Assessing animal condition, nutrition, and stress from urine in snow

A critical view *David Saltz, Gary C. White, and Richard M. Bartmann*

Response *Glenn D. DelGiudice, Michael R. Riggs, L. David Mech, and Ulysses S. Seal*

David Saltz and colleagues offer alternative views to Glenn DelGiudice's review of using urine in snow to index nutritional restriction. DelGiudice and his colleagues respond with counterpoints. Ungulate managers and researchers take note!

A critical view

Managing wild populations depends on correctly assessing their future reproductive success and survival (Downing 1980). Many morphological and physiological indices have been offered for assessing future performance of populations (Franzmann 1985). A widely used physiological index has been urea nitrogen (Harder and Kirkpatrick 1994). However, concerns about its reliability for assessing performance of individuals or populations have been repeatedly raised (Kirkpatrick et al. 1975, Kie et al. 1983, Brown 1984, Saltz et al. 1992, Harder and Kirkpatrick 1994).

These concerns were mostly based on the fact that high urea nitrogen may indicate good forage or advanced starvation. Nevertheless, the use of urea nitrogen as a physiological index has been continuously advocated (DelGiudice 1995 and citations therein). Specifically, DelGiudice (1995) advocated the use of urea nitrogen:creatinine (UN:C) ratios from urine in snow to assess nutritional condition and nutritional deprivation of deer (*Odocoileus* spp.) populations on winter range. We contend that, with the present state of knowledge, metabolites from urine in snow, specifically UN:C ratios, cannot reliably be used for such assessments. We support this contention by ad-

Address for David Saltz: Science Division, Nature Reserves Authority, 78 Yirmeyahu Street, Jerusalem 94467, Israel. Gary C. White: Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins, CO 80523, USA. Richard M. Bartmann: Research Section, Colorado Division of Wildlife, 317 West Prospect Road, Fort Collins, CO 80526, USA. Glenn D. DelGiudice: Forest Wildlife Populations and Research, Group, Minnesota Department of Natural Resources, Grand Rapids, MN 55744, USA. Michael R. Riggs: Section of Wildlife, Minnesota Department of Natural Resources, St. Paul, MN 55155, USA. L. David Mech: U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, MD 20708, USA. Ulysses S. Seal: Captive Breeding Specialist Group, World Conservation Union, Apple Valley, MN 55124, USA.

dressing the issue at 3 levels: (1) at the definition level, we argue that urea nitrogen is not a direct index of nutritional condition, (2) at the level of the individual animal, we identify problems associated with the patterns in urea nitrogen during the starvation process that limit its applicability as an index, and (3) at the population level, we point out limitations in assessing nutritional deprivation or condition inherent to sampling urine in snow.

Urea nitrogen and definitions of condition, nutrition, and stress

Many physiological variables offered for assessing future survival and performance of animals have been labeled as indices of either condition, nutrition, or stress. The term condition is frequently used in wildlife and animal science literature in a vague and undefined manner, so there is a need for precise, operational, and quantifiable definition (Murphy and Noon 1991).

Many researchers attempted to define condition (Caughley 1971, Owen and Cook 1977, Hanks 1981, Taber et al. 1979). The resultant definitions were sometimes considerably different and, consequently, condition has been used loosely and interchangeably with nutrition and stress. To clarify and narrow existing definitions, the adjective nutritional has been used in conjunction with condition (Parker et al. 1993a, DelGiudice et al. 1994). However, only recently did Grubb (1995) clearly define nutritional condition as: "The state of body components controlled by nutrition and which in turn influence an animal's fitness." This definition was adopted by Harder and Kirkpatrick (1994) in the recent techniques manual published by The Wildlife Society.

Grubb's (1995) definition has several attributes that make it superior to others: (1) it clearly identifies nutritional condition as a state variable, (2) the term fitness clearly describes how condition effects the animal and, therefore, why it is important to the wildlife manager, and (3) if the term nutrition is removed, a clear definition of condition still remains (i.e., the state of body components which influence an animal's fitness). Because there are many body components and their state can be evaluated in many ways (e.g., physical damage, functional disorder, disease, etc.), the definition of condition must be narrowed so it addresses only the nutritional aspect, hence nutritional condition. We do, however, recommend substituting the term future fitness for fitness because condition does not affect past reproductive success.

In contrast to condition, nutrition, as defined by Robbins (1983) and rephrased by Grubb (1995), is a variable that describes the rate at which assimilable energy and nutrients are ingested and processed. Similarly, stress is a variable that describes the instantaneous rate at which energy and protein reserves are depleted (Saltz and White 1991a). The relationship between nutrition, nutritional condition, and stress can be illustrated in a simple conceptual model (Fig. 1). In this model, nutritional condition is a state variable and nutrition and stress are rate variables affecting it; nutrition has a positive and stress a negative effect. It is within this context that we must evaluate physiological indices of an animal's current and future well being.

As pointed out by DelGiudice (1995), urea is the product of amino acids from 2 sources: ingested protein and lean body tissue. Four factors affect blood urea nitrogen (BUN) and urinary UN:C ratios (Warren et al. 1982): protein intake, energy intake, tissue catabolism, and urea recycling. There is a direct relationship between protein intake and BUN or UN:C ratios (Franzmann 1985). During prolonged starvation, however, urea nitrogen levels rise after fat reserves are exhausted and lean body tissue is more heavily used for energy (DeCalesta et al. 1975, Torbit et al. 1985). Therefore, when diet is adequate, urea nitrogen is an index of nutrition, while in starving animals that are catabolizing lean body tissue, it is an index of stress reflecting the rate at which lean body tissue is consumed. Condition (nutritional or otherwise) is not a process and is not measured by rates; it is a state. Thus, at no time does urea nitrogen index nutritional condition, except possibly as a boolean (yes or no) indicator of fat reserves which, as we shall show later, has limited value.
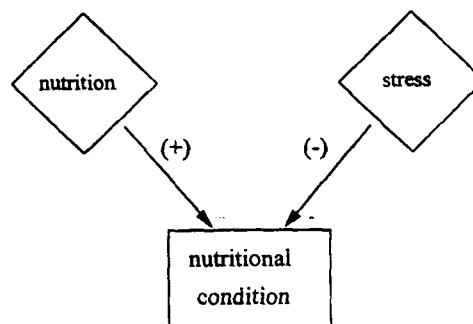

Fig. 1. A conceptual model describing the relationship between nutrition, nutritional condition, and stress in an individual animal. Nutritional condition is a state variable. Nutrition and stress are rate variables with positive and negative affects, respectively, on nutritional condition.

