# Sampling recommendations to assess nutritional restriction in deer

*Stephen S. Ditchkoff and Frederick A. Servello*

**Abstract** Chemical analysis of urine collected from snow for urinary urea nitrogen (N):creatinine (C) ratios has received increasing use to determine the degree of nutritional restriction of populations of white-tailed deer (*Odocoileus virginianus*) in winter. However, statistically based sampling requirements for designing effective field studies or monitoring programs have not been described. We calculated power curves using urea N:C ratio data from 1,383 urine samples collected from white-tailed deer during winter in Maine to predict sample sizes needed to detect effect sizes in urea N:C data where variances are expected to be low, high, or incapable of being predicted. To detect effect sizes of biological significance that may occur in early-mid or mid–late winter, or when sample variances are low, approximately 15–20 samples would be required per wintering area. However, during late winter or when sample variances are great, 20–25 samples would be required. When using a chi-square to detect differences between proportions of deer with urea N:C values $\geq$3.5 mg/mg, approximately 35 samples would be required to detect proportional differences $\geq$0.22. Our data suggest that a combination of analysis of variance and chi-square analysis is suitable to determine spatial and temporal differences in levels of urinary urea N:C collected from wintering white-tailed deer.

**Key words** creatinine, nutritional restriction, *Odocoileus virginianus,* power analysis, sample size, undernutrition, urea nitrogen, white-tailed deer

DelGiudice et al. (1987) reported a technique based on analyzing urine collected from snow to classify undernutrition of populations of white-tailed deer (*Odocoileus virginianus*) during winter. Urea N:C ratios measured in urine become elevated because of increased catabolism of body tissues during periods of nutritional restriction (DelGiudice et al. 1987) and can indicate a progressive deterioration in deer nutrition during winter (DelGiudice 1995). A major advantage of this technique is that large numbers of samples from multiple populations can be collected to study spatial and temporal variation in deer nutrition (DelGiudice 1995). Although this technique has been used for free-ranging white-tailed deer (DelGiudice et al. 1990), black-tailed deer (*O. hemionus*, Parker et al. 1993), moose (*Alces alces*; DelGiudice et al. 1991*a*, 1997), bison (*Bison bison*, DelGiudice et al. 1994*b*), and elk (*Cervus elaphus*, DelGiudice et al. 1991*b*), it has received some criticism (Saltz et al. 1995, White et al. 1995*b*). Saltz et al. (1995) argued that urea N:C ratios cannot accurately differentiate between deer populations under varying levels of nutritional restriction due to inherent variability in urea N:C values of individual deer. Using power analysis, they calculated that >60 samples would be required to detect a difference of 0.268 mg/mg in mean urea N:C between populations or sample periods, thus making this technique impractical. DelGiudice et al. (1995) argued that a difference of 0.268 mg/mg between mean urea N:C ratios is small from a nutritional perspective and therefore a sampling requirement of 60 is inappropriate. They estimated that only 11 samples would be necessary to detect a change of 1.0 mg/mg in mean urea N:C ratios. Given the

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increasing use of this technique in field studies, a comprehensive analysis of sampling issues is needed. Using urine samples from white-tailed deer collected in 10 wintering areas in Maine and information from the literature, we generated power curves that can be used to predict sample sizes required to statistically detect temporal and spatial differences in most urea N:C data sets. We provide sample size estimates to aid in designing studies to monitor changes in nutritional status of an individual population or to compare level of nutritional restriction of multiple populations. In addition, these power curves are applicable to sampling scenarios where expected variability in urea N:C data is low, high, or unknown.

## Study area

We collected deer urine samples ($n$=1,383) in 4 deer wintering areas (DWA) in central Maine and 6 DWAs in northern Maine. Landscape patterns within Maine DWAs are variable and range from spruce (*Picea* spp.)-balsam fir (*Abies balsamea*)-dominated stands with little understory vegetation on upland sites to northern white-cedar (*Thuja occidentalis*)-dominated stands with dense understories along watercourses. Most DWAs in Maine are harvested selectively with diameter-limit patch cuts to create a mosaic of uneven-aged stands. This harvest regime commonly results in dense spruce-fir regeneration of recently harvested areas (<10 years old). Dominant conifer species of the overstory in Maine DWAs are balsam fir, spruce, hemlock (*Tsuga canadensis*), northern white-cedar, and white pine (*Pinus strobus*, Marston 1986). In central Maine during January-March 1993, mean snow depths ranged from 7 to 66 cm and mean temperatures from -12 to 0°C. Mean snow depths in northern Maine ranged from 30 to 80 cm and mean temperatures from -21 to -2°C (Maine Department of Inland Fisheries and Wildlife, unpublished data).

