three male and 1 female offspring were born the following spring. Antler development of the males will be measured throughout their lives to assess heritability of antler characteristics. Direct sequencing of a portion of the cytochrome-b gene revealed no differences among 5 Alaskan moose. Sequences from 2 Siberian moose were identical but differed from Alaskan sequences by 2 third-position transitions. Comparison of these sequences with a published sequence from a Newfoundland moose shows 8 nucleotide substitutions, which indicates a greater time separation between eastern and western North American moose than between Alaskan and Siberian moose. We believe that inconsistencies in the base scoring of the published sequence from Newfoundland account for the observed differences, rather than evolutionary change.

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

CONTENTS

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 truly understand the impact 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, there is a paucity of information concerning population genetics of moose. 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, we must describe the potential effects which antler-based management strategies may have on genetics.

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 (*see* Hedrick and Miller 1992) and subsequent investigations have demonstrated that information gained from such analysis can be useful to managers (*see* 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 decreased 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 two 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 can have a dramatic effect on genetic structure, and 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 extremely important 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 over time in frequency 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 probably is 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 (*see* Goss 1983). The limited data available indicate that 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.

H_{30}: 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.

H_{50}: 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.

H_{70}: 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, as well as kidney, liver, and heart tissue if possible, will be collected from as many animals as possible, utilizing harvested animals as well as road kills. If animals in populations of interest are scheduled to be collared for other projects, we will attempt to collect whole blood and ear tissue samples from those animals.

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

This job will be conducted at the MRC using moose acquired from different parts of the state that are known to have different antler forms. 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 will be placed in a large pen and fed a formulated ration ad libitum. Females will be retained to be bred to their fathers as yearlings and 2-yr-olds. Male offspring will be weighed weekly in September, and their antlers will be removed, weighed and measured. Weights and antler measurements will be analyzed 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 4. CALCULATE CHANGES IN COMPOSITION IN POPULATIONS SUBJECT TO SHS AND CONTROL POPULATIONS UNDER GENERAL HUNTS.

A modeling exercise was conducted to examine the effect of nutrition on antler characteristics and population parameters in populations under 1) a selective harvest system or 2) no hunting. The model was similar to that reported by Hundertmark et al. (1993) but allowed for greater phenotypic expression of antler size resulting from better nutrition characteristic of populations below nutritional carrying capacity (K). The model was run for populations below K and at K. A manuscript describing this exercise is in preparation and will be included in the next annual report.

JOB 5. LABORATORY ANALYSIS OF TISSUE SAMPLES.

Electrophoretic analysis of tissue samples is scheduled to be conducted at the Savannah River Ecology Lab in 1996. Number of loci examined will be determined by the types of tissue samples obtained, but at a minimum the 20 loci examined by Hundertmark et al. (1992) will be scored. Additionally, the Idh and Acp loci will be scored (Hartl et al. *in press*). Genetic variability 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).

Liver and kidney tissue from a moose collected by a hunter near Tok, Alaska was subjected to a cesium chloride density gradient centrifugation for isolation of mitochondrial DNA (mtDNA). This isolation step is necessary for confirmation of the mitochondrial origin of the sequences derived from other tissue samples. The mtDNA was purified via millipore filtration. A portion of cytochrome b was selected for amplification via the polymerase chain reaction (PCR) using conserved primers MVZ04 and MVZ05. Amplification of the heavy strand was successful, and the PCR product was purified and subjected to dideoxy sequencing on a vertical polyacrylamide gel using the commercial product Sequenase and radiolabeled nucleotides. The gel was dried and was exposed to autoradiographic film for a period of 7 days.

Using a salt extraction technique, we extracted genomic DNA from tissue samples from 30 additional moose, including 2 collected in Siberia. Primers MVZ04 and MVZ05 were used to amplify both strands of the targeted section of cytochrome-*b*. We analyzed sequences of the purified PCR product with an automated sequencer (Applied Biosystems, Inc. Model 373A, using MVZ05 as the sequencing primer. Sequences of 5 Alaskan and 2 Siberian moose were edited at the time of this writing, and these were compared to the published sequence.