Urea nitrogen and starvation in individual animals

The starvation process is commonly divided into 3 phases (Young and Scrimshaw 1971, Torbit et al. 1985). In Phase I, fat and protein reserves (mainly alanine) are catabolized. As alanine reserves are exhausted, the body relies mostly on fat reserves (Phase II). When fat reserves dwindle, the animal enters Phase III, which is characterized by rapid catabolism of lean body tissue. These 3 phases are clearly reflected in BUN levels and urinary UN:C ratios (Cahill 1970, DeCalesta et al. 1975, Warren et al. 1982, Torbit et al. 1985, Saltz and White 1991a, Cherel et al. 1994, DelGiudice et al. 1994), forming what has been termed the "U-shaped" curve (Harder and Kirkpatrick 1994).

The problems with differentiating between high UN:C ratios of Phase I and those of Phase III have been pointed out previously (Brown 1984, Harder and Kirkpatrick 1994). Possibly, these problems may be resolved by measuring other urinary metabolites such as cortisol (Saltz and White 1991a) or potassium (DelGiudice 1995). However, the major drawback of urea nitrogen as an index to nutrition, nutritional condition, or stress is due to a different problem—the extended duration of Phase II. This phase, characterized by low and relatively unchanged BUN levels and urinary UN:C ratios, can extend over several months and even the entire winter in mature deer (DeCalesta et al. 1975, Saltz and White 1991a, DelGiudice et al. 1994). During this time the animal's weight (nutritional condition) continues to decline. This is probably the biggest failure of urea nitrogen as an index. The pattern is evident in other homeotherms (Young and Scrimshaw 1971, Cherel et al. 1994), but should be especially pronounced in deer and other species that recycle urea (Robbins et al. 1974). Low UN:C ratios indicate nutritional deprivation but do not provide much insight to nutritional condition. Rather, the question here is not whether deer on northern winter ranges are nutritionally deprived (they all are), but how severe is this deprivation.

Phase III, in which BUN and UN:C ratios are elevated, is relatively short. Because the amount of energy/unit mass in lean body tissue is far less than in fat, lean body tissue is rapidly consumed and death follows shortly after onset of this phase. Also, rapid loss of lean body tissue reduces the animal's ability to move around and forage, which quickens this process (Saltz and White 1991b).

By combining data from Phase II and Phase III and regressing BUN or UN:C ratios on time or body mass, a significant but spurious positive relationship is produced. A good example of this can be found in DelGiudice et al. (1994:Fig. 4b). They concluded that "The stronger and significant relationship between percent mass loss and urinary UN:C ratios in our captive deer lends confidence to the potential of this characteristic as an index of nutritional condition." However, scrutiny of this figure reveals that residuals around the linear regression were not normally distributed and that linear regression is an inappropriate model for these data. To highlight the consequences involved, consider that most data points for weight losses of 20-25% corresponded to UN:C ratios of ≤5. But according to the regression function, an animal with a UN:C ratio of 5 would have lost only 10% of its mass, less than half the actual loss. Also in Fig. 4b, 21 of 25 data points for weight losses of 10-25% fell below the regression line (i.e., their mass loss based on UN:C ratios was underestimated by the line). A log transformation or a power function would improve the fit of the model to the data. However, given the distribution pattern of points in Fig. 4b (DelGiudice et al. 1994), we suspect that even after transformation, the 95% confidence bands (on the regression line and mean UN:C ratios) would be such that, in an inverse regression (calibration), UN:C ratio would be a poor predictor of percent cumulative mass losses.

Assessing population performance through urine in snow

We assume that populations experiencing pronounced nutritional deprivation will have a higher proportion of animals in Phase III of the starvation process than populations facing mild nutritional deprivation. Because Phase III is synonymous with depleted fat reserves, this boolean (yes or no) index may be used as an index of population nutritional condition. However, making such assessments from urine in snow poses several problems. First is the between age- and sex-class variation. We agree with DelGiudice (1995) that fawns and adult does in the same area would not be expected to have the same pattern of UN:C or cortisol:creatinine (Co:C) ratios. In contrast to fawns, adults have greater access to food, lower metabolic rates, and larger fat reserves that allow them to survive longer. There is no practical, cheap method to determine age class from urine, so this inter-class difference will increase the variance of any index and greatly reduce the power of statistical tests to detect differences through time.

Second, there is individual heterogeneity within age and sex classes. Such variation would be large because the probabilities of survival of individuals within an age and sex class would not be identical (i.e., all animals within an age class do not die simul-

taneously). Their weight loss patterns and survival depend on their mass when entering winter as well as access to forage and other factors (Saltz and White 1991*a*, Bartmann et al. 1992). Obviously, sampling urine in snow precludes controlling for these individual covariates. The result is that large sample sizes are needed to obtain precise estimates of the mean index level. Age- and sex-class variation and individual heterogeneity within age and sex classes mask over-winter trends in the indices and, hence, reduce power of statistical procedures to detect trends through time.

Third, there is a serious problem associated with sampling over time. To visualize this, imagine a bell-shaped curve that depicts the probability-density function of the UN:C index in urine-snow samples at the first sampling time (Fig. 2, curve A). A second sample is then taken later in the winter (Fig. 2, curve B). The right tail of this distribution will be truncated by animals that die. As winter progresses, the distribution shifts to the right from increased undernutrition as more animals die. Censoring of individuals from the population (dead deer don't leave urine in snow) causes the means of UN:C at times 1 and 2 to be nearly identical, even though the second distribution is shifted to the right from the first. The right-censoring effect of mortality decreases the effectiveness of the index for detecting trends in the time series of samples. Thus, once again, it appears that the UN:C ratio can only be used as a "yes or no" index of fat reserves. Because of lower survival rates for fawns (Bartmann et al. 1992), the percent of the total snow–urine samples from fawns will decrease as winter progresses. Adults may well exhibit low UN:C values prior to major fawn mortality, so the declining

number of fawns will cause a decline in the percent of samples with elevated UN:C. These phenomena could lead to the mistaken conclusion that population condition is improving.