## Methods

We collected snow urine samples during 6 periods (1-15 Jan, 16-31 Jan, 1-15 Feb, 16-28 Feb, 1-15 Mar, and 16-31 Mar) in 1993 to generate a large and variable data set capable of producing accurate pooled variance estimators (MSE) for power analyses. Handling and storage procedures followed those described by DelGiudice et al. (1988, 1989). We analyzed snow urine for N and C by spectrophotometry using test kits (66-UV and 555, Sigma Chemical Co., St. Louis, Mo.).

We used power analyses to determine sample sizes necessary to statistically detect differences in mean urea N:C ratios. Because urea N:C data can vary spatially and temporally (DelGiudice et al. 1989, Ditchkoff 1994), we generated power curves that would predict sample sizes for studies where urea N:C data is expected to have low, high, or unknown variability. Our model was a 1-way analysis of variance (ANOVA) designed to represent a single DWA sampled during 4 time periods or 4 DWAs sampled during one time period. Urea N:C values for periods 2 and 3 were kept constant (K) and the values for periods 1 and 4 were varied below and above K by the same increment ($\lambda$) to generate effect sizes ($2\lambda$, maximum difference between periods) ranging from 0.1 to 1.0 mg/mg urea N:C. We chose this design because power analyses with $n$ groups will yield a conservative value when groups 1 and $n$ vary from the mean of the groups by the same increment ($\lambda$, Nicewander and Price 1997). To determine indices of the difference between means (Cohen 1988:275) to be used in power calculations, we used error mean squares from nested ANOVAs calculated with our field data. The error mean square for the low variability model was obtained from urea N:C data ($n$=391) collected during 16 January-15 February. This period is characterized by urea N:C data with low means and variability due to consistent snow cover and nutrition of deer. Our data for 16 January-15 February had means and variances similar to those reported by DelGiudice et al. (1989), the only other published study of urea N:C values for free-ranging deer. We calculated the error mean square for the high-variability model using urea N:C data ($n$=507) collected during 1-31 March. These data provide the only example of urea N:C data with inflated variances and where deer are experiencing extreme nutritional restriction (Ditchkoff 1994). During this time period, deer are most susceptible to nutritional insult because of harsh conditions, low food availability, and deteriorated fat reserves (Mautz 1978*b*). As a result, urea N:C data can vary significantly during this period (Ditchkoff 1994). We used urea N:C data ($n$=1,383) collected from 1 January to 31 March to generate the error mean square for the unknown-variability model. These data incorporate data sets used for the low- and high-variability models and likely provide variance estimates that can be used to predict sample

Table 1. Power (1-β) of a chi-square[a] to detect differences between proportions (2λ) of urine samples with urinary urea nitrogen:creatinine values ≥3.5 mg/mg when *n* ranges from 10 to 60 and α=0.10.

| | Sample size (*n*) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2λ | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 |
| 0.10 | 0.359 | 0.403 | 0.454 | 0.499 | 0.529 | 0.563 | 0.612 | 0.643 | 0.661 | 0.719 | 0.739 |
| 0.12 | 0.366 | 0.419 | 0.473 | 0.522 | 0.555 | 0.594 | 0.637 | 0.671 | 0.701 | 0.746 | 0.781 |
| 0.14 | 0.384 | 0.432 | 0.493 | 0.549 | 0.600 | 0.646 | 0.671 | 0.710 | 0.747 | 0.789 | 0.813 |
| 0.16 | 0.405 | 0.452 | 0.528 | 0.590 | 0.636 | 0.677 | 0.720 | 0.760 | 0.789 | 0.824 | 0.854 |
| 0.18 | 0.418 | 0.487 | 0.557 | 0.626 | 0.674 | 0.726 | 0.767 | 0.799 | 0.837 | 0.864 | 0.885 |
| 0.20 | 0.445 | 0.511 | 0.588 | 0.670 | 0.718 | 0.766 | 0.810 | 0.843 | 0.866 | 0.893 | 0.917 |
| 0.22 | 0.460 | 0.544 | 0.624 | 0.706 | 0.759 | 0.809 | 0.847 | 0.883 | 0.904 | 0.934 | 0.946 |
| 0.24 | 0.485 | 0.580 | 0.673 | 0.745 | 0.797 | 0.845 | 0.882 | 0.916 | 0.938 | 0.952 | 0.966 |
| 0.26 | 0.502 | 0.604 | 0.706 | 0.784 | 0.839 | 0.882 | 0.918 | 0.938 | 0.957 | 0.971 | 0.979 |
| 0.28 | 0.530 | 0.650 | 0.747 | 0.816 | 0.876 | 0.913 | 0.942 | 0.962 | 0.975 | 0.982 | 0.991 |
| 0.30 | 0.543 | 0.689 | 0.781 | 0.860 | 0.908 | 0.940 | 0.966 | 0.977 | 0.986 | 0.991 | 0.995 |
| 0.32 | 0.591 | 0.717 | 0.812 | 0.889 | 0.935 | 0.963 | 0.978 | 0.988 | 0.993 | 0.995 | 0.998 |
| 0.34 | 0.616 | 0.759 | 0.855 | 0.918 | 0.960 | 0.977 | 0.989 | 0.994 | 0.997 | 0.998 | 0.999 |
| 0.36 | 0.636 | 0.785 | 0.884 | 0.939 | 0.973 | 0.989 | 0.994 | 0.998 | 0.998 | 0.999 | 0.999 |
| 0.38 | 0.680 | 0.816 | 0.914 | 0.962 | 0.986 | 0.996 | 0.998 | 0.999 | 0.999 | 0.999 | 0.999 |

