



Sampling and analysis methods for snow-urine nutritional assays
by Andrew Charles Pils

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Fish and Wildlife Management
Montana State University
© Copyright by Andrew Charles Pils (1997)

Abstract:

Investigations with captive and free-ranging cow elk have demonstrated that urinary allantoin:creatinine (A:C) ratios can be used as an index of dietary intake.

Practical management applications of the technique, however, would require random collections of snow-urine, resulting in unknown proportions of samples from various sex-age classes. The goal of this research was to develop sampling protocols and statistical methods for analyzing A:C ratios derived from random collections of snow-urine. Snow-urine samples ($n=2,051$) were collected from cow, calf, and bull elk in the Madison-Firehole drainage of Yellowstone National Park, during five consecutive winters from 1991-92 through 1995-96. Significant differences were detected in mean A:C ratios among sex-age classes. Computer simulations using these data demonstrated that mean A:C ratios derived from random sample collections were highly variable and consistently overestimated the true mean A:C ratio of cow elk. These problems were somewhat alleviated by the use of trimmed means. Trimming 15 % of the right tail and 20% of the left tail of the ordered sample distribution resulted in mean A:C ratios with consistently less bias, lower variability of sample means, and smaller confidence interval widths than did untrimmed means. Additional simulations and field experience suggested that a realistic sampling goal would be the collection of 20 samples at two-week intervals throughout the winter. This sampling protocol and the use of trimmed means was tested during the winter of 1996-97 by collecting a total of 1,008 samples from six elk herds that were managed for three different goals and were expected to have different nutritional levels. More differences in average overwinter A:C ratios were detected with trimmed means than with untrimmed means. Differences in average overwinter A:C ratios that were consistent with expected differences in the nutritional levels among herds were also detected among the six herds, indicating that when used with the sampling protocol and statistical methods detailed in this paper, A:C ratios were sensitive enough to detect important differences in the nutritional levels among herds.

SAMPLING AND ANALYSIS METHODS FOR SNOW-URINE NUTRITIONAL
ASSAYS

by

Andrew Charles Pils

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Fish and Wildlife Management

MONTANA STATE UNIVERSITY-BOZEMAN
Bozeman, Montana

May 1997

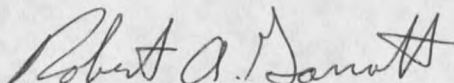
N378
P6479

APPROVAL

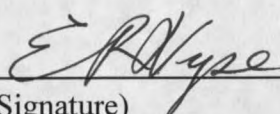
of a thesis submitted by

Andrew Charles Pils

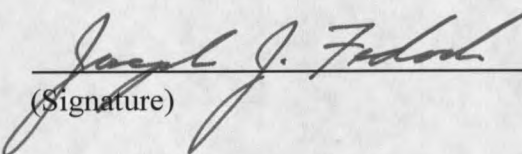
This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Robert A. Garrett  2/19/98
(Signature) Date

Approved for the Department of Biology

Ernest R. Vyse  2/19/98
(Signature) Date

Approved for the College of Graduate Studies

Joseph J. Fedock  2/25/98
(Signature) Date

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University-Bozeman, I agree that the Library shall make it available to borrowers under rules of the Library.

If I have indicated my intent to copyright this thesis by including a copyright notice page, copying is allowed only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Signature

Andrew Charles Lib

Date

2-19-98

ACKNOWLEDGEMENTS

This research was funded by the Rob and Bessie Welder Wildlife Foundation, the Rocky Mountain Elk Foundation, and Montana Fish, Wildlife and Parks. Polaris Industries, Inc., Lloyd Laboratories, and Tubbs Snowshoe Co. donated equipment and field supplies. I thank J. McDonald, S. Yu, P.J. White, and M. Becker for assistance with sample collections in Yellowstone National Park; R. Abbeglen and National Park Service staff, and S. Carsley and Yellowstone Alpen Guides for logistical support in Yellowstone National Park; J. Vore, K. Hamlin, and E. Schmidt of Montana Fish, Wildlife, and Parks, W. Clark and J. Mack of the National Park Service, and J. Bailey for logistical support, assistance with sample collections, information on the study areas, and reviewing the manuscript; J. Berardinelli and R. Adair for use of laboratory facilities and technical support for urine assays; L. Jackiw for assistance with urine assays; D. McDonald, P. J. White, H. Picton, and T. McMahon for reviewing the manuscript; J. Borkowski for assistance and advice on statistical analysis; and R.A. Garrott for support, guidance, and advice on the project.

TABLE OF CONTENTS

	Page
APPROVAL PAGE.....	ii
STATEMENT OF PERMISSION TO USE.....	iii
ACKNOWLEDGMENTS.....	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
ABSTRACT.....	ix
INTRODUCTION.....	1
METHODS.....	4
Tests For Differences in Mean A:C Ratios Among Sex-age Classes.....	4
Study Area.....	4
Sample Collection and Statistical Analysis.....	5
Computer Simulations of Random Sample Collections.....	7
Random Sampling Protocol Design and Test.....	10
Study Areas.....	11
Sampling Protocol Design.....	14
Statistical Analysis.....	16
RESULTS.....	18
Tests For Differences in Mean A:C Ratios Among Sex-age Classes.....	18
Computer Simulations of Random Sample Collections.....	21
Random Sampling Protocol Design and Test.....	26

DISCUSSION.....	32
Tests For Differences in Mean A:C Ratios Among Sex-age Classes.....	32
Computer Simulations of Random Sample Collections.....	33
Random Sampling Protocol Design and Test.....	35
MANAGEMENT IMPLICATIONS.....	40
LITERATURE CITED.....	42

LIST OF TABLES

Table	Page
1. Snow-urine samples collected from cow, calf, and bull elk in the Madison-Firehole drainage of Yellowstone National Park for five consecutive winters.....	19
2. Calf:cow ratios and allantoin:creatinine (A:C) ratio sample sizes from cow and calf elk in the Madison-Firehole drainage of Yellowstone National Park during five consecutive winters, used in computer simulations of random sample collections.....	22
3. A comparison of untrimmed and trimmed mean allantoin:creatinine (A:C) ratios derived from computer simulations of random sample collections of size 10, 20, and 30, iterated 1,000 times, from cow and calf elk.....	23
4. Sample sizes of allantoin creatinine (A:C) ratios and average snow depths at snow-urine sample collection sites for six study areas during the winter of 1996-97.....	27
5. Point estimates with their associated errors and Bonferonni multiple comparison test groupings of average overwinter untrimmed and trimmed mean allantoin:creatinine (A:C) ratios for six study areas during the winter of 1996-97.....	29

LIST OF FIGURES

Figures	Page
1. Predicted allantoin:creatinine (A:C) ratios for three sex-age classes of elk occupying the Madison-Firehole drainage of Yellowstone National Park during five consecutive winters.....	20
2. Relative frequency histograms of untrimmed and trimmed mean allantoin:creatinine (A:C) ratios from computer simulations of random sample collections	24
3. Relative frequency histograms of 95% confidence interval widths for untrimmed and trimmed mean allantoin:creatinine (A:C) ratios derived from computer simulations of random sample collections (n=20) from cow and calf elk in Yellowstone National Park.....	25
4. Predicted allantoin:creatinine (A:C) ratios derived from random snow-urine sample collections for five free-ranging elk herds during the winter of 1996-97.....	30

ABSTRACT

Investigations with captive and free-ranging cow elk have demonstrated that urinary allantoin:creatinine (A:C) ratios can be used as an index of dietary intake. Practical management applications of the technique, however, would require random collections of snow-urine, resulting in unknown proportions of samples from various sex-age classes. The goal of this research was to develop sampling protocols and statistical methods for analyzing A:C ratios derived from random collections of snow-urine. Snow-urine samples (n=2,051) were collected from cow, calf, and bull elk in the Madison-Firehole drainage of Yellowstone National Park, during five consecutive winters from 1991-92 through 1995-96. Significant differences were detected in mean A:C ratios among sex-age classes. Computer simulations using these data demonstrated that mean A:C ratios derived from random sample collections were highly variable and consistently overestimated the true mean A:C ratio of cow elk. These problems were somewhat alleviated by the use of trimmed means. Trimming 15 % of the right tail and 20% of the left tail of the ordered sample distribution resulted in mean A:C ratios with consistently less bias, lower variability of sample means, and smaller confidence interval widths than did untrimmed means. Additional simulations and field experience suggested that a realistic sampling goal would be the collection of 20 samples at two-week intervals throughout the winter. This sampling protocol and the use of trimmed means was tested during the winter of 1996-97 by collecting a total of 1,008 samples from six elk herds that were managed for three different goals and were expected to have different nutritional levels. More differences in average overwinter A:C ratios were detected with trimmed means than with untrimmed means. Differences in average overwinter A:C ratios that were consistent with expected differences in the nutritional levels among herds were also detected among the six herds, indicating that when used with the sampling protocol and statistical methods detailed in this paper, A:C ratios were sensitive enough to detect important differences in the nutritional levels among herds.

INTRODUCTION

Winter nutrition has important effects on ungulate populations in many temperate and high latitude areas. Forage availability and quality are at an annual low, while energetic demands are high (Mautz 1978). Inadequate winter nutrition can result in decreased survival (Clutton-Brock et al. 1985, Guinness et al. 1978) and reproductive rates (Thorne et al. 1976). Biologists responsible for managing ungulate populations are therefore often interested in evaluating winter nutrition. Analysis of urinary metabolite ratios from urine-saturated snow samples is a technique for indexing winter nutritional restriction of ungulates (DelGuidice et al. 1989). The technique is non-invasive and samples are often relatively easy to collect. However, rigorous collection protocols are essential for meaningful interpretations of data (White et al. 1995).