Another problem associated with sampling over time can be seen in the winter storm example discussed by DelGiudice (1995). He recommended sampling urine in snow immediately following snow fall for obvious logistical reasons. Unfortunately, this period is also when deer are the most stressed because of not feeding during the snow storm. The effect is to increase variation across time. To adequately sample this variation, more samples across time would be required as well as larger sample sizes at each time period.

Finally, the link between the indices discussed and the survival rate of the population is weak (Saltz et al. 1992, 1993). DelGiudice (1995) discredited the attempts by Saltz et al. (1992) to link fawn survival to urine indices because only yearlings were used. Although we had data on fawns (Saltz and White 1991*a*), we did not use them. The reason was that after losing 15% of their weight, fawns were removed from the experiment and fed to keep them from dying. This removal, which emulated mortality in the wild, caused mean UN:C and Co:C ratios for the remaining animals to drop after each removal. By using only yearlings, we avoided this truncation problem and minimized the inter-age class variation (i.e., inter-age variation was zero). Further, the yearlings were paired to minimize the difference in indices between the 2 treatments. Finally, yearlings were more likely than adults to be stressed but not suffer the censoring effects of mortality like fawns. Thus, in response to DelGiudice's (1995) criticism, we argue that we maximized our chances of detecting a relationship between the urine indices and over-winter survival. Still, we found that the variation across animals masked the theoretical result we expected until individual-specific covariates were included in the statistical models.

To examine the necessary sample sizes to detect changes in UN:C and Co:C between 2 sampling periods, we used the data for yearlings from Saltz et al. (1992). We estimated the within-period variance with density effect removed (representing individual variation) as 0.202 for UN:C and 0.00139 for Co:C, and the between-period variance (representing changes through time) as 0.072 for UN:C and 0.0000455 for Co:C. Hence, effect sizes of 0.268 and 0.00675 represent a 1 SD change in UN:C and Co:C across time, respectively. To detect an increase in UN:C from a population mean of 0.1 (representing a typical mid-winter value for these yearlings) to 0.368
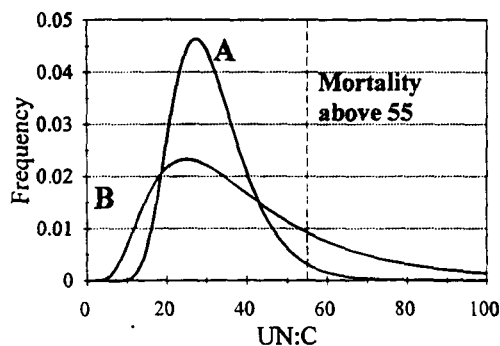

Fig. 2. Mortality in a population can censor UN:C to cause the same mean UN:C at 2 times for live animals, but with different proportions of the population assumed dead. Animals with UN:C values >55 are assumed dead. At time 1 (curve A), only a small proportion of the population has died and the mean of UN:C of live animals is approximately 30. Later at time 2 (curve B), a much larger proportion of the population has died, but mean UN:C of live animals is still approximately 30.

would require $n = 60$ for each sample with $\alpha = 0.05$ and $\beta = 0.10$. However, the estimated variance of 0.202 is much smaller than we would expect under field conditions where snow dilution will increase variance and where multiple age classes would be encountered. Assume that the variance would double (i.e., Var[UN:C] = 0.404). Then $n = 119$ for each sample. The same calculations for Co:C are even less encouraging, as detecting an increase in Co:C from 0.05 to 0.05675 requires $n = 588$. Doubling the variance doubles the necessary sample size. Thus, we conclude that sampling urine in snow is an inefficient process for detecting changes in the condition of a population. Based on our previous work, we believe that the same amount of effort and cost would be better directed at monitoring over-winter fawn survival where the relation of this parameter to population condition is obvious.

Conclusions

Existing knowledge of animal physiology and specific data on UN:C ratios indicate urea nitrogen is a poor index of nutritional condition and nutritional deprivation for individual free-ranging animals. Furthermore, while some indices correlate with nutritional condition or nutritional deprivation of individuals, DelGiudice (1995) presented no evidence that these indices, when based on snow-urine samples, explained variation in the condition of the population. Rather, he expected the reader to accept the individual measures as reliable population parameters. As shown above, this leap of logic is not well founded. The index UN:C has not been validated against the true parameter (condition) that it supposedly indexes (see Rotella and Ratti 1986, Guthery 1989, Ratti and Rotella 1989 for an example of such an evaluation) and represents the house of cards so eloquently discredited by Romesburg (1981). Finally, even if we accept that these indices are valid and relate to population condition, large variation in the population will require large sample sizes to detect critical differences in these parameters. We believe that this effort could be more efficiently spent monitoring over-winter animal survival.

Response

DelGiudice (1995) discussed "...considerations, potential, and limitations..." of using urine in snow (snow-urine) to assess nutritional restriction of northern deer (*Odocoileus* spp.). The author's intent was to temper expectations with realities and to encourage additional research aimed at refining this technique (Ditchkoff 1994, White et al. 1995a). In this counterpoint, Saltz et al. expressed an opposing view concerning the potential of this technique. However, we submit that the basis of the argument of Saltz et al. is fundamentally unsound. Specifically, it misrepresents and ignores many of DelGiudice's (1995) most important points, it relies on a physiological model of starvation which has not been validated for deer, and its conclusions are based on a discussion of blood or serum urea nitrogen (SUN) and urinary urea nitrogen:creatinine (UN:C). Their argument is compromised further by frequent, injudicious citing of literature. The result is a highly skewed and misleading interpretation of DelGiudice (1995) and of the use and potential of the snow-urine technique.

Background—Setting the record straight

At the outset, Saltz et al. stated (p. 694) "Specifically, DelGiudice (1995) advocated the use of urea nitrogen:creatinine (UN:C) ratios from urine in snow to assess nutritional *condition* ..." (emphasis added). This is not so; DelGiudice (1995:689, 690) clearly stated that it is "...most valuable for general assessment of nutritional *restriction*," (emphasis added) and that "Presently, nutritional condition...may not be assessed directly with this technique...." DelGiudice's conclusions were prefaced by a discussion that emphasized the distinction between the often loosely used terms "nutritional restriction" and "nutritional condition." Thus, we believe that much of Saltz et al.'s criticism is inappropriate.