RESULTS AND DISCUSSION

JOB 1. COLLECT TISSUE SAMPLES FROM MOOSE POPULATIONS ACROSS THE STATE. Tissue samples were collected from moose populations across Alaska as well as other parts of the United

States and Canada (Table 1). These samples represent mostly hunter-killed animals but some are tissues collected from wild animals handled during collaring programs.

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

Two yearling bulls captured in Galena were placed in separate enclosures along with 3 females each. The outcome of these matings and the status of all offspring are listed in Table 2.

JOB 4. CALCULATE CHANGES IN COMPOSITION IN POPULATIONS SUBJECT TO SHS AND CONTROL POPULATIONS UNDER GENERAL HUNTS.

Preliminary data analysis for the modeling exercise are complete. Results are being written as a manuscript intended for publication. A draft abstract of the manuscript is contained in Appendix A.

JOB 5. LABORATORY ANALYSIS OF TISSUE SAMPLES.

The 5' end of mtDNA was chosen for amplification because reliable universal primers exist that have been successful on a variety of mammals (Irwin et al. 1991), and the only published mtDNA sequence for a moose consists of 307 base pairs from this region (Carr and Hughes 1993). This published sequence was derived from a moose in Newfoundland, providing an immediate comparison between moose from eastern and western North America. Although moose were introduced into Newfoundland, the stock from which they were transplanted was of eastern Canadian origin.

A sequence of 163 nucleotides was scored from the autoradiograph. Sequences derived from the automated sequencer consisted of 381 nucleotides beginning at the 5' end of the gene. No nucleotide substitutions were detected among the Alaskan sequences. Two third-position transitions distinguish the Siberian sequences from those from Alaska. The single sequence of the Newfoundland moose differed from the Alaskan samples by 8 transitions, 7 of which were third position and 1 a first position, which resulted in an amino acid substitution. Such a high degree of sequence divergence between Alaskan and Newfoundland moose was unexpected. We believe it is likely that the moose sequence published by Carr and Hughes (1993) is incorrect at these sites, as our own sequences of moose from New Hampshire agree with those from Alaska. Carr (pers. commun.) stated that several third-position nucleotides probably were misidentified due to ambiguities between different commercial sequencing kits. However, he did not identify which positions were incorrect.

Sections of cytochrome-b and the hypervariable section of the mitochondrial control region were amplified via PCR with conserved primers (Table 3) and were sequenced with an ABI 373A automated sequencer. These sequences must be edited manually before they can be analyzed.

RECOMMENDATIONS

Electrophoretic and mtDNA analyses will continue during the next reporting period and electrophoretic analyses of allozymes will be conducted. Tissue samples from moose in the populations sampled in 1994 will be solicited from successful hunters in 1995 to increase sample sizes. The captive moose will be allowed to breed during 1995 according to the schedule in Table 2. 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 examining genetic processes at the population level.

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ATG ACC AAC ATC CGA AAA ACC CAC CCA TTA ATA AAA ATT GTA AAT AAC GCA TTT
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

ATT GAT CTT CCA GCC CCA TCA AAT ATT TCA TCA TGA TGA AAT TTT GGT TCC CTA
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                                                     .C ..C ... ..T ...

TTA GGA GTT TGC TTA ATC TTA CAA ATC CTT ACA GGA CTA TTC CTA GCA ATA CAT
... ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ...
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

TAT ACA TCT GAT ACA ATA ACA GCA TTC TCC TCT GTC ACC CAT ATC TGC CGA GAT
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
... ... C.C ..C ... ... ... ... ... ... ... ... ... ..C ... ... ... ...

GTA AAT TAC GGC TGA ATC ATT CGA TAT ATG CAT GCA AAC GGA GCC TCA ATA TTT
... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C

TTC ATC TGC TTA TTT ATA CAT GTA GGA CGA GGA CTA TAC TAC GGA TCC TAT ACT
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TTT CTA GAA ACA TGA AAC ATC GGA GTG ATC CTT CTA TTT ACA GTA ATA GCC ACA
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GCA
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Figure 1. A 381-nucleotide consensus sequence of the mitochondrial cytochrome-*b* gene from five Alaskan moose (top sequence), compared with Siberian moose (*n* = 2, second sequence), and a Newfoundland moose (third sequence). Dots in the bottom sequences indicate identity with the top sequence.