[a] Power estimates were calculated using a 2 × 4 chi-square with equal sample sizes.

size when accurate estimates of variance are unavailable. Field data were $\log_e$ transformed prior to all analyses (DelGiudice et al. 1989, White et al. 1995*a*), but results of power analyses are described as nontransformed to ease interpretation. We set α=0.10 and 1-β=0.8 for all power analyses.

DelGiudice et al. (1995) suggested analyzing the proportion of individuals exhibiting urea N:C values ≥3.5 mg/mg in addition to analyzing mean urea N:C data to lend additional insight into the overall level of nutritional restriction of the population. They contend that individuals with urea N:C values ≥3.5 mg/mg represent the portion of the population of concern because these animals are experiencing severe nutritional restriction (DelGiudice et al. 1987, 1994*a*; Saltz and White 1991). We used power analysis to determine sample sizes necessary to detect differences between proportions of urinary urea N:C values ≥3.5 mg/mg. A relatively simple analysis to examine proportions in this context is the chi-square test. A contingency table of size 2 × T (T=number of sampling periods) can be constructed by converting the proportions into binary data. Those values ≥3.5 mg/mg receive a value of 1, and any remaining data become 0. We constructed contingency tables of size 2 × 4 to represent 4 sampling periods (or 4 DWAs) and the number of urine samples with urea N:C values ≥ or <3.5 mg/mg. We varied the sample size of each sampling period from 10 to 60 by increments of 5 during each set of replicates. Sampling periods 2 and 3 had a proportion of samples with urea N:C values ≥3.5 mg/mg as 0.20. This point was approximately the midpoint of the range of values (0.00–0.44) for proportions of deer with urea N:C values ≥3.5 mg/mg for the DWAs sampled in this study (Ditchkoff 1994). The proportions for sampling periods 1 and 4 were then varied below and above 0.20 by the same increment (λ). We varied λ by increments of 0.01 from 0.05 to 0.19. We then generated binomial data randomly for each of the 4 sampling periods using a random number generator designed to create a random binomial distribution around the proportion for that sampling period (RANBIN, SAS Institute, Inc., 1985). Each permutation of sample sizes and proportions was replicated 10,000 times, and we calculated a chi-square value and significance level (*P*) for each replicate. The power (1-β) of each test was the percentage of replications with $P \leq \alpha$. Unless otherwise stated, all sample-size estimates are based on power analyses with α=0.10.

## Results and discussion

Number of urine samples required to detect differences in mean urea N:C ratios decreased as the difference between mean values became greater (Figure 1). Approximately 25 samples per sampling period would be necessary to detect a difference between urea N:C values of 0.3 mg/mg using the

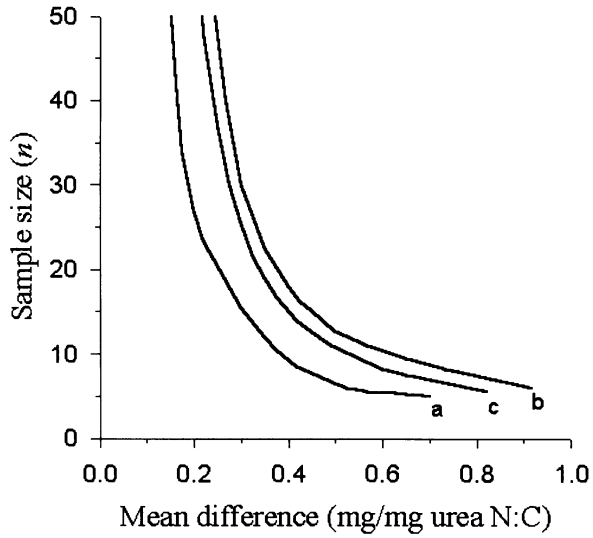
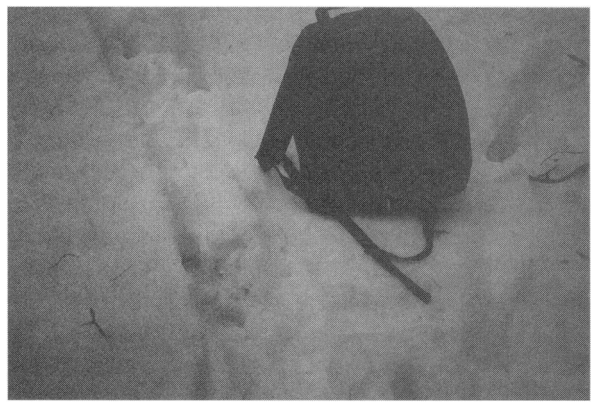