Most urinary metabolite research has focused on urea nitrogen:creatinine (UN:C) ratios. The premise of this research was that during periods of prolonged nutritional restriction, animals deplete their fat reserves. Catabolism of lean body mass then accelerates, leading to a rise in urinary urea nitrogen excretion, which is reflected by increased UN:C ratios (Moen and DelGuidice 1997). However, White et al. (1995) found substantial differences in mean UN:C ratios among sex-age classes. During random snow-urine sample collections, such differences could significantly confound interpretation of mean UN:C ratio data because the proportion of samples represented by

each sex-age class will usually be unknown. White et al. (1995) simulated random sample collections from a population of cow and calf elk (*Cervus elaphus*) and found relatively high variability in simulated mean UN:C ratios because of chance differences in the proportion of cow to calf samples collected. Therefore, differences in mean UN:C ratios are difficult to interpret because they could reflect differences in the proportion of each sex-age class represented by the samples between collections or actual differences in the nutritional status of the animals sampled. This problem was further magnified as calf mortality changed the sex-age composition of the population as the winter progressed, which changed the probability of sampling animals from each group. Although mean UN:C ratios should have been rising in response to increased nutritional stress, mean UN:C ratios from simulated random collections fell as calf mortality censored these animals from the sampling population, because calves had higher mean UN:C ratios than cows (White et al. 1995).

Allantoin is another urinary metabolite that has been investigated by animal scientists interested in the nutrition of domestic ruminants (Balcells et al. 1991, Chen et al. 1990, 1995). Recent research has demonstrated that A:C ratios have potential for use on free-ranging ungulates. Vagnoni et al. (1996) showed a positive, curvilinear relationship between A:C ratios and digestible dry matter intake in captive elk fed controlled diets. Garrott et al. (1997) performed a similar investigation, using a broader range of dietary treatments. They detected a significant linear relationship between A:C ratios and kilojoules of metabolizable energy per kilogram of metabolic body mass, similar to results reported by Vagnoni et al. (1996). They concluded that A:C ratios were

a robust index of dietary intake over a broad range of dietary treatments, including submaintenance diets that would be realistic for wintering elk. Garrott et al. (1996) were able to detect differences in mean A:C ratios of free-ranging adult female elk that were correlated with winter severity and overwinter calf mortality (as indexed by changes in calf:cow ratios), indicating that the technique was sensitive enough to detect actual differences in nutrition. However, the sampling design used in their study involved repeatedly sampling individual radio-collared cows (Garrott et al. 1996). Realistic management scenarios would require random sample collection from animals of unknown sex-age class. If mean A:C ratios are different among sex-age classes, the same sampling problems encountered in previous studies which employed UN:C ratios may be introduced.

In this paper, the potential of A:C ratios for assessing overwinter nutrition of free-ranging elk populations is explored. Specific objectives were to: 1) determine if differences in mean A:C ratios exist among three sex-age classes of elk: adult females (cows), young-of-the-year (calves), and adult males (bulls); 2) use computer simulations to determine if differences in mean A:C ratios among sex-age classes could confound the interpretation of data derived from random sample collections, and to explore statistical techniques for analyzing A:C ratio data; and 3) design and test a sampling protocol for random sample collections.

METHODS

Tests For Differences in Mean A:C Ratios Among Sex-age Classes

Study Area

The study area was located in the Madison-Firehole drainage of Yellowstone National Park, Wyoming, and was described by Craighead et al. (1973) and Garrott et al. (1996). This high elevation (2,250-2,800 m) winter range of approximately 27,000 ha supports a herd of 600-800 non-migratory elk (R. Garrott, Montana State University, unpublished data). The area includes the Gibbon, Firehole, and upper Madison Rivers, and several geyser basins. Lodgepole pine (*Pinus contorta*) forests dominate, with scattered pockets of Engelmann spruce (*Picea engelmanni*), subalpine fir (*Abies lasiocarpa*), and Douglas fir (*Pseudotsuga menziesii*). Wet and dry meadows and sagebrush (*Artemisia* spp.) grasslands occur in the non-forested areas associated with rivers. In 1988, widespread fires burned approximately half of the forested area. The winter climate is severe, with typical peak snow depths of 115-150 cm. However, thermal activity associated with geyser basins prevents deep snow from accumulating in some areas.

Sample Collection and Statistical Analysis

During the winters of 1991-92 through 1995-96, snow-urine samples were collected from elk of known sex-age class from December through April each winter. Sequential samples were collected from 22-27 radio-collared cow elk following the restricted randomized design described by Garrott et al. (1996). Samples were collected when the target animal was observed urinating or could be backtracked to a urination spot. The number of cows, calves, and bulls associated with the instrumented animal was recorded. Monthly calf:cow ratios were calculated by aggregating group composition data for each month with at least 80 group observations. Samples were also collected opportunistically from bulls and calves that were observed voiding. After collection, samples were frozen and stored until they were assayed at Montana State University. Allantoin and creatinine assays were performed on each sample in duplicate as described by Garrott et al. (1996). Samples with a coefficient of variation $>5\%$ between duplicates were re-assayed. Allantoin:creatinine ratios were calculated as described by Vagnoni et al. (1996). Ratios >1.0 were excluded from the analysis, based on Vagnoni et al.'s (1996) observation that 1.0 was approximately the upper limit for A:C ratios when captive elk were fed a high quality diet ad libitum.

The spatial power function feature of the SAS PROC MIXED procedure (SAS Institute 1992) was used to generate a model for predicting A:C ratios. The model was similar to that developed by Garrott et. al (1996), with the addition of a categorical variable for sex-age class, and can be expressed as:

$$A:C = b_0 + Y_j + S_i + (YS)_{ij} + b_{1j}(season) + b_{2j}(season^2) + b_{3i}(season) + b_{4i}(season^2) + b_{5i}(season) + b_{6i}(season^2)$$

where:

I = sex-age class (cow, calf, or bull)

j = year (1991-92, 1992-93, 1993-94, 1994-95, or 1995-96)

$season$ = a term corresponding to a date within a year

Y_j = categorical variable which adjusts predicted A:C ratios for differences in mean A:C ratios across years

S_i = categorical variable effect which adjusts predicted A:C ratios for differences in mean A:C ratios among sex-age classes

$(YS)_{ij}$ = interaction effect which adjusts predicted A:C ratios for differences in mean A:C ratios among sex-age classes across years

$b_{1j}(season)$ = linear trend effect in A:C ratios estimated at a specified time within a year

$b_{2j}(season^2)$ = quadratic trend effect in A:C ratios estimated at a specified time within a year

$b_{3j}(season)$ = interaction effect which specifies a potentially different linear trend in A:C ratios for each year

$b_{4j}(season^2)$ = interaction effect which specifies a potentially different quadratic trend for each year

$b_{5i}(season)$ = interaction effect which adjusts predicted A:C ratios for differences in the linear trend of A:C ratios among sex-age classes

$b_{6i}(season^2)$ = interaction effect which adjusts predicted A:C ratios for differences in the quadratic trend of A:C ratios among sex-age classes

Because individual calves and bulls could not be identified during sampling, they were modeled under the assumption that all samples came from a single animal. Separate variance components were used for cows versus calves and bulls. The variability associated with calves and bulls would be larger than that for cows, where samples came from repeatedly-sampled individuals, and the variation between animals could be accounted for. Therefore, tests were conservative, because if sex-age class differences were detected with the inflated variance estimates for calves and bulls, there was a greater likelihood that they really existed. Type III sums of squares were used to compute F-test statistics for all significance tests.

Computer Simulations of Random Sample Collections

The effects of differences in mean A:C ratios among sex-age classes on mean A:C ratios derived from random sample collections were investigated using computer simulations. Simulations were also used to explore techniques for analyzing A:C ratios derived from random sample collections, given that differences in mean A:C ratios exist among sex-age classes. The goal was to develop statistical methods that provide an accurate index of cow nutrition. An index of cow nutrition would be useful for management biologists. The largest proportion of ungulate populations is typically comprised of adult females. In polygynous organisms, such as ungulates, adult females are essentially the reproductive unit of the population. Therefore, winter nutrition of adult females has important effects on population dynamics.

The A:C ratio data derived from samples collected in the Madison-Firehole drainage of Yellowstone National Park during five consecutive winters as described earlier in this paper was split into two subsets. One subset was created from A:C ratios of either cows or calves for each month and year that data were available. Bulls were excluded from simulations because A:C ratio data from bulls were limited and because they are typically segregated from cow and calf groups during winter (Main et al. 1996). Thus, the chances of sampling bulls during random collections was minimal. Simulations were not performed for months with calf:cow ratio estimates derived from less than 80 group observations and without at least three A:C ratios for each sex-age class. Simulations were performed using the MATLAB computer software package (MathWorks, Inc. 1993). For each simulated collection, random numbers were first selected from the interval [0-1]. Each random number determined if a sampled animal was from the cow or the calf data subset. The probability of sampling a cow or calf was determined by the proportion of each group represented in the population, as indexed by monthly calf:cow ratios. For example, if the calf:cow ratio was 1:4, there was an 80% chance of selecting a cow and a 20% chance of selecting a calf. Thus, if the random number was between 0 and 0.8, a cow was selected. Otherwise, a calf was selected. Once the sex-age class for each animal to be sampled was determined, A:C ratios were randomly selected, with replacement, from the appropriate data set. Collections of 10, 20, and 30 samples were simulated. The standard mean and approximate 95% confidence interval, and the associated confidence interval width from each simulated collection were calculated. The average of all A:C ratios from cows for each month was calculated

to obtain the true cow mean for that month.

As an alternative to standard means, trimmed means were also considered.