Table 1. Number of moose from which tissue samples were obtained for genetic analysis, grouped by state or province and by Game Management Unit (GMU).

	State/Province											
GMU	AK	CO	MB	MI	MN	NB	ND	NH	NS	ON	(blank)	Total
01A	1	0	0	0	0	0	0	0	0	0	0	1
01B	17	0	0	0	0	0	0	0	0	0	0	17
01C	12	0	0	0	0	0	0	0	0	0	0	12
01D	40	0	0	0	0	0	0	0	0	0	0	40
06	1	2	0	0	0	0	0	0	0	0	0	3
06A	11	0	0	0	0	0	0	0	0	0	0	11
06B	29	0	0	0	0	0	0	0	0	0	0	29
06C	8	0	0	0	0	0	0	0	0	0	0	8
07	2	0	0	0	0	1	0	0	0	0	0	3
13A	3	0	0	0	0	0	0	0	0	0	0	3
13B	4	0	0	0	0	0	0	0	0	0	0	4
13C	2	0	0	0	0	0	0	0	0	0	0	2
13E	3	0	0	0	0	0	0	0	0	0	0	3
14	0	0	0	0	0	4	0	0	0	0	0	4
14A	50	0	0	0	0	0	0	0	0	0	0	50
14C	1	0	0	0	0	0	0	0	0	0	0	1
15	1	0	0	0	0	0	0	0	0	0	0	1
15A	17	0	0	0	0	0	0	0	0	0	0	17
15B	9	0	0	0	0	0	0	0	0	0	0	9
15C	4	0	0	0	0	0	0	0	0	0	0	4
16	0	1	0	0	0	0	0	0	0	0	0	1
16A	47	0	0	0	0	0	0	0	0	0	0	47
16B	1	0	0	0	0	0	0	0	0	0	0	1
17	0	4	0	0	0	0	0	0	0	0	0	4
171	0	17	0	0	0	0	0	0	0	0	0	17
17B	1	0	0	0	0	0	0	0	0	0	0	1
20A	1	0	0	0	0	0	0	0	0	0	0	1
20B	2	0	0	0	0	0	0	0	0	0	0	2
20C	1	0	0	0	0	0	0	0	0	0	0	1
21B	85	0	0	0	0	0	0	0	0	0	0	85
26A	3	0	0	0	0	0	0	0	0	0	0	3
A2	0	0	0	0	0	0	0	1	0	0	0	1
MRC	13	0	0	0	0	0	0	0	0	0	0	13
(blank)	34	3	5	18	101	0	20	20	1	12	20	234
Total	403	27	5	18	101	5	20	21	1	12	20	633

Table 2. Breeding history of moose used in the antler heritability study.

Year of Breeding	Sire	Dam	Offspring	Offspring status
1994	Plum	Scarlet	male	alive
		Dodger	stillbirth	
		Mustard	male	alive
	Green	Blue	female	died Dec 1995
		Caitlin	stillbirth	
		Laurel	male	alive
1995	Plum	Blue		
		Laurel		
		Caitlin		
	Green	Scarlet		
		Dodger		
		Mustard		
		Hillary		
		Sabrina		

Table 3. Sections of the mitochondrial genome of moose amplified via PCR and sequenced, and the appropriate primers.

Gene	Primer 1	Primer2
Cytochrome b	MVZ04	MVZ05
	MVZ14	MVZ23
	MVZ16	MVZ37
Control Region	LGL283	ISM015
	LGL283	CST039
	TDKD	LmPro