Saltz et al.'s argument concerning urea nitrogen and the starvation process relies on a proposed relationship of SUN and urinary UN:C to a 3-phase model of starvation documented for humans (Young and Scrimshaw 1971) and postulated for mule deer (*O. hemionus*, Torbit et al. 1985). Methods of DelGiudice (1995) require sequential collection and chemical analysis of deer urine in snow with an emphasis on the temporal variation of urinary UN:C ratios. Aside from critical differences in logistical considerations (e.g., blood-sampling requires the capture of deer), we favor use of urinary UN:C over SUN because SUN concentrations are affected markedly by the dehydration concomitant with nutritional restriction (Coles 1980:245, Benjamin 1981:177, DelGiudice et al. 1994). Interpretation of a series of urinary UN:C ratios over time are not confounded by this problem (DelGiudice et al. 1994). Yet Saltz et al. discuss SUN and urinary UN:C as if they are interchangeable and cite studies that examined only SUN as a nutritional index (DeCalesta et al. 1975, Kirk-

patrick et al. 1975, Kie et al. 1983). Further, they attempted to support their view with reviews by Brown (1984) and Harder and Kirkpatrick (1994), who reasonably recommended caution in interpreting urinary UN:C data (i.e., the "U-shaped curve") but did not discuss in-depth actual values or new data to extend our understanding of the issues. These 2 reviews considered only the earliest research on deer urinalysis (Warren et al. 1981, 1982; DelGiudice et al. 1987a, 1989).

The 3-phase process of starvation described by Saltz et al. oversimplifies the behavior of urinary UN:C (and SUN) profiles in deer. The original description of this conceptual model (Cahill 1970, Young and Scrimshaw 1971) was based primarily on experimental data from humans and monogastrics that is expanded by Newsholme and Leech (1983:536-561) for human physiology. However, its strict application to ruminants (e.g., deer) and urinary UN:C ratios remains problematic for the following reasons.

First, the digestive and metabolic physiology of ruminants and monogastric animals is different in fed and undernourished conditions. For example, humans obtain much of their glucose from dietary carbohydrates. However, during intermediate and prolonged starvation, serum ketones increase markedly and replace glucose as energy for the brain and other tissues. Consequently, the rates of gluconeogenesis and protein catabolism are reduced, thereby conserving nitrogen (Newsholme and Leech 1983:547-549). Conversely, in ruminants, dietary carbohydrates are fermented in the rumen to produce volatile fatty acids (VFA's), from which glucose ultimately is derived via gluconeogenesis (Bergman 1984). In fed ruminants, proprionic acid (a VFA) is the major precursor of gluconeogenic glucose. During starvation or chronic undernutrition, contribution of proprionic acids can decrease to nil and up to 50% of an individual's glucose is produced by catabolism of endogenous protein (Bergman 1984). Further, there is no reduction of the brain's glucose requirement in undernourished ruminants (Bergman 1984), and ketosis is difficult to induce in deer and other ruminants at or below maintenance feeding (Bergman 1984, Card et al. 1985).

Second, human starvation was characterized as complete nutritional deprivation or fasting (Cahill 1970, Young and Scrimshaw 1971). However, free-ranging deer most commonly experience varied degrees of chronic nutritional restriction, not true starvation (Mautz 1978). Furthermore, an ungulate's physiological response to winter nutritional restriction varies with the degree of deprivation, from sim-

ply reduced intake of a cultivated or manufactured high protein-high energy diet to actual starvation (Warren et al. 1981; Torbit et al. 1985; DelGiudice et al. 1987a,b; 1990, 1994).

Torbit et al. (1985) did not validate the 3-phase model of starvation referred to by Saltz et al., but Torbit (1981) demonstrated that low dietary energy (i.e., severe restriction) induces a more rapid and continuous rate of body protein catabolism than mild or moderate dietary energy deprivation. This is consistent with subsequent findings of continuously high urinary UN:C ratios (≥4 mg:mg) in fasted deer versus deer experiencing progressive, moderate nutritional restriction (mean UN:C declined to 1 mg:mg by 12 weeks) (DelGiudice et al. 1987a, 1990). Even 3-day severe nutritional restriction will induce dramatic increases in urinary UN:C and apparent catabolism in deer (DelGiudice et al. 1994:Table 1).

These findings reflect part of the physiological basis for assessing the degree of nutritional restriction (not condition) over time from urinary UN:C ratios. As fat reserves are exhausted and restriction progresses, glucose is increasingly derived from catabolism of body protein and UN:C ratios rise accordingly (DelGiudice et al. 1994). Varying rates of body protein catabolism during winter have been documented for free-ranging, northern deer (Parker et al. 1993b).

Our main point is that the degree of winter nutritional restriction will have the major influence on *shape* (e.g., U-shaped versus sigmoid) and *scale* of the UN:C curve in wild ungulate populations. For example, as referred to by Saltz et al., the transverse arm (16 weeks long) of the UN:C curve of winter-pastured deer (Saltz and White 1991a) was defined by mean values of approximately 0.05-0.2 mg:mg, whereas in the severely nutritionally restricted, captive deer of DelGiudice et al. (1994), mean values of the transverse arm (12 weeks) were 3.7-6.1 mg:mg.

Saltz et al. (p. 696) support their adoption of the 3-phase starvation model for deer with inappropriate references. They stated that the 3 phases of starvation "...are clearly reflected in BUN levels and urinary UN:C ratios (Cahill 1970, DeCalesta et al. 1975, Warren et al. 1982, Torbit et al. 1985,...)." Cahill (1970) addressed this subject in humans, not ruminants, and DeCalesta et al. (1975) reported SUN concentrations but not urinary UN:C. Warren et al. (1982) did not include starvation or chronic undernutrition; their deer groups all gained weight while under observation. Torbit et al. (1985) included no measurements of urinary UN:C.