Trimmed means are a statistical procedure whereby a fixed percentage of the data are deleted from the right and left tails of the ordered sample distribution, prior to calculation of the mean (Ruppert 1988). Trimmed means can be used to improve the robustness and efficiency of estimation and statistical testing. They are most useful when data comes from distributions with a non-normal, skewed tail. The data points comprising the skewed tail occur as part of the normal sampling variability and are not necessarily statistical outliers. Trimmed means should be applicable for analysis of random sample collection data, because the sample will typically include a mixture of observations coming from different sex-age classes, which may each have separate distributions. Trimming could be used to censor calf and bull measurements from the sample, so that the trimmed mean approximates the mean of the cow measurements.

Trim percentages used to calculate trimmed means were selected based on empirical evidence. The goal was to select trim percentages that would yield an accurate approximation of the true cow mean A:C ratio, with small confidence interval widths, and that were also robust to both changes in mean A:C ratios among sex-age classes, and changes in the proportion of each sex-age class that was sampled. Various trim percentages were evaluated, including 15%-left tail / 20%-right tail, 5%-left tail / 20% right tail, 5%-left tail / 30%-right tail, 15%-left and right tails, and 20%-left tail / 15%-right tail. The percentile bootstrapping method (Efron and Tibshirani 1993) was used to generate an approximate 95% confidence interval of trimmed mean A:C ratios and its

associated interval width. Two-hundred bootstrap samples of the appropriate sample size were selected from the data. Trimmed mean A:C ratios were calculated for each of the 200 bootstrap samples. The width of the middle 95% of the ordered bootstrap distribution of trimmed mean A:C ratios was the bootstrap confidence interval width. Coverage percentages of standard (untrimmed) and trimmed means for each simulation were determined by calculating the frequency with which the approximate 95% confidence intervals of untrimmed and trimmed mean A:C ratios captured the associated mean A:C ratio of cows. The entire procedure was repeated 1,000 times, yielding 1,000 sets of untrimmed and trimmed mean A:C ratios and their associated 95% confidence interval widths and coverage percentages. The average of each set of 1,000 untrimmed and trimmed mean A:C ratios, standard deviations of the 1,000 untrimmed and trimmed means, 95% confidence interval widths, and coverage percentages were then calculated.

Random Sampling Protocol Design and Test

A random snow-urine sampling protocol was designed and tested during the winter of 1996-97 on six different free-ranging elk herds. These herds are managed for three different goals. Differences in nutritional level among the herds were expected to accompany differences in management goals. The Madison-Firehole and Northern Range herds, within Yellowstone National Park, Wyoming, are managed under the National Park Service's policy of natural regulation (Houston 1982). Winter mortality of calves due to malnutrition is a main regulating factor for these populations (Singer et al. 1997, R. Garrott, Montana State University, unpublished data). Densities of elk were expected

to be high relative to resources for these herds, so low nutritional levels were predicted. Wall Creek Wildlife Management Area and the Hungry Horse Study Area both support herds managed by the Montana Department of Fish, Wildlife, and Parks for sustained yield. Elk are managed at moderate densities relative to resources. Winter nutrition of these herds was expected to be substantially higher than for the Yellowstone herds. The National Elk Refuge and Gros Ventre winter ranges, near Jackson, Wyoming, support herds that are also harvested. However, they were supplementally fed for much of the winter. Nutritional levels were expected to be highest for these herds.

Study Areas

The Madison-Firehole study area was described earlier in the text.

The Northern Range herd has been estimated at 17,000-22,000 animals, and winters on approximately 140,000 ha. of land in the Lamar, Yellowstone, and Gardiner River drainages (Houston 1982, Singer et. al 1997). Upland grassland and shrub steppe communities, dominated by bluebunch wheatgrass (*Agropyron spicatum*) and Idaho fescue (*Festuca idahoensis*), along with big sagebrush (*Artemisia tridentata*), are the most widespread vegetation types. Pockets of open-canopy Douglas-fir forest with bluebunch wheatgrass understory are scattered throughout the area. Stands of aspen (*Populus tremuloides*), cottonwood (*P. spp.*), and willow (*Salix spp.*) also occur, although their distributions are limited. Winter conditions are highly variable, ranging from warm, "open" winters with light snow cover, to long, cold winters with deep snow. The upper portion of the winter range located in the Lamar River valley (elevation 2,000-2,600 m)

typically experiences the harshest conditions, with snow depths usually approximately twice that of the Gardiner area (Singer et al. 1997). The upper portion of the winter range from Soda Butte to the Lamar Canyon was selected as the sampling area to represent the most severe climatic conditions. Samples would therefore be collected from a group of animals wintering under relatively similar conditions with similar nutrition. By mid-February, the original sampling area was expanded to include the Blacktail Plateau because severe conditions caused most of the elk to vacate the original sampling area as they moved to lower elevations.

The 2,770 ha. Wall Creek Wildlife Management Area located on the west side of the Madison River valley in southwestern Montana was described by Alt et al. (1992). Elevation ranges from 1,680-2,010 m. Strong winds usually keep the north and northeast facing slopes and flat benches that dominate the topography relatively snow-free for most of the winter. Vegetation of the area is primarily open grasslands, dominated by bluebunch wheatgrass and Idaho fescue. A rest-rotation cattle grazing system is used on this area, which is maintained primarily to provide winter range for elk. Elk typically begin arriving on the winter range in late-November to early December, and remain there until snowmelt in the surrounding higher elevation areas allows them to leave. During the winter of 1996-97, approximately 2,500 elk were estimated on the winter range (K. Hamlin, Montana Department of Fish, Wildlife, and Parks, personal communication).

The Hungry Horse Study Area is located west of the continental divide near Firefighter Mountain in the Flathead Range of northwestern Montana. The elk winter range lies along approximately 15 km. of the northeastern shore of Hungry Horse

Reservoir, and ranges in elevation from 1,100 m. at the reservoir to about 1,524 m. The climate has a strong maritime influence, with snow and/or rain common. Winter snowpacks are large and persist from mid-November through mid-March, with typical snow depths of 120-180 cm. Gentle to steep west and southwest facing slopes dominate the topography. Closed-canopy coniferous forests, consisting primarily of lodgepole pine with scattered pockets of Douglas-fir, and shrub understory, dominate the vegetation. Huckleberry (*Vaccinium occidentale*) is the primary understory shrub. The canopy is broken in places by clearcuts and natural shrubfields, the latter dominated primarily by serviceberry (*Amelanchier alnifolia*). Beargrass (*Xerophyllum tenax*) is a ubiquitous ground cover plant. The area supports two small elk herds during the winter, totaling approximately 140 animals (J. Vore, Montana Department of Fish, Wildlife, and Parks, personal communication).

The National Elk Refuge near Jackson, WY, was described by Cole (1969), Boyce (1989), and Smith et al. (1997). Elevation at the Jackson Hole valley floor, where the 9,800 ha. refuge is located, is approximately 1,900 m. The climate of the area is characterized by short, cool summers, and cold winters. The average January maximum snow depth at Jackson is approximately 43 cm (Boyce 1989). Composition of the vegetation is primarily irrigated grasslands, shrublands, and mixed conifer-aspen forest. Approximately 65% of the elk in the Jackson herd unit, which summers in the surrounding areas of southern Yellowstone and Grand Teton National Parks and the Bridger Teton National Forest, winters each year on the National Elk Refuge. As elk congregate on the refuge in late October to early December, they utilize natural forage

until supplemental feeding of pelleted alfalfa at the rate of 2.7 kg./elk/day is initiated. Snow depth and crusting, precipitation during the previous summer, and density of elk are used to determine when supplemental feeding is initiated. During the winter of 1996-97, supplemental feeding of an estimated 10,700 elk began on 29 January, and continued through the sampling period (J. Bailey, University of Wyoming, personal communication).

The Gros Ventre study area is located on the southeastern edge of the Jackson Hole valley along the Gros Ventre River, near Jackson, WY. Elevation is approximately 2230 m. The climate of the area is similar to that of the nearby National Elk Refuge although snowfall is greater at the Gros Ventre study area, due to its higher elevation. Vegetation is dominated by dry sagebrush/bunchgrass meadows, with stands of cottonwood trees along the flat to moderately-sloping river floodplain. The Wyoming Department of Fish and Game operates three feedgrounds on the study area which serves as winter range for approximately 12% of the elk in the Jackson herd unit. Supplemental feeding of alfalfa hay to wintering elk begins when snow depth criteria are reached. During the winter of 1996-97, feeding of an estimated 1,950 elk began on 27 December and continued throughout the sampling period (J. Bailey, University of Wyoming, personal communication). Samples were collected only at one of the three feedgrounds so that sampling would be consistent.

Sampling Protocol Design

Garrott et al. (1997) determined that A:C ratios index the nutrition of animals for

only a short period of several days immediately before the urine sample was voided. To index overwinter nutrition, an adequate number of samples must be collected periodically throughout the entire winter. Therefore, the winter was divided into 2-week sampling periods: 14-28 Dec (period 1), 28 Dec-10 Jan (period 2), 11-24 Jan (period 3), 25 Jan-7 Feb (period 4), 8-21 Feb (period 5), 22 Feb-7 Mar (period 6), 8-21 Mar (period 7), and 22 Mar-4 Apr (period 8). Given the logistic constraints of collecting samples and based on results of computer simulations which indicated that a sample size of 20 provides an accurate approximation of mean A:C ratios for cow elk, the goal was to collect a minimum of 20 samples per period. Urination spots scattered throughout areas where groups of elk were observed or where their recent presence could be inferred from fresh sign were selected for collection. The age of samples was estimated by either observation of groups of elk or by determining if the snow in which the samples had been voided had fallen within the past two days. Samples estimated at older than 2 days were not collected because they may not have been representative of conditions being indexed. Collection, storage, and assay procedures were the same as those described earlier in the text.