Figure 1. Number of deer urine samples required per sample period to detect differences between mean urea nitrogen:creatinine values using analysis of variance when sample variance is expected to be a) low, b) high, or c) incapable of being predicted. For all calculations, $(1-\beta)=0.80$ and $\alpha=0.10$.

unknown-variance model. However, approximately 15 and 30 samples would be suitable to detect this same difference when there is low and high sample variance, respectively. Number of urine samples required to statistically detect a difference between means ≤0.2 mg/mg was near 30 according to the low-variance model, but was >50 when sample variance was greater. Power analysis also indicated that required sample sizes decreased as the difference between proportions increased (Table 1). Approximately 25 samples would be necessary to detect differences when the proportional difference is 0.30. However, when the proportional difference was ≤0.20, power analysis indicated that ≥50 urine samples per sampling period are required.

Nutritional restriction of deer populations in northern regions follows a general pattern as winter progresses (DelGiudice et al. 1989, Ditchkoff 1994). Healthy deer populations during early-mid winter are typically characterized by adequate fat reserves (Mautz 1978*b*,Torbit et al. 1985). Although deer consume forages during winter to reduce their rate of body fat catabolism (Mautz 1978*b*), winter forages are usually low in protein (Short et al. 1966, Mautz 1978*a*) and deer conserve N by recycling urea and minimizing excretory losses (Robbins et al. 1974). DelGiudice et al. (1989) found that deer populations experiencing moderate nutritional restriction commonly associated with early-mid winter exhibited mean urea N:C levels ranging from 0.3 to 2.0 mg/mg. Ditchkoff (1994) documented mean urea N:C values ranging from 0.7 to 3.7 mg/mg in early and mid winter; however, 90% of those values were <2.5 mg/mg. DelGiudice et al. (1991*c*) found that an elk population had mean urea N:C values ≤1.5 mg/mg during early-mid winter. With low variability in mean urea N:C values during early-mid winter, detecting mean differences (e.g., 0.3 mg/mg) among populations would require >15 urine samples per DWA. Because most deer exhibit low to moderate levels of nutritional restriction (urea N:C <3.5) during this period, an effort to compare proportions of deer experiencing elevated levels of nutritional restriction would yield little information.

Wintering white-tailed deer experience a wide range of conditions during mid-late winter. In some cases, early-mid winter conditions continue throughout winter and urea N:C values for these populations remain relatively low. In other cases, populations experience high levels of nutritional restriction (e.g., limited forage intake, high use of body fat reserves, catabolism of body protein) due to severe climatic conditions (Kelsall 1969, Parker et al. 1984) or competition for limited food (Ozoga 1972, Mautz 1978*a*). Nutritional restriction also can vary among sex and age classes (e.g., fawns, mature males), which can increase variability in urea N:C ratios of populations (DelGiudice and Seal 1988). When systemic N levels rise from catabolism of body proteins, deer begin excreting excess urea, causing levels of urinary urea N:C to increase (DelGiudice et al. 1987). Ditchkoff (1994) found that deer had mean urea N:C values ranging from 0.7 to 5.6 mg/mg during mid-late winter, whereas DelGiudice et al. (1991*a*) reported mean urea N:C



Deer urine in snow with backpack for comparison.

Deer trails in snow.

values ranging from 2.9 to 7.7 mg/mg for moose populations and noted that means were related to nutritional constraints of the habitat. At high mean urea N:C levels, the proportion of individuals exhibiting urea N:C values ≥3.5 mg/mg (prolonged undernutrition, DelGiudice and Seal 1988) increases. Ditchkoff (1994) found that proportions of deer experiencing prolonged undernutrition ranged from 0.00 to 0.44 during mid-late winter. DelGiudice et al. (1997) reported that the proportion of moose experiencing severe nutritional restriction ranged from 0.10 to 0.90 during late winter.

During mid-late winter, variability between urea N:C profiles of wintering deer populations