APPENDIX

EFFECTS OF POPULATION DENSITY ON ANTLER PHENOTYPE AND SELECTIVE HARVEST IN A SIMULATED MOOSE POPULATION

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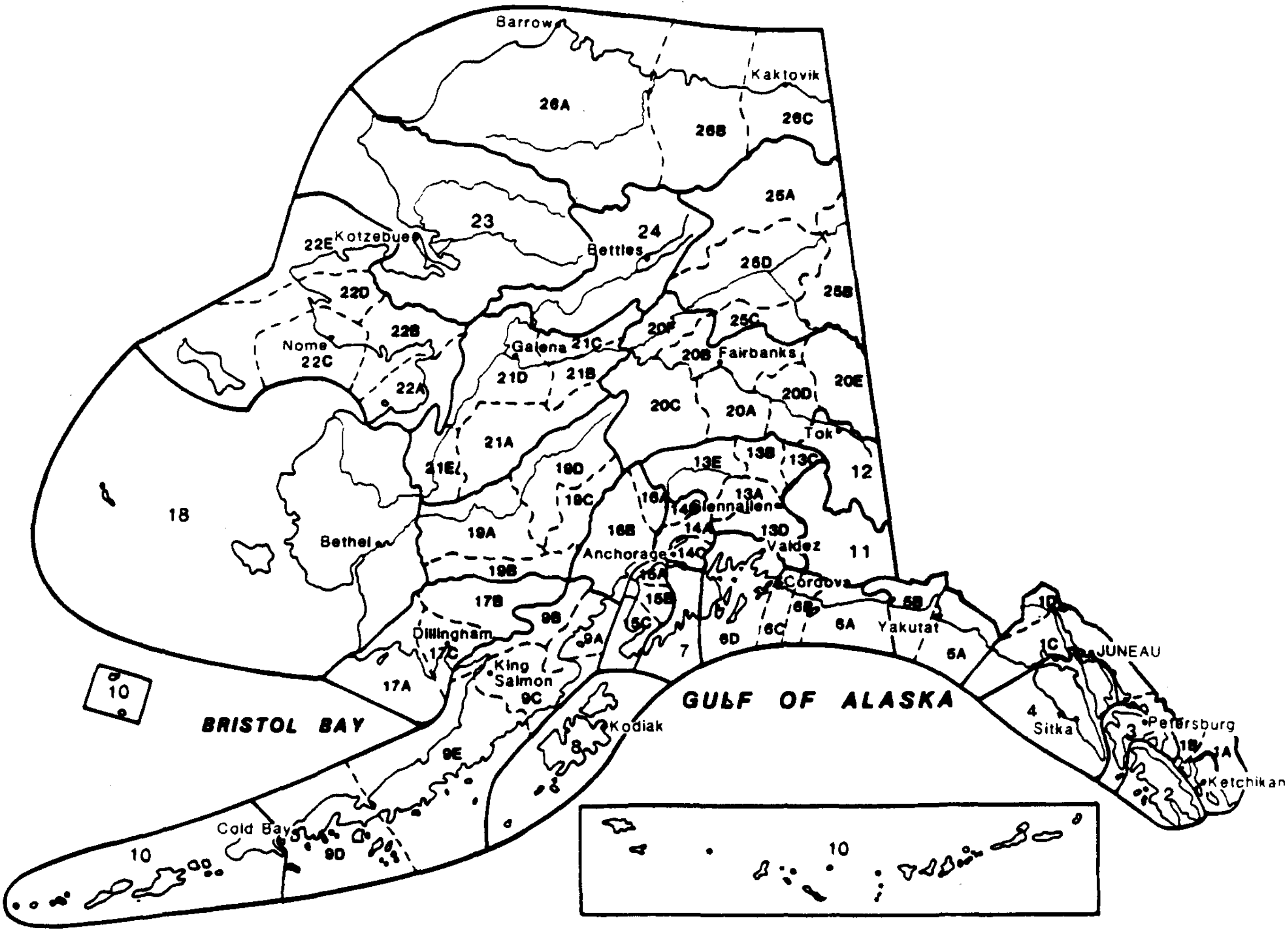
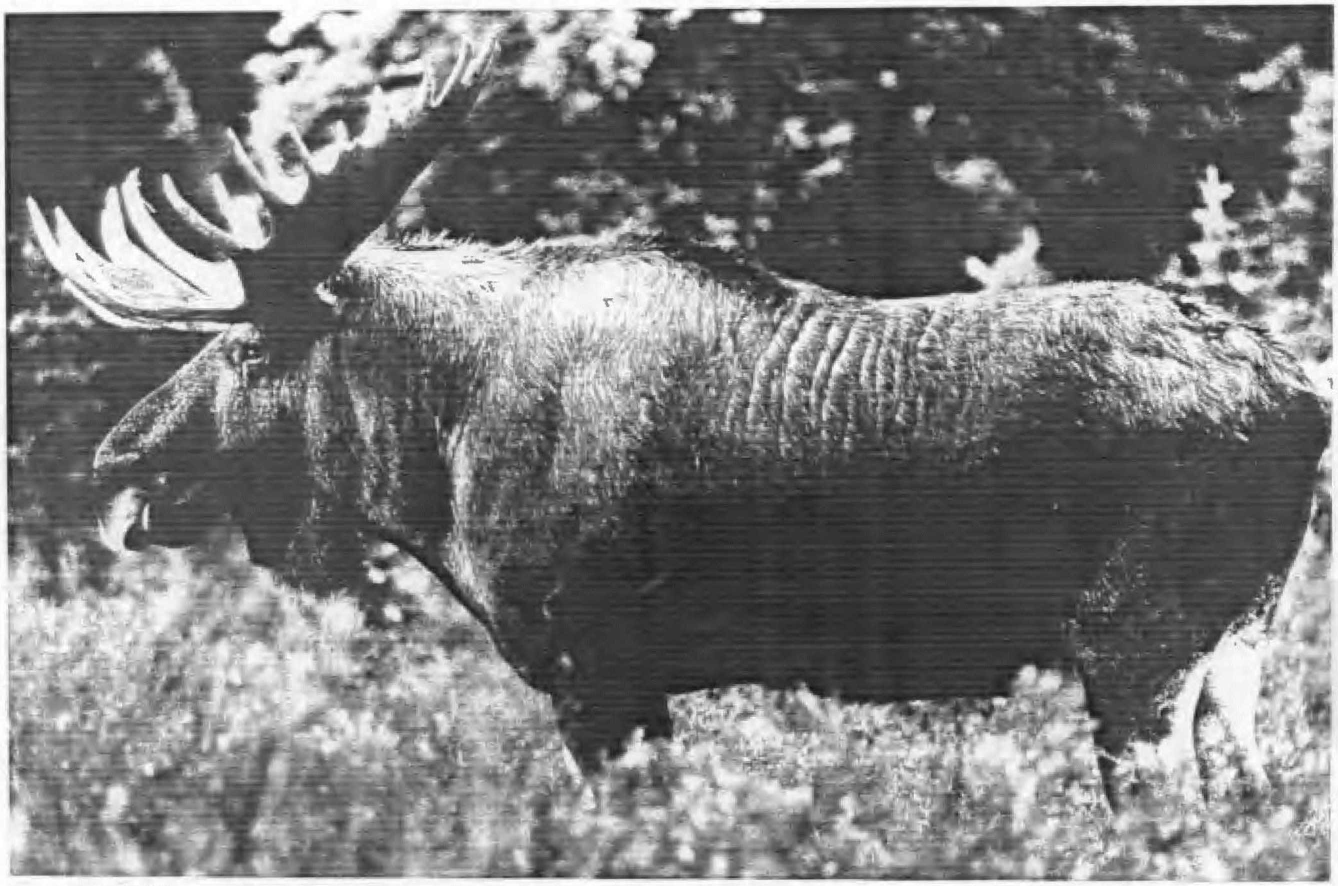
ABSTRACT: We simulated moose (*Alces alces*) populations held either at or below carrying capacity (K) to determine the effect of population density on harvest rate and frequency of alleles favoring antler growth under a selective harvest system. A Ricker model of density-dependent population growth was created to achieve stable populations with no hunting. Recruitment was reduced by 25% to achieve the population held below K. Each population was subjected to a harvest plan that defined legal bulls as those with either a spike-fork antler as yearlings or with an antler spread of 50 inches (127 cm) or greater. Harvest, population composition, and frequency of alleles favorable to antler growth were monitored throughout the simulations. At K, harvest consisted of both spike/fork yearlings and large bulls, and the frequency of favorable antler alleles declined slightly from that obtained with no hunting. When the population was reduced below K, only large bulls were harvested because young bulls expressed antlers larger than spike/fork due to better nutrition. Also, the frequency of favorable alleles declined steadily, likely to fixation for unfavorable alleles. Ratios of all bulls:100 cows and large bulls:100 cows responded similarly under each scenario, indicating that these variables do not always reflect the status of populations. The effect of nutrition on the potential for antler growth must be considered when predicting the outcome of antler-based selective harvests.

Alaska's Game Management Units



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.

WILDLIFE RESTORATION



PAT COSTELLO