Snow depths associated with each group of elk sampled were measured. The center of the area used by each group of elk was estimated, and snow-depth measurements were recorded at 5-pace intervals in each of the 4 cardinal directions, for a total of 20 measurements per group. All snow depth measurements for a given collection area and period were averaged.

Statistical Analysis

To make valid comparisons of mean A:C ratios among herds, the seasonal effects discussed earlier in the paper were considered. Therefore, models were constructed to predict untrimmed and trimmed mean A:C ratios for each study area using SAS PROC GLM (SAS Institute Inc. 1992). Each model was similar to that used earlier in this paper, except that the year and sex-age variables, and their associated interaction effects, were not necessary. The models can be stated as:

$$\hat{A:C} = b_0 + A_i + b_1(\text{season}) + b_2(\text{season}^2) + b_{3i}(\text{season}) + b_{4i}(\text{season}^2)$$

where:

I = study area (Wall Creek, Hungry Horse, Madison-Firehole, Northern Range, Gros Ventre, or National Elk Refuge)

A_i = categorical variable which adjusts predicted A:C ratios for each study area

b_0 = y-intercept

season = a term corresponding to a date within a year

$b_1(\text{season})$ = linear trend effect in A:C ratios estimated at a specified time within a year

$b_2(\text{season}^2)$ = quadratic trend effect in A:C ratios estimated at a specified time within a year

$b_{3i}(\text{season})$ = interaction effect which specifies a potentially different linear trend in A:C ratios for each study area

$b_{4i}(\text{season}^2)$ = interaction effect which specifies a potentially different quadratic trend in A:C ratios for each study area

Computer simulations indicated that sample sizes of at least 10 can be trimmed to provide an index of cow elk nutrition, so this was the minimum number of ratios required

from each study area and time period for inclusion in the analysis. When there were >20 ratios for a given study area and time period, 20 ratios were randomly selected for use in the analysis, so that comparisons among herds were made using similar sample sizes.

Functions were defined for the two curves (untrimmed and trimmed) generated for each study area, using the parameters incorporated into the models. Each function was integrated using the model parameter estimates, which yielded the area under the curve of the functions. The integration values were then divided by the total number of days in the sampling period, to provide the average overwinter A:C ratio for each study area.

Bonferroni multiple comparison ($\alpha = 0.05$) tests were then performed to test for differences in untrimmed and trimmed mean A:C ratios among study areas.

RESULTS

Tests for Differences in Mean A:C Ratios Among Sex-age Classes

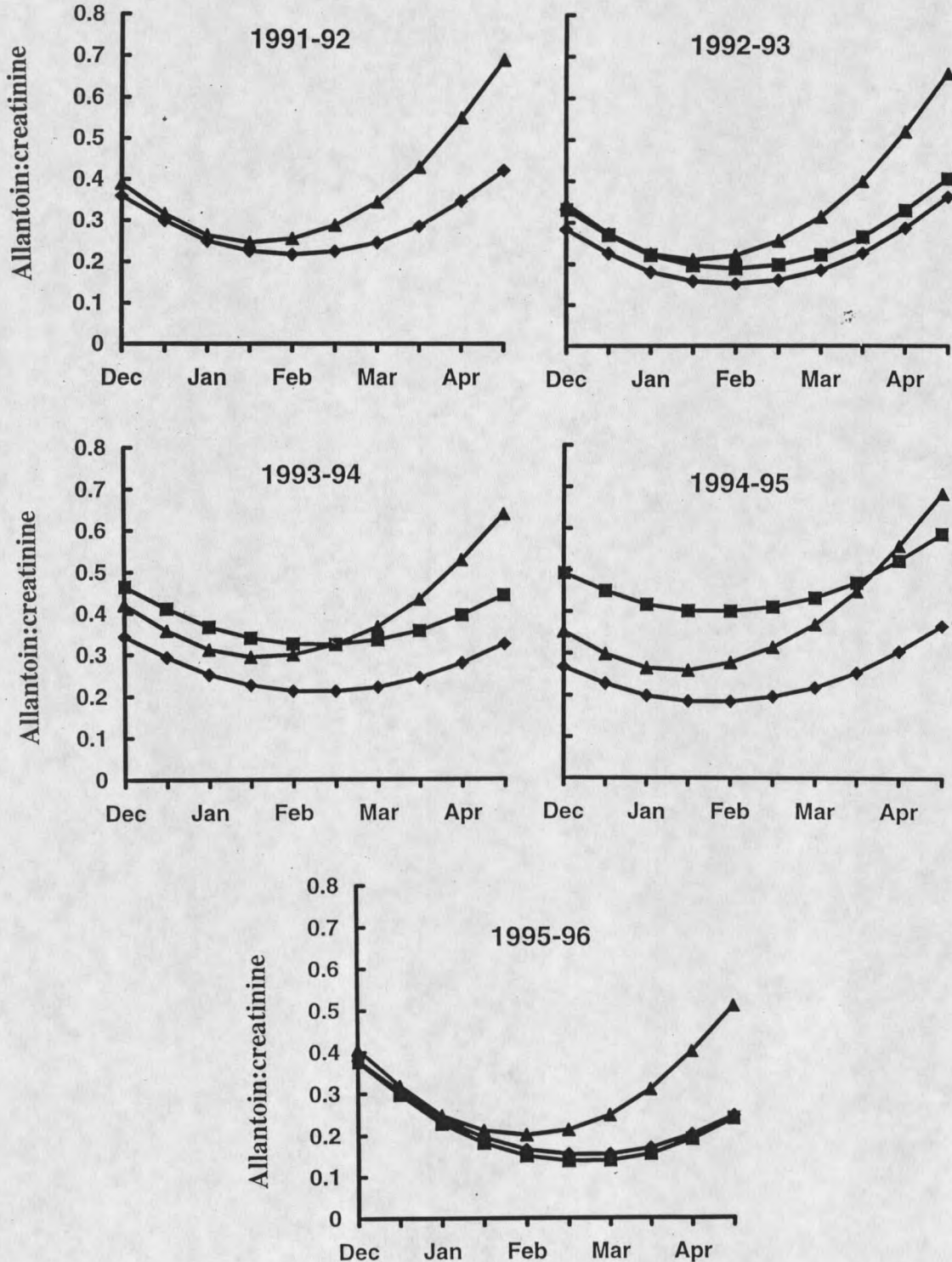
A total of 2,051 samples was collected from the Madison-Firehole winter range over the five years. Seventy-five samples were too dilute to assay for metabolite concentrations or of insufficient quantity to assay. Twenty-four A:C ratios >1.0 were omitted from the analysis. A total of 1,952 ratios was used for the analysis (Table 1).

Differences were found in mean A:C ratios across years ($P < 0.01$) and among sex-age classes ($P = 0.02$) (Fig. 1). Cows typically had the lowest ratios throughout a given winter, except during 1995-96 when predicted ratios for bulls were slightly lower. The curve of predicted A:C ratios for bulls during the winter of 1991-92 was not shown because predicted ratios were so high that they were off the scale. When A:C ratios from all sex-age classes and years were aggregated, there was no linear trend in A:C ratios across time ($P = 0.68$). However, a quadratic trend in mean A:C ratios was detected ($P < 0.01$). When A:C ratios for each year were considered separately, there was a linear component to their curves ($P < 0.01$), with a different slope for each year, indicating that A:C ratios were not constant across time. The quadratic trends in A:C ratios between years were not significantly different ($P = 0.46$). However, both the linear ($P < 0.01$) and quadratic ($P < 0.01$) components of the curves varied between sex-age classes, indicating

Table 1. Snow-urine samples collected from cow, calf, and bull elk in the Madison-Firehole drainage of Yellowstone National Park for five consecutive winters. Samples were used to test for differences in mean allantoin:creatinine (A:C) ratios among the three sex-age classes, and to explore questions of sampling and analysis using computer simulations.

Sex-age	Year					Total
	1991-92	1992-93	1993-94	1994-95	1995-96	
Cows	215	347	400	296	183	1441
Calves	69	45	137	68	29	348
Bulls	31	25	41	50	16	163
Total	315	417	578	414	228	1952

Figure 1. Predicted allantoin:creatinine ratios for three sex-age classes of elk occupying the Madison-Firehole drainage of Yellowstone National Park during five consecutive winters. Calves and bulls were sampled opportunistically, while radio-collared cows were repeatedly sampled. ▲ = calves, ◆ = cows, ■ = bulls



that the curves for each sex-age class had a different shape. Curves of predicted A:C ratios for cows and bulls were generally similar in shape, although the curves for calves typically rose much more sharply in late February or early March than the curves for cows and bulls. The interaction effect between year and sex-age class was non-significant ($P = 0.26$), indicating that differences in mean A:C ratios among sex-age classes were relatively constant from year to year. However, visual inspection of Fig. 1 showed that differences between the cow and bull curves were more variable than differences between the cow and calf curves.

Computer Simulations of Random Sample Collections

Simulations were performed on the data for the 17 months that had adequate snow-urine samples and calf:cow ratio estimates (Table 2). The best trimming percentages were determined to be 15%-left tail / 20%-right tail because these percentages consistently yielded trimmed mean A:C ratios that were closest to the true cow mean A:C ratio and had smaller confidence interval widths than the other percentages considered. Therefore, trim percentages of 15%-left tail / 20% right tail were used for all reported trimmed means.