Finally, Saltz et al. criticized a relationship between urinary UN:C and percent cumulative mass loss (a

measure of physical condition) of captive deer (Del-Giudice et al. 1994:Fig. 4B) as "spurious." As stated originally and reinforced by DelGiudice (1995), this is considered a preliminary finding. However, we suggest, counter to Saltz et al., that the physical condition of deer occurs along a continuum (Moen 1973:5-7, Robbins 1983:6-7). The UN:C-mass loss relationship is not spurious simply because data reflect 2 phases of a human construct. The 2 deer groups were fed the same low protein-low energy diet but were subjected to 2 regimes of nutritional restriction. We originally described the relationship by fitting a quadratic regression model to the data, overlaying the regression line on the data, and reporting the R^2 value. We did not report P values or confidence limits because we deemed the sample ($n = 7$) too small to represent a natural deer population and because the repeated measures data were too unbalanced to permit analysis by standard ANOVA (analysis of variance) or MANOVA (multivariate analysis of variance) methods. Our intent was to confine strict inference to the sample. However, Associate Editor G. C. White for *The Journal of Wildlife Management* insisted that P values be calculated prior to publication. This could only be done by using methods that circumvented the repeated measures problem. Therefore, the regression was fit to the means rather than to the individual data points. A simple linear model provided the best fit to the means. This is the line which Saltz et al. criticized as being ill-fitting.

With newly available software (PROC MIXED, SAS Inst., Inc. 1992), we used restricted maximum likelihood to fit a generalized, linear, mixed model to the repeated measures data of DelGiudice et al. (1994). This approach is especially useful for estimating effects from incomplete repeated measures data (Laird and Ware 1982, Ware 1985). We fit the model to untransformed and log-transformed data. Once again, a quadratic provided the best fit to the raw data while a linear regression best fit the transformed data (Fig. 3). Not surprisingly, the plot of the geometric means of the log-regression was quite similar to the mean regression line (DelGiudice et al. 1994:Fig. 4B); both techniques minimize the influence of extreme values.

We maintain that the quadratic model is appropriate and that the P values on what was essentially a pilot study are meaningless. The data in Figure 3, from deer in the transverse (Fig. 3, open symbols) and ascending (Fig. 3, solid symbols) arms of the UN:C curve, demonstrate a trend in UN:C with declining body mass. This trend appears to be curvilinear in our small sample. As indicated by DelGiudice et al. (1994), these results do not definitively show that
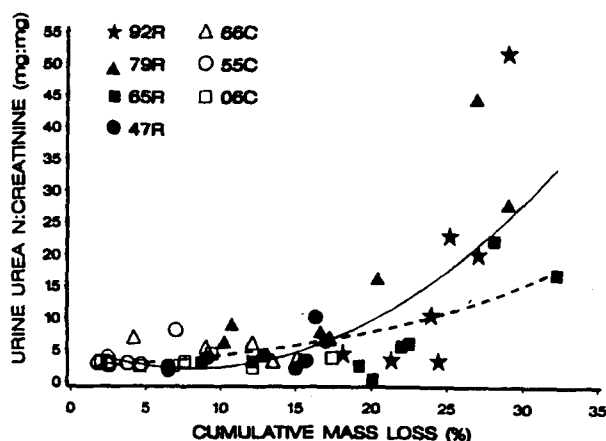

Fig. 3. Urinary urea nitrogen:creatinine (UN:C) ratios versus percent cumulative mass loss of 3 captive, adult white-tailed deer fed a low protein-low energy commercial diet ad libitum (open symbols) and in 4 deer fed restricted amounts (solid symbols), Grand Rapids, Minnesota, 4 February-5 May 1988. The solid line is the generalized linear mixed model (GLMM) estimate of the mean response; broken line is the GLMM estimate of the geometric mean response of the pooled data from DelGiudice et al. (1994:Fig. 4B).

UN:C is useful as an index of nutritional or physical *condition*, nor can they form the basis for a generally applicable inverse regression model. However, they are consistent with much of our field data and with our contention that mean UN:C values provide a promising tool for monitoring the nutritional restriction of wild ungulates. Given the logistic, ethical, and political problems, it is unlikely that anyone will ever complete a captive study on the scale required for a firm statistical inference to a larger population.

Individual and population inferences

From a physiological perspective, the primary concern when using urinary UN:C for assessing nutritional restriction or condition (DelGiudice 1995) is that values may be affected by dietary protein and energy (DelGiudice 1995, Saltz et al.). Citing Warren et al. (1982), Saltz et al. listed tissue catabolism and urea recycling as *additional* factors influencing UN:C ratios; in fact, both are components of the physiological mechanisms linking dietary protein and energy metabolism to the dynamics of urinary UN:C ratios. As dietary protein decreases, renal urea recycling increases and urinary UN decreases (Mould and Robbins 1981); however, as dietary energy becomes deficient, body protein catabolism may accelerate, with a concomitant increase in urinary UN:C (DelGiudice et al. 1987a, 1994).

As in the medical field, interpretation of urinary data (e.g., UN:C) from individual deer, whether under controlled or natural conditions, relies on the current knowledge of many areas of research (e.g., ani-

mal and plant ecology, nutrition, renal physiology, clinical biochemistry) and reference values of urinary characteristics established under conditions of known nutrition (e.g., mild to severe chronic nutritional deprivation or fasting). This approach is analogous to interpretation of human medical diagnostics, where physicians routinely diagnose a variety of pathologies by simple qualitative comparison of patient profiles to established normal ranges. Issues of statistical inference do not arise in such situations, nor does the problem of index validation discussed by Romesburg (1981).

The situation becomes much more complex and uncertain when we attempt to assess nutritional restriction of an entire population from samples of snow-urine specimens. However, because individuals in wild populations usually compete for the same finite resources and are influenced by the same environmental factors, mean nutritional status of animals will be much more strongly correlated with survival than would be expected in human populations. On the other hand, survival in animal populations tends to be age- or age-and-sex-specific. Thus, we can reasonably expect the mean level of nutritional restriction of individuals in a sample to reflect the restriction of the typical individual animal in the target population, only insofar as the sample is representative of the population age and sex distribution.

As DelGiudice (1995) and Saltz et al. indicated, several problems potentially confound the interpretation of mean population UN:C values from samples of snow-urine: (1) age and sex of the "donors" usually will be unknown, (2) UN:C values averaged over individuals from the transverse and ascending arms of a U-shaped UN:C curve may indicate a state not representing any individual in the sample, and (3) it is difficult to interpret time-series data from open populations without information on immigration–emigration or birth–death. While we acknowledge these difficulties, we believe that Saltz et al. have ignored some promising remedies, overstated some of the sampling problems, and introduced several irrelevant statistical issues.