Untrimmed mean A:C ratios were typically biased high, compared to the true cow mean (Table 3). Trimmed mean A:C ratios consistently approximated the true cow mean more closely than untrimmed means, had lower variability (Fig. 2), and smaller confidence interval widths (Fig. 3). Coverage percentages for untrimmed and trimmed means were comparable. Variability of untrimmed and trimmed means and their

Table 2. Calf:cow ratios and allantoin:creatinine ratio sample sizes from cow and calf elk in the Madison-Firehole drainage of Yellowstone National Park, during five consecutive winters, used in computer simulations of random sample collections.

Month		Year				
		1991-92	1992-93	1993-94	1994-95	1995-96
December	Calves/100 cows		47			40
	No. calf samples		26			10
	No. cow samples		77			39
January	Calves/100 cows		40	48	41	34
	No. calf samples		8	47	24	11
	No. cow samples		89	87	87	63
February	Calves/100 cows	31	29	40	27	31
	No. calf samples	10	9	45	19	14
	No. cow samples	61	104	127	68	59
March	Calves/100 cows	20	19	38	23	15
	No. calf samples	4	7	14	14	4
	No. cow samples	46	72	101	91	51
April	Calves/100 cows			38		
	No. calf samples			20		
	No. cow samples			51		

Table 3. A comparison of untrimmed and trimmed mean allantoin:creatinine (A:C) ratios derived from computer simulations of random sample collections of size 10, 20, and 30, iterated 1000 times, from cow and calf elk. Mean difference was the average difference of untrimmed or trimmed mean A:C ratios from the mean A:C ratio of all cow samples.

Sample size		December - January		February - April	
		Untrimmed mean	Trimmed mean	Untrimmed mean	Trimmed mean
n = 10	Mean difference	+0.02	+0.01	+0.04	+0.01
	Standard deviation of means	0.03	0.02	0.06	0.03
	Mean 95% C.I. width	0.11	0.09	0.20	0.15
	Mean 95% coverage percentage	92	89	94	90
n = 20	Mean difference	+0.02	0.00	+0.04	0.00
	Standard deviation of means	0.02	0.02	0.04	0.02
	Mean 95% C.I. width	0.08	0.02	0.14	0.08
	Mean 95% coverage percentage	88	89	91	89
n = 30	Mean difference	+0.02	0.00	+0.04	0.00
	Standard deviation of means	0.02	0.01	0.03	0.02
	Mean 95% C.I. width	0.07	0.04	0.12	0.06
	Mean 95% coverage percentage	81	87	85	87

Figure 2. Relative frequency histograms of untrimmed and trimmed mean allantoin:creatinine (A:C) ratios from computer simulations of random sample collections for January (top panels) and April (bottom panels), 1994. A:C ratios from cow and calf elk in Yellowstone National Park were randomly selected ($n=20$) for each simulated collection. Collections were iterated 1,000 times for each month.

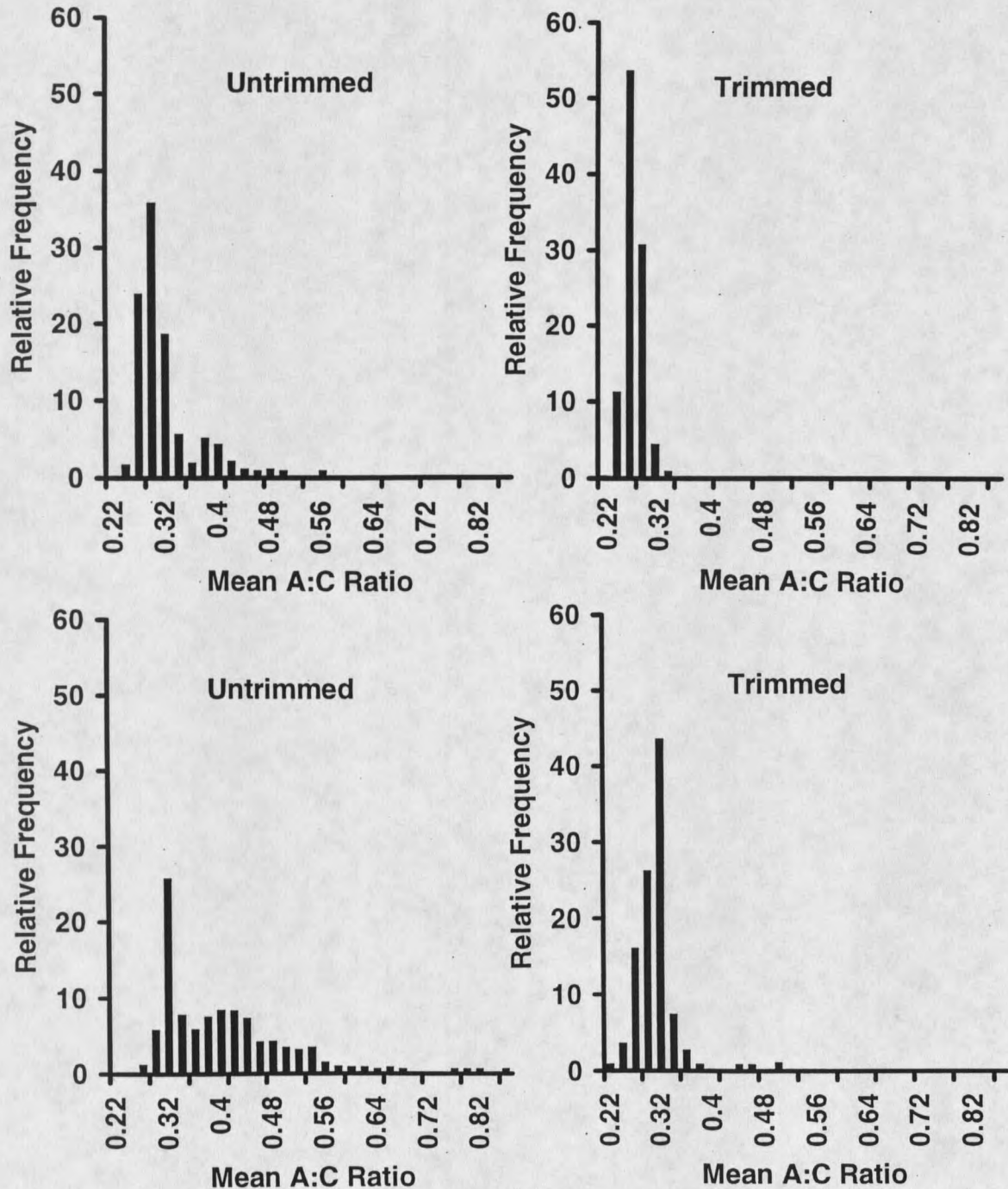
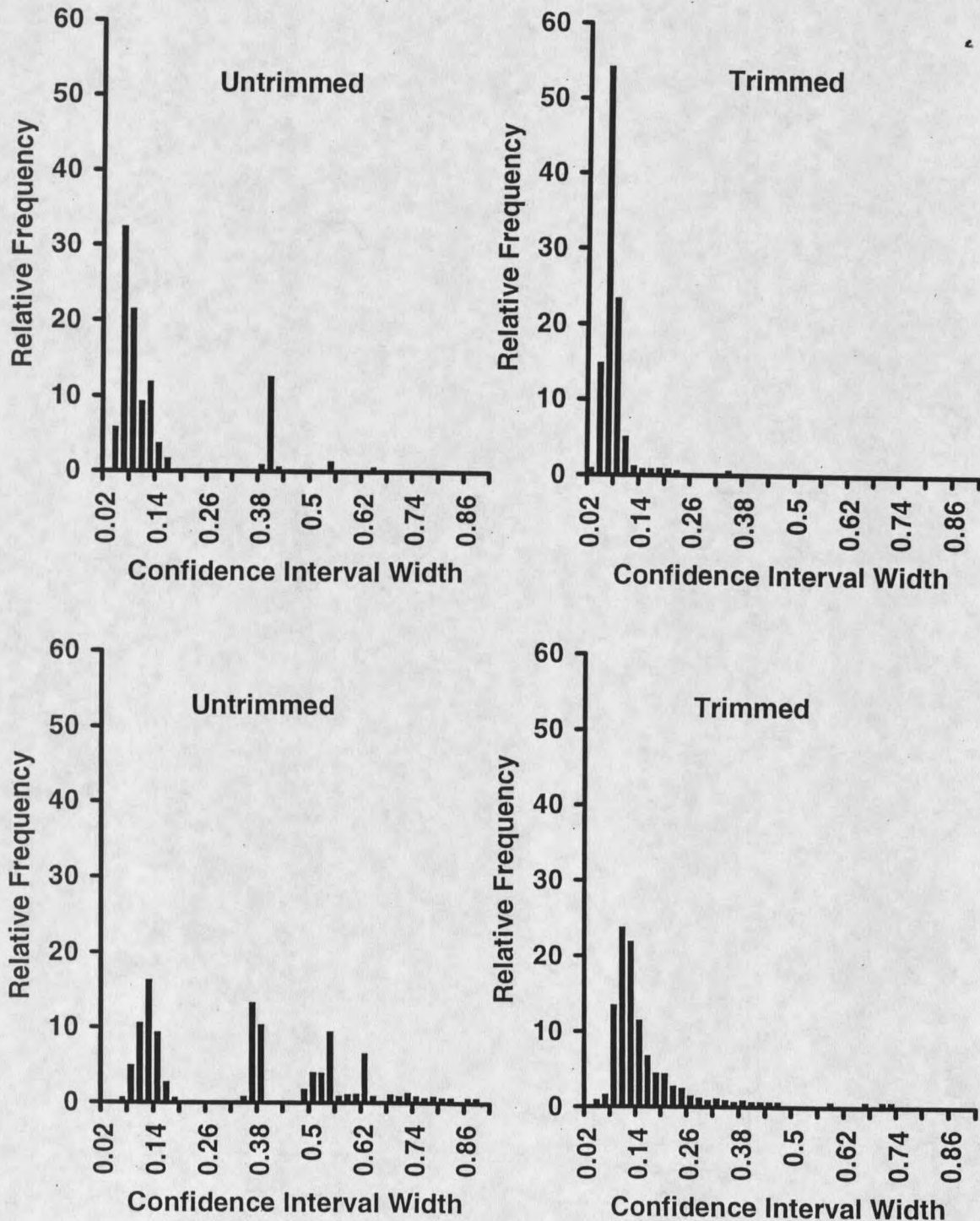


Figure 3. Relative frequency histograms of 95% confidence interval widths for untrimmed and trimmed mean allantoin:creatinine (A:C) ratios derived from computer simulations of random sample collections ($n=20$) from cow and calf elk in Yellowstone National Park for January (top panels) and April (bottom panels), 1994. Confidence intervals for untrimmed means were calculated by standard methods, while those for trimmed means were calculated by bootstrapping.



associated confidence interval widths increased from early to late winter. A larger decrease was observed in the bias from the true cow mean, standard deviation of the sample mean, and confidence interval width when sample size was increased from 10 to 20 than when sample size was increased from 20 to 30, for both untrimmed and trimmed means.