The key to all 3 issues is that strict inference must be confined to smaller finite populations (e.g., deer in a specific winter yard), based on an entire series of sequential samples, collected under comparable conditions. Saltz et al. misunderstood the purpose of DelGiudice's (1995) winter storm example. It was used to illustrate the sensitivity of urinary UN:C of free-ranging deer to environmental events causing severe nutritional restriction. To clarify, only a minimum amount of fresh snow (i.e., a dusting) is actually necessary to detect fresh deer tracks and urine speci-

mens. We recommend that sample collections be made within 2–3 days of light snowfalls, not following severe snowstorms.

All individual deer must progress along the tissue catabolism gradient on their way to death by undernutrition, albeit at different rates. Thus, as winter progresses, mean UN:C will rise as body protein catabolism accelerates in the individuals at greatest risk (e.g., fawns). If suddenly, as in Saltz et al.'s example, several deer in the ascending arm of the UN:C curve die, a "dip" will occur in mean UN:C. Unless supplemental food is supplied (which DelGiudice [1995] stated must be known), this dip could only be interpreted as animals have died. However, that a dip in mean UN:C ratios would occur in a natural, free-ranging population (as opposed to the enclosed, controlled population of Saltz and White 1991a) remains to be demonstrated. In pooling UN:C data from adult cow and calf elk (*Cervus elaphus*) from White et al. (1995b), mean UN:C increased over time despite a drop from 74 calves:100 cows to 27 calves:100 cows from early to late winter.

Saltz et al. postulated a scenario in which several deaths occur between consecutive samples with similar UN:C means. In this situation, one would infer correctly that the mean level of nutritional restriction had not changed much. Of course, this would be due to the loss (by death) of the individuals whose UN:C had ascended precipitously in the interim. Saltz et al. erroneously refer to this as a problem of censored data. We are not examining an event–time distribution; rather we are estimating a mean time-series from sequential cross-sectional samples; thus, there is no censoring (Lee 1992:1–4). Problems arise not in estimating such a series, but in inferring its genesis. One remedy is to shrink the sampling intervals (i.e., increase sampling occasions) to where both the rise and subsequent dip in UN:C will be recorded. Due to the vagaries of snowfall patterns this may not always be possible. However, like the physician reviewing a patient's urine profile, researchers and managers should examine all the signs and symptoms. A sudden die-off will produce bodies; in addition, other urinary parameters, (e.g., potassium:creatinine) may help to clarify the pattern presented by the UN:C series (DelGiudice et al. 1987a).

The inability of Saltz et al. (1992) to relate urinary UN:C ratios of 2 small samples ($n_1 = n_2 = 6$) of trained yearling mule deer (specimens collected from individuals in metabolic cages) in separate pastures (100% winter survival) to the weekly survival of 101 fawns (5% winter survival) illustrated the importance of obtaining a representative sample of snow-urine to

assess nutritional restriction of a population. Mean UN:C ratios of the yearlings remained low (overall $\bar{x}$ = 0.25 mg:mg) throughout winter (Saltz and White 1991a, Saltz et al. 1992, 1993), whereas Saltz and White (1991a,b) showed that UN:C ratios of severely undernourished and dying fawns were markedly elevated (overall $\bar{x}$ = 5.2 mg:mg) compared to the yearlings.

Clearly, the fawns of Saltz et al. (1992) were experiencing accelerated catabolism and began dying (weeks 3–16) when the yearling UN:C ratios were still low. Had Saltz et al. (1992) measured yearling and fawn UN:C ratios, the curve of the means would have reflected the relative proportions of deer in the transverse (primarily yearlings) and ascending (fawns) arms of the UN:C curve. The proportion of deer in the ascending arm, and consequently the UN:C curve, would have risen over the first 10 weeks and then declined as most of the fawns died and left the population. In weeks 16–19 the curve would have come up again as protein catabolism accelerated in the yearlings.

Saltz et al. (1992) did not monitor fawn UN:C because (1) they did not want to introduce additional heterogeneity into the data, and (2) they did not want to engage the open population problem by measuring individuals that actually died during the 19 weeks of observation. In adopting this strategy, they threw out the baby out with the bath water. As managers and scientists, we want to know what is happening to those individuals in the population that are at greatest risk. Thus, we should seek diagnostic parameters that closely reflect observed heterogeneity in the patterns of risk.

Because no yearlings died, whereas approximately 95% of the fawns died, Saltz et al.'s (1992) 2 age-classes were clearly responding to different age-specific hazards. To focus on the yearling data because they were easier to obtain and better behaved than those of the major population at risk is methodologically hazardous. If one wants to predict the behavior of a population, one needs to sample that population. Saltz et al.'s (1992) homogeneous samples of convenience excluded the very individuals (fawns) that carried the greatest information.

The question of variability of UN:C values within and across sampling occasions is important. Using variance estimates from their 1992 study, Saltz et al. indicated that a sample size of 60 would be required to detect a change of 0.268 in UN:C. We have 3 objections to this calculation. First, it is based on a t-test between consecutive samples. The need, however, is to identify trends over all the samples. This requires much more powerful model-based trend tests

using a pooled variance estimator (MSE) from all the data (Agresti 1990:97–100), not just from 2 samples.

Second, Saltz et al. set α = 0.05 and $1 - \beta$ = 0.90, criteria which we believe are unreasonably conservative (Tacha et al. 1982, Cohen 1990, Buckland et al. 1993). We believe most wildlife managers and field biologists would accept 90% and 80% chances of *not* making a Type I or Type II error, respectively. For the two-sample t-test proposed by Saltz et al., these criteria are met with n = 35.

Finally, our field work indicates much smaller coefficients of variation (CV, %) than Saltz et al. cited. Mean CV of UN:C from 29 collections (mean n = 21 specimens/collection) of snow-urine (winters 1984–1985, 1993–1994) by DelGiudice et al. (1989, unpubl. data) in Minnesota was 52%. Only 3% (1/29) of the samples had CV >100%, and 72% (21/29) had CV < 55%. By contrast, 44% (15/34) of collections (n = 6) of Saltz et al. (1992) had CV > 100%, and only 12% (11/34) had CV < 55%; mean CV was 96%. Thus, relative variances reported by Saltz et al. (1992) averaged 2 times higher than those which we have observed in our field data. Furthermore, the minimum effect size (0.268) which Saltz et al. specified is far too small to be of physiologic significance. Values of UN:C reported by Saltz et al. (1992) in 12 tame mule deer (0.05–0.99) were at the extreme low end of the range of winter UN:C of Minnesota white-tailed deer (0.3–10.0). Thus, we believe that 1.0 is a more meaningful minimum effect size (mean standard deviation observed in our 29 collections was 0.938). Minimum sample size required to detect this difference with α = 0.05 and $1 - \beta$ = 0.90 is 19 and for α = 0.10 and $1 - \beta$ = 0.80 is only 11.