Random Sampling Protocol Design and Test

A total of 1,008 samples were collected at the six study areas during 1996-97 (Table 4), 765 of which were included in the analysis. Snow depths varied considerably across the study areas, and were substantially greater for the Hungry Horse study area than for the other areas (Table 4). The Madison-Firehole and Gros Ventre study areas had comparably high snow depths, while those for the Northern Range were lower. The National Elk Refuge and Wall Creek study areas had the lowest snow depth measurements.

The model for predicting A:C ratios of trimmed samples explained a much higher percentage ($R^2 = 0.61$) of the variability in A:C ratios than the model using untrimmed samples ($R^2 = 0.24$). Variability in mean A:C ratios was much higher for the Gros Ventre herd than for the other herds. Because the model-based curve of predicted A:C ratios for the Gros Ventre fit the data poorly, it was not reported. Significant differences were found in untrimmed ($P < 0.01$) and trimmed ($P < 0.01$) samples among study areas. Linear trend effects in A:C ratios were detected for both untrimmed ($P < 0.01$) and trimmed samples ($P < 0.01$), indicating that A:C ratios were not constant

Table 4. Sample sizes of allantoin:creatinine ratios and average snow depths at snow-urine sample collection sites for six study areas during the winter of 1996-97. Twenty measurements were taken at each site. All snow measurements for a given collection period were averaged, and expressed in centimeters. Sampling period dates were: Per.1 = 14-27 Dec, per. 2 = 28 Dec-10 Jan, Per. 3 = 11-24 Jan, Per. 4 = 25 Jan-7 Feb, Per. 5 = 8-21 Feb, Per. 6 = 22 Feb-7 Mar, Per. 7 = 8-21 Mar, and Per. 8 = 22 Mar-4 Apr.

Study area	Sampling period															
	1		2		3		4		5		6		7		8	
	n	Snow	n	Snow	n	Snow	n	Snow	n	Snow	n	Snow	n	Snow	n	Snow
Wall Creek	25	6.4			25	9.0	25	8.0	25	6.1	25	8.9	39	4.3	20	5.1
Hungry Horse	23	90.9	45	91.4	42	96.5	33	94.3	43	89.5			11	90.2	38	68.0
Northern Range	21	32.7	26	15.1	26	33.5	18		20	26.9			30	23.0		
Madison-Firehole	18	34.1	25	40.7	27	44.4	26	46.4	24	38.9	25	44.4	14	35.2		
Gros Ventre	22	43.7	20	38.1	16	39.4	19	53.3	15	50.8	33	31.5	19	25.4	16	28.3
Nat'l. Elk Refuge	21	8.5	19	5.1			43	31.0	23	16.0	23	10.2				
Totals	130		135		136		164		150		106		113		74	

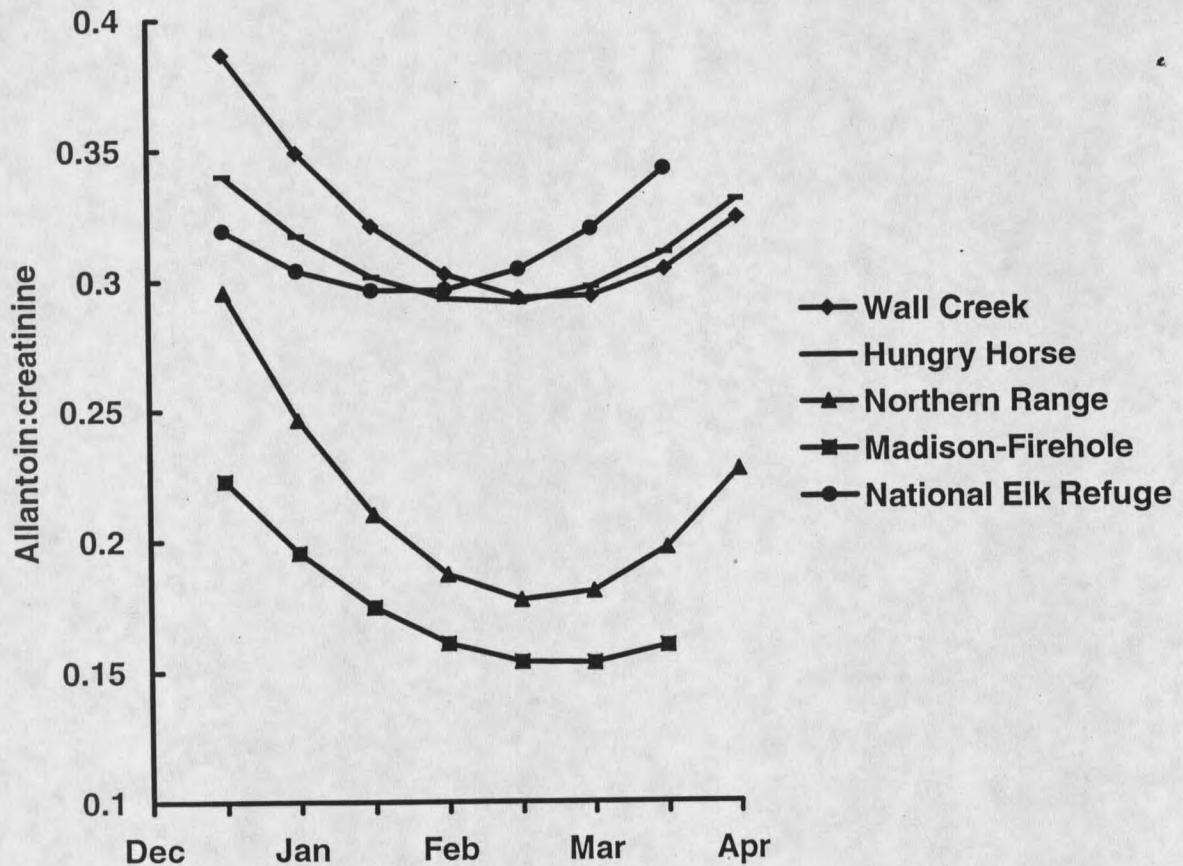
across time. There was a quadratic effect for both untrimmed ($P = 0.04$) and trimmed ($P < 0.01$) samples as well. When A:C from each study area were considered separately, there was a linear trend effect for both untrimmed ($P < 0.01$) and trimmed ($P < 0.01$) samples, with a different slope for each study area. In addition, there was a separate quadratic trend effect in untrimmed ($P < 0.01$) and trimmed ($P < 0.01$) samples for each study area.

More differences in average overwinter A:C ratios among study areas were detected with trimmed samples than with untrimmed samples. Bonferonni multiple comparison tests found a significant difference for trimmed, but not untrimmed samples, in the average overwinter A:C ratios between the National Elk Refuge and Northern Range herds (Table 5). The curves of predicted A:C ratios for the Northern Range and the National Elk Refuge were clearly separated from each other for the entire winter (Fig. 4). Average overwinter A:C ratios for the Wall Creek, Hungry Horse, Gros Ventre, and National Elk Refuge herds were not significantly different for either untrimmed or trimmed samples. These herds had the highest average overwinter A:C ratios, followed by the Northern Range and the Madison-Firehole herds. However, the curve of predicted A:C ratios for the National Elk Refuge showed an increase that coincided with the initiation of supplemental feeding (Jan. 29), while predicted A:C ratios for the other herds were still decreasing (Fig. 4). Curves of predicted A:C ratios for the Northern Range and Madison-Firehole herds were much lower than those for the other herds. Average overwinter A:C ratios for the Madison-Firehole herd were significantly lower than for the other herds with both untrimmed and trimmed samples. Average overwinter A:C ratios

Table 5. Point estimates with their associated errors and Bonferonni multiple comparison test groupings of average overwinter untrimmed and trimmed mean allantoin:creatinine (A:C) ratios for six study areas during the winter of 1996-97. Curves of predicted A:C ratios for each study area were calculated, and the average overwinter A:C value for each study area was determined by integrating to find the average value of the function defining each curve. Study areas with the same group letter (a, b, or c) had no significant differences in average overwinter A:C ratios among the areas, but were significantly different from study areas with a different letter.