Many of Saltz et al.'s comments suggest that they have missed the point of DelGiudice (1995). The objective was to describe patterns in UN:C over time that will help wildlife managers or researchers identify major trends or shifts in nutritional restriction in local populations of wild ungulates. As discussed by DelGiudice ("Future research," 1995), additional research is needed. Nevertheless, we believe the snow-urine technique has potential and exhibits many of the desirable characteristics of an index of nutritional status as outlined by Harder and Kirkpatrick (1994:276).

At present, these monitoring techniques are not intended to yield precise estimates of percent body mass loss and fat depletion or provide formal tests of statistical hypotheses concerning *population condition*. Deer populations are open and heterogeneous in ways that affect survival. But this is true of nearly all populations and does not mean we should not attempt to monitor them. Physiological assessments are important to a thorough characterization and un-

derstanding of population condition (Hanks 1981, Harder and Kirkpatrick 1994, DelGiudice 1995).

Clearly, assessing winter nutritional restriction and overwinter survival of ungulates would complement each other and provide greater insight into the role of nutrition in linking environmental variation and population variation. Currently, we are in the sixth year of a 10-year deer study that includes such an approach on 4 study sites. Recently, we began counting and measuring bed sizes of sampled individuals in free-ranging doe-fawn groups in an attempt to relate herd composition to assessments of nutritional restriction by snow-urine chemistry. We disagree with Saltz et al. not because we are naive about remaining problems, but because we are optimistic about solving them.

Literature cited

Agresti, A. 1990. Categorical data analysis. John Wiley and Sons, New York, N.Y. 558pp.

Bartmann, R. M., G. C. White, and L. H. Carpenter. 1992. Compensatory mortality in a Colorado mule deer population. Wildl. Monogr. 121. 39pp.

Benjamin, M. M. 1981. Outline of veterinary clinical pathology. The Iowa State Univ. Press, Ames. 351pp.

Bergman, E. N. 1984. Disorders of carbohydrate and fat metabolism. Pages 412-422 in M. J. Swenson, ed. Duke's physiology of domestic animals. Comstock Publ. Assoc., Div. Cornell Univ. Press. Ithaca, N.Y.

Brown, R. D. 1984. The use of physical and physiological indices to predict the nutritional condition of deer—a review. Pages 52-63 in P. R. Krausman and N. S. Smith, eds. Deer in the southwest: a workshop. School of Renewable Natural Resour., Univ. Ariz.. Tucson.

Buckland, S. T., D. R. Anderson, K. P. Burnham, and J. L. Laake. 1993. Distance sampling: estimation of abundance of biological populations. Chapman Hall, New York, N.Y. 446pp.

Cahill, G. F., Jr. 1970. Starvation in man. New Engl. J. Med. 282:668-675.

Card, W. C., R. L. Kirkpatrick, K. E. Webb, Jr., and P. F. Scanlon. 1985. Nutritional influences on NEFA, cholesterol, and ketones in white-tailed deer. J. Wildl. Manage. 49:380-385.

Caughley, G. 1971. Demography, fat reserves and body size of a

population of red deer *Cervus elaphus* in New Zealand. Mammalia 35:369-383.

Cherel, Y., J. Gilles, Y. Handrich, and Y. Le Maho. 1994. Nutrient reserve dynamics and energetics during long-term fasting in the king penguin (*Aptenodytes patagonicus*). J. Zool. (Lond.) 234:1-12.

Cohen, J. 1990. Things I have learned (so far). Am. Psychol. 45:1,304-1,312.

Coles, E. H. 1980. Veterinary clinical pathology. W.B. Saunders Co., Philadelphia. Pa. 562pp.

DeCalesta, D. S., J. G. Nagy, and J. A. Bailey. 1975. Starving and refeeding mule deer. J. Wildl. Manage. 39:663-669.

DelGiudice, G. D. 1995. Assessing winter nutritional restriction of northern deer with urine in snow: considerations, potential, and limitations. Wild. Soc. Bull. 23:687-693

DelGiudice, G. D., L. D. Mech, and U. S. Seal. 1989. Physiological assessment of deer populations by analysis of urine in snow. J. Wildl. Manage. 53:284-291.

DelGiudice, G. D., L. D. Mech, and U. S. Seal. 1990. Effects of winter undernutrition on body composition and physiological profiles of white-tailed deer. J. Wildl. Manage. 54:539-550.

DelGiudice, G. D., L. D. Mech, and U. S. Seal. 1994. Undernutrition and serum and urinary urea nitrogen of white-tailed deer during winter. J. Wildl. Manage. 58:430-436.

DelGiudice, G. D., L. D. Mech, U. S. Seal, and P. D. Karns. 1987a. Winter fasting and refeeding effects on urine characteristics in white-tailed deer. J. Wildl. Manage. 51:860-864.

DelGiudice, G. D., L. D. Mech, U. S. Seal, and P. D. Karns. 1987b. Effects of winter fasting and refeeding on white-tailed deer blood profiles. J. Wildl. Manage. 51:865-873.

Ditchkoff, S. S. 1994. Nutritional ecology of white-tailed deer in Maine during winter. M.S. Thesis, Univ. Maine. Orono. 85pp.

Downing, R. L. 1980. Vital statistics of animal populations. Pages 247-268 in S. D. Schemnitz, ed. Wildlife management techniques manual. Fourth ed. The Wildl. Soc., Washington, D.C.

Franzmann, A. W. 1985. Assessment of nutritional status. Pages 239-259 in R. J. Hudson and R. G. White, eds. Bioenergetics of wild herbivores. CRC Press Inc., Boca Raton, Fla.

Grubb, T. C., Jr. 1995. On induced anabolism, induced caching and induced construction as unambiguous indices of nutritional condition. In R. Yosef and F. E. Lorer, eds. Shrikes of the world: biology and conservation. Western Found. of Vert. Zool, Los Angeles, Calif.

Guthery, F. S. 1989. Test of a density index assumption for gray partridge: a comment. J. Wildl. Manage. 53:1,132-1,133.