Study area	Untrimmed			Trimmed		
	Estimated A:C ratio	Std. error	Group	Estimated A:C ratio	Std. error	Group
Wall Creek	0.36	0.01	a	0.32	0.01	a
Hungry Horse	0.32	0.01	a	0.31	0.01	a
Gros Ventre	0.32	0.01	a	0.31	0.01	a
National Elk Refuge	0.32	0.02	a,b	0.32	0.01	a
Northern Range	0.27	0.01	b	0.22	0.01	b
Madison-Firehole	0.19	0.01	c	0.18	0.01	c

Figure 4. Predicted allantoin:creatinine (A:C) ratios derived from random sample collections for five free-ranging elk herds during the winter of 1996-97. Samples were collected during eight sampling periods from 14 Dec-4 Apr. For each sampling period, the largest 20% and the smallest 15% of A:C ratios were trimmed from the data set prior to analysis.



differed considerably between untrimmed and trimmed samples for the Wall Creek and Northern Range herds (Table 5). In both cases, estimates were lower for trimmed samples.

DISCUSSION

Tests For Differences in Mean A:C Ratios Among Sex-age Classes

Results presented in Fig. 1 are consistent with those reported by Garrott et al. (1996) for repeatedly-sampled cows. Both studies detected a U-shaped pattern in A:C ratios for each winter, with highest values typically occurring in December and April. In both cases, differences in mean A:C ratios were found across winters.

Differences in mean A:C ratios among sex-age classes were detected. Segregation of cows and bulls during winter may help explain the observed differences in mean A:C ratios between these two groups. Sexual segregation in winter is commonly observed among elk, along with many other north-temperate cervids and bovids (Main et al. 1996). Bulls frequently band together in small groups separate from larger cow/calf groups (Peek and Lovaas 1968). Several investigators have found that winter diets of females were higher in crude protein than diets of males, suggesting that males segregate to poorer quality habitat (Main and Coblentz 1990). However, Staines et al. (1982) found that red deer (*Cervus elaphus*) stags probably compensated for a lower quality diet by increasing forage intake relative to body size. Clutton-Brock et al. (1982) reported that although feeding rates for the two sexes were similar during winter, stags spent more time feeding each day than hinds. Stags also avoided areas that were grazed heavily by hind groups, in

favor of areas supporting a higher biomass of lower-quality forage (Clutton-Brock et al. 1987, Staines et al. 1982). Higher intake rates by males could increase their metabolizable protein and energy intake, translating into the higher observed A:C ratios.

Several investigators have reported that calf elk have higher forage intake rates during winter relative to body size than do adults. Murie (1951) found that elk calves fed hay consumed a daily average of 31.1 g / kg of body weight, compared to 25.0 g / kg of body weight for adults. Likewise, Hungerford (1952) fed unrestricted diets of hay to cow and calf elk held in feeding pens. Calves consumed a daily average of 23.0 g / kg of body weight, while cows ate 20.6 g / kg of body weight. In a similar study, Geis (1954) reported that calf elk consumed hay at a daily average rate of 25.9-26.6 g / kg of body weight, compared to 20.4-20.9 g / kg of body weight for cows. Differences in the excretion of creatinine between cows and calves should also influence A:C ratios. Creatinine is excreted in constant proportion to muscle mass (Coles 1980). Because they have less muscle tissue, snow-urine samples from calves would be expected to exhibit lower concentrations of creatinine relative to allantoin, than those from cows. Greater forage intake rates along with lower excretion of creatinine should combine to produce higher average A:C ratios for calves than for cows.

Computer Simulations of Random Sample Collections

Because there are differences in mean A:C ratios between cows and calves, A:C ratios of cow and calf elk have separate distributions. Trimming 20% from the right tail of ordered A:C ratio distributions appears to effectively censor calf A:C ratios. However,

because the right tail of the cow distribution and the left tail of the calf distribution sometimes overlap, trimming 20% from the right tail of the sample distribution also removes a variable number of the largest cow A:C ratios. Trimming 15% from the left tail of the sample distribution compensates for the cow A:C ratios trimmed from the right tail. As a result, trimmed means of random sample collections closely approximate the true cow mean.

Results from computer simulations of random sample collections from animals of unknown sex-age class are similar to those reported by White et al. (1995) for UN:C. Untrimmed mean A:C ratios may have substantial bias and variability (Fig. 2) due to differences in the proportion of each sex-age class sampled between collections and differences in mean A:C ratios among sex-age classes. Large confidence intervals sometimes associated with untrimmed mean A:C ratios (Fig. 3) would limit the power to detect differences in the nutritional level of populations. However, the use of trimmed means of A:C ratios derived from random sample collections appears to provide an accurate index of cow nutrition with increased power to detect differences in the nutritional level of populations.

Changes in calf:cow ratios across and within winters would influence the effectiveness of trimmed means. Early winter calf:cow ratios tend to be relatively constant among years. In populations which experience significant juvenile mortality, calf:cow ratios typically decrease as winter progresses, with lower calf:cow ratios during winters of greater severity (Garrott et al. 1996). Therefore, there would be a greater probability of sampling calves in late winter or early spring during mild rather than severe

winters. Trimmed means would be most useful in late winter or early spring when differences in mean A:C ratios among sex-age classes are greatest (Fig. 1), especially during mild winters with low juvenile mortality and a higher probability of sampling calves.

A threshold value for sample size was suggested by the simulations. A marked decrease in the bias, variability, and confidence interval width associated with sample means was observed when the sample size was increased from 10 to 20, while there was little change in those same statistics when the sample size was increased from 20 to 30. Therefore, 20 samples per collection period should be obtained during random sample collections in order to maximize the accuracy and power to detect differences in nutritional levels of populations, while minimizing the time and costs of collecting samples.

Random Sampling Protocol Design and Test

The analysis of average overwinter A:C ratios for the six study areas during the winter of 1996-97 indicated that the use of trimmed samples provided greater ability to detect differences in the nutrition of free-ranging elk populations. No significant difference was found in average overwinter A:C ratios between the Northern Range and the National Elk Refuge herds when untrimmed samples were compared, yet a significant difference was detected when trimmed samples were used. Because it is probably reasonable to assume that the nutrition of a supplementally-fed population would be substantially higher than that of a naturally-regulated population, it appears that the use of

trimmed samples provided a better assessment of overwinter nutrition than did untrimmed samples. Average overwinter A:C ratios for the Wall Creek and Northern Range herds were considerably lower when trimmed samples were used, compared to untrimmed samples. Results of computer simulations suggested that untrimmed mean A:C ratios sometimes overestimate the true cow mean A:C ratio by a substantial margin. As a result, the use of trimmed mean A:C ratios provided a closer and more robust approximation of the true cow mean A:C ratio. This suggests that untrimmed predicted A:C ratios from these areas may have been an overestimate of the true cow mean A:C ratio, perhaps due to sampling a high percentage of calves and/or bulls.

Average overwinter A:C ratios of the Northern Range and Madison-Firehole herds in Yellowstone National Park followed the expected pattern. Management for natural regulation predicts that populations would sometimes increase to high densities, and mortality due to malnutrition would be an important regulating factor (Houston 1982). Average overwinter A:C ratios for these herds were significantly lower than for any of the other herds, indicating that nutrition for cow elk in these populations was substantially lower than for the two herds managed for sustained yield and the two herds which were supplementally fed. In addition, the average overwinter A:C ratio of the Madison-Firehole herd was significantly lower than that for the Northern Range herd, as was expected. Environmental conditions during winter are typically much more severe at the higher-elevation Madison-Firehole area. For example, measured snow depths were greater for the Madison-Firehole area than for the Northern Range (Table 4) throughout the winter, which probably resulted in decreased access to forage.

As expected, mean overwinter A:C ratios from the Wall Creek and Hungry Horse herds were significantly higher than those from the Northern Range or Madison-Firehole herds. However, curves of predicted A:C ratios for the Wall Creek and Hungry Horse herds were similar (Fig. 4) and not significantly different from each other. Snow depths for the Hungry Horse area were much greater than for any of the other areas, while those for Wall Creek were among the lowest of the six study areas (Table 4). Because snow was much deeper at Hungry Horse and access to forage should have been much more restricted, it might have been expected that A:C ratios from this herd would have been substantially lower than those for Wall Creek. However, the Hungry Horse herd was at much lower densities than the Wall Creek herd. Also, formation of a hard crust in early January at the Hungry Horse area allowed elk to walk on top of the snowpack for much of the winter, and they foraged extensively on high-quality browse plants, lichens, and mosses, in addition to conifer needles and tips (J. Vore, Montana Department of Fish, Wildlife, and Parks, personal communication). These factors may have combined to produce a level of nutrition in the Hungry Horse herd similar to that of the Wall Creek herd. Although A:C ratios from both herds indicated a fairly high nutritional level for cow elk in these populations as compared to the two Yellowstone herds, predicted A:C ratios over most of the winter were below those reported by Garrott et al. (1997) for maintenance-level diets.

No significant difference was found in average overwinter A:C ratios between the National Elk Refuge herd and the Wall Creek and Hungry Horse herds, even though animals at the National Elk Refuge were supplementally fed for much of the winter.

Predicted A:C ratios for the National Elk Refuge herd before initiation of feeding were lower than those for the Wall Creek and Hungry Horse herds (Fig. 4). Unlike the Wall Creek and Hungry Horse herds, supplemental feeding maintains the National Elk Refuge herd at higher densities than the natural forage base could support without sustaining long-term damage. Prior to initiation of feeding, high elk densities may decrease the amount of forage available per animal for the National Elk Refuge herd compared to the Wall Creek and Hungry Horse herds, which are managed at much lower densities. Once feeding was initiated, the nutritional level of the National Elk Refuge herd appeared to increase above that of the Wall Creek and Hungry Horse herds (Fig. 4). However, the difference in predicted A:C ratios between the National Elk Refuge herd after initiation of feeding and the Wall Creek and Hungry Horse herds was not as great as was expected.

Mean A:C ratios associated with the Gros Ventre herd were much more variable than those from any of the other herds. As a result, the only inference that could be made about the nutritional level of this herd was that it was higher than the nutritional level of the two Yellowstone herds. Several factors could help explain the observed variability in mean A:C ratios for this herd. There may be substantial variability in the quantity of hay ingested among animals. For example, some elk do not come out to feed immediately after hay is put out. These animals may not consume as much hay as those that begin feeding immediately. Also, periodic winter thaws allow elk to utilize natural vegetation in addition to supplemental feed, adding to variability in nutrition between sampling periods. Garrott et al. (1997) reported that A:C ratios quickly respond to changes in digestible dry matter intake, so short-term changes in forage intake would likely be

reflected by changes in mean A:C ratios.

The degree of winter severity may have influenced the results of the comparisons of average overwinter A:C ratios among the six herds. All study areas were subjected to a severe winter during 1996-97, with near-record snowpacks, extended periods of cold temperatures, and heavy crust formation on the snowpack in many areas. Such conditions undoubtedly affected the nutritional levels of individual populations, as well as comparisons of nutritional levels among different populations. This study should be repeated during winter(s) of more average severity to provide greater insight into the sensitivity of mean A:C ratios derived from random sample collections for indexing the winter nutrition of free-ranging elk.

MANAGEMENT IMPLICATIONS

When the sampling protocol and statistical analysis approach presented in this paper were used, mean A:C ratios derived from random sample collections appeared to provide a promising non-invasive technique for indexing the nutrition of free-ranging elk populations. Significant differences in average overwinter A:C ratios were detected among several herds. More subtle differences in nutrition, although not statistically significant, were suggested by plots of predicted A:C ratios. However, the type of comparison made in this paper may not be the most useful kind. While A:C ratios provided insight into the nutrition of different populations relative to each other, interpretation of such comparisons was difficult. Animal densities, habitat types, snowpack, and climate varied considerably among study areas. All of these factors probably had major effects on elk nutrition, and confounded attempts to interpret results of such comparisons. A more useful application of the technique might be to make comparisons of nutrition on a single area or several similar areas across winters, which would reduce the number of confounding factors and simplify interpretation of results. Biologists might use this type of comparison for such purposes as evaluating the effects of habitat manipulations or the quality of winter ranges, or determining appropriate population densities for winter ranges.

Before A:C ratios can be used to index the nutrition of ungulate species other than elk, preliminary research must be performed to determine the importance of allantoin relative to other purine derivatives for indexing dietary intake for each species (Garrott et al. 1996). The response of A:C ratios to dietary intake by other ungulate species should be similar to that observed for elk. Differences in mean A:C ratios among sex-classes, similar to those discussed for elk in this paper, would be expected to exist for other ungulate species, as well.

LITERATURE CITED

- Alt, K.L., M.R. Frisina, and F.J. King. 1992. Coordinated management of elk and cattle, a perspective - Wall Creek Wildlife Management Area. *Rangelands* 14:12-15.
- Balcells, J., J.A. Guada, C. Castrillo, and J. Gasa. 1991. Urinary excretion of allantoin and allantoin precursors by sheep after different rates of purine infusion into the duodenum. *Journal of Agricultural Science* 116:309-317.
- Boyce, M.S. 1989. The Jackson elk herd: intensive wildlife management in North America. Cambridge University Press, Cambridge, U.K. 306 pp.
- Chen, X.B., F.D. DeB. Hovell, E.R. Ørskov, and D.S. Brown. 1990. Excretion of purine derivatives by ruminants: effect of exogenous nucleic acid supply on purine derivative excretion by sheep. *British Journal of Nutrition* 63:131-142.
- Chen, X.B., A.T. Mejia, D.L. Kyle, and E.R. Ørskov. 1995. Evaluation of the use of the purine derivative:creatinine ratio in spot urine and plasma samples as an index of microbial protein supply in ruminants: studies in sheep. *Journal of Agricultural Science* 125:137-143.
- Clutton-Brock, T.H., F.E. Guinness, and S.D. Albon. 1982. Red deer: behavior and ecology of two sexes. University of Chicago Press, Chicago, Illinois. 378 pp.

- Clutton-Brock, T.H., M. Major, and F.E. Guinness. 1985. Population regulation in male and female red deer. *Journal of Animal Ecology* 54:831-846.
- Clutton-Brock, T.H., G.R. Iason, and F.E. Guinness. 1987. Sexual segregation and density-related changes in habitat use in male and female red deer (*Cervus elaphus*). *Journal of Zoology* 211:275-289.
- Cole, G.F. 1969. The elk of Grand Teton and Southern Yellowstone National Parks. National Park Service Resource Report GRTE-N-1. Washington, D.C. 80 pp.
- Coles, E.H. 1980. Veterinary clinical pathology. W.B. Saunders Co., Philadelphia, Pennsylvania. 562 pp.
- Craighead, J.J., F.C. Craighead, Jr., R.L. Ruff, and B.W. O'Gara. 1973. Home ranges and activity patterns of nonmigratory elk of the Madison drainage herd as determined by biotelemetry. Wildlife Monograph Number 33. 50pp.
- DelGuidice, G.D., M.L. Mech, and U.S. Seal. 1989. Physiological assessment of deer populations by analysis of urine in snow. *Journal of Wildlife Management* 53:284-291.
- Efron, B. and R.J. Tibshirani. 1993. An introduction to the bootstrap. Chapman and Hall, Inc., London, Great Britian. 436 pp.
- Garrott, R.A., P.J. White, D.B. Vagnoni, and D.M. Heisey. 1996. Purine derivatives in snow-urine as a dietary index for free-ranging elk. *Journal of Wildlife Management* 60:735-743.

Garrott, R.A., J.G. Cook, J.G. Berardinelli, P.J. White, S. Cherry, and D.B. Vagnoni.

1997. Evaluation of urinary allantoin:creatinine ratios as a nutritional index for elk. *Canadian Journal of Zoology* 75:1519-1525.

Geis, A.F. 1954. The food requirements and relative digestibility of a variety of winter diets fed to elk (*Cervus canadensis nelsoni*) under controlled conditions. M.S.

Thesis, University of Montana, Missoula. 68 pp.

Guinness, F.E., T.H. Clutton-Brock, and S.D. Albon. 1978. Factors affecting calf mortality in red deer (*Cervus elaphus*). *Journal of Animal Ecology* 47:817-832.

Houston, D.B. 1982. The northern Yellowstone elk. MacMillan Publishing, New York, New York. 474 pp.

Hungerford, C.R. 1952. The food consumption and weight response of elk (*Cervus canadensis nelsoni*) under winter conditions. M.S. Thesis, University of Montana, Missoula. 60 pp.

Main, M.B., F.W. Weckerly, and V.C. Bleich. 1996. Sexual segregation in ungulates: new directions for research. *Journal of Mammology* 77:449-461.

Main, M.B., and B.E. Coblentz. 1990. Sexual segregation among ungulates: a critique. *Wildlife Society Bulletin* 18:204-210.

MathWorks, Inc. 1993. *MATLAB Software*. MathWorks, Inc., Natick, Massachusetts.

Mautz, W.W. 1978. Nutrition and carrying capacity. Pages 321-348 in J.L. Schmidt and D.L. Gilbert, eds. *Big game of North America: ecology and management*. Stackpole Books, Harrisburg, Pennsylvania.

- Moen, R., and G. D. DelGuidice. 1997. Simulating nitrogen metabolism and urinary urea nitrogen:creatinine ratios in ruminants. *Journal of Wildlife Management* 61:881-894.
- Murie, O.J. 1951. *The elk of North America*. Stackpole Books, Harrisburg, Pennsylvania. 376 pp.
- Peek, J.M., and A.L. Lovaas. 1968. Differential distribution of elk by sex and age on the Gallatin winter range, Montana. *Journal of Wildlife Management* 32:553-557.
- Ruppert, D. 1988. Trimming and Winsorization. Pages 348-353 in C.B. Read, S. Kotz, and N.L. Johnson, eds. *Encyclopedia of Statistical Sciences*, Vol. 9. John Wiley and Sons, New York, New York.
- SAS Institute Inc. 1992. SAS technical report P-229. SAS Institute Inc., Cary, North Carolina. 620 pp.
- Singer, F.J., A. Harting, K.K. Symonds, and M.B. Coughenour. 1997. Density dependence, compensation, and environmental effects on elk calf mortality in Yellowstone National Park. *Journal of Wildlife Management* 61:12-25.
- Smith, B.L., R.L. Robbins, and S.H. Anderson. 1997. Early development of supplementally fed, free-ranging elk. *Journal of Wildlife Management* 61:26-38.
- Staines, B.W., J.M. Crisp, and T. Parish. 1982. Differences in the quality of food eaten by red deer (*Cervus elaphus*) stags and hinds in winter. *Journal of Applied Ecology* 19:65-77.

- Thorne, E.T., R.E. Dean, and W.G. Hepworth. 1976. Nutrition during gestation in relation to successful reproduction in elk. *Journal of Wildlife Management* 40:330-335.
- Vagnoni, D.B., R.A. Garrott, J.G. Cook, P.J. White, and M.K. Clayton. 1996. Urinary allantoin:creatinine ratios as a dietary index for elk. *Journal of Wildlife Management* 60:728-734.
- White, P.J., R.A. Garrott, C.A. Vanderbilt White, and G.A. Sargeant. 1995. Interpreting mean chemical ratios from simple random collections of snow-urine samples. *Wildlife Society Bulletin* 23:705-710.

MONTANA STATE UNIVERSITY LIBRARIES



3 1762 10274914 8