Hanks, J. 1981. Characterization of population condition. Pages 47-73 in C. W. Fowler and T. D. Smith, eds. Dynamics of large mammal populations. John Wiley and Sons, Inc., New York, N.Y.

Harder, J. D., and R. L. Kirkpatrick. 1994. Physiological methods in wildlife research. Pages 275-306 in T. A. Bookhout, ed. Research and management techniques in wildlife and habitats. Fifth ed. The Wildl. Soc., Bethesda, Md.

Kie, J. G., M. White, and D. L. Drawe. 1983. Condition parameters of white-tailed deer in Texas. J. Wildl. Manage. 47:583-594.

Kirkpatrick, R. L., D. E. Buckland, W. A. Abler, P. F. Scanlon, J. B. Whelan, and H. E. Burkhart. 1975. Energy and protein influences on blood urea nitrogen of white-tailed deer in Texas. J. Wildl. Manage. 39:692-698.

Laird, N. M., and J. H. Ware. 1982. Random-effects models for longitudinal data. Biometrics 38:963-974.

Lee, E. T. 1992. Statistical methods for survival data analysis. John Wiley and Sons, New York, N.Y. 482pp.

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, Pa.

MOEN, A. N. 1973. Ecology: an analytical approach. W.H. Freeman and Co., San Francisco, Calif. 458pp.

MOULD, E. D., AND C. T. ROBBINS. 1981. Nitrogen metabolism in elk. J. Wildl. Manage. 45:323–334.

MURPHY, D. D., AND B. D. NOON. 1991. Coping with uncertainty in wildlife biology. J. Wildl. Manage. 55:773–782.

NEWSHOLME, E. A., AND A. R. LEECH. 1983. Biochemistry for the medical sciences. John Wiley and Sons, New York, N.Y. 952pp.

OWEN, M., AND W. A. COOK. 1977. Variation in body weight, wing length and condition of mallards *Anas platyrhynchos platyrhynchos* and their relationship to environmental changes. J. Zool. (Lond.) 183:377–395.

PARKER, K. L., G. D. DELGIUDICE, AND M. P. GILLINGHAM. 1993*a*. Do urinary urea nitrogen and cortisol ratios of creatinine reflect body-fat reserves in black-tailed deer? Can. J. Zool. 71:1,841–1,848.

PARKER, K. L., M. P. GILLINGHAM, T. A. HANLEY, AND C. T. ROBBINS. 1993*b*. Seasonal patterns in body mass, body composition, and water transfer rates of free-ranging and captive black-tailed deer (*Odocoileus hemionus sitkensis*) in Alaska. Can. J. Zool. 71:1,397–1,404.

RATTI, J. T., AND J. J. ROTELLA. 1989. Test of a density index assumption for gray partridge: a reply. J. Wildl. Manage. 53:1,133–1,134.

ROBBINS, C. T. 1983. Wildlife feeding and nutrition. Academic Press, New York, N.Y. 343pp.

ROBBINS, C. T., R. L. PRIOR, A. N. MOEN, AND W. J. VISEK. 1974. Nitrogen metabolism of white-tailed deer. J. Anim. Sci. 38:186–191.

ROMESBURG, H. C. 1981. Wildlife science: gaining reliable knowledge. J. Wildl. Manage. 45:293–313.

ROTELLA, J. J., AND J. T. RATTI. 1986. Test of a critical density index assumption: a case study with gray partridge. J. Wildl. Manage. 50:532–539.

SALTZ, D., AND G. C. WHITE. 1991*a*. Urinary cortisol and urea nitrogen responses to winter stress in mule deer. J. Wildl. Manage. 55:1–16.

SALTZ, D., AND G. C. WHITE. 1991*b*. Urinary cortisol and urea nitrogen responses in irreversibly undernourished mule deer fawns. J. Wildl. Dis. 27:41–46.

SALTZ, D., G. C. WHITE, AND R. M. BARTMANN. 1992. Urinary cortisol, urea nitrogen excretion, and winter survival in mule deer fawns. J. Wildl. Manage. 56:640–644.

SALTZ, D., G. C. WHITE, AND R. M. BARTMANN. 1993. Corrigendum. J. Wildl. Manage. 57:422.

SAS INSTITUTE, INC. 1992. SAS/STAT software: changes and enhancements, SAS Tech. Rep. P-229, Release 6.07, Cary, N.C. 620pp.

TABER, R. D., K. L. WHITE, AND N. S. SMITH. 1979. The annual cycle of condition in Rattlesnake. Montana, mule deer. Proc. Mont. Acad. Sci. 19:72–78.

TACHA, T. C., W. D. WARDE, AND K. P. BURNHAM. 1982. Use and interpretation of statistics in wildlife journals. Wildl. Soc. Bull. 10:355–362.

TORBIT, S. C. 1981. In vivo estimation of mule deer body composition. Ph.D. Diss., Colorado State Univ., Fort Collins. 98pp.

TORBIT, S. C., L. H. CARPENTER, D. M. SWIFT, AND A. W. ALLDREDGE. 1985. Differential loss of fat and protein by mule deer during winter. J. Wildl. Manage. 49:80–85.

WARE, J. H. 1985. Linear models for the analysis of longitudinal studies. Am. Stat. 39:95–101.

WARREN, R. J., R. L. KIRKPATRICK, A. OELSCHLAEGER, P. F. SCANLON, AND F. C. GWAZDAUSKAS. 1981. Dietary and seasonal influences on nutritional indices of adult male white-tailed deer. J. Wildl. Manage. 45:926–936.

WARREN, R. J., R. L. KIRKPATRICK, A. OELSCHLAEGER, P. F. SCANLON, K. E. WEBB, JR., AND J. B. WHELAN. 1982. Energy, protein, and seasonal influences on white-tailed deer fawn nutritional indices. J. Wildl. Manage. 46:302–312.

WHITE, P. J., R. A. GARROTT, AND D. M. HEISEY. 1995*a*. Variability in snow-urine assays. Can. J. Zool. 73:427–432.

WHITE, P. J., R. A. GARROTT, C. A. VANDERBILT WHITE, AND G. A. SARGEANT. 1995*b*. Interpreting mean snow-urine ratios from simple random collections. Wildl. Soc. Bull. 23:705–710.

YOUNG, V. R., AND N. S. SCRIMSHAW. 1971. The physiology of starvation. Sci. Am. 225:14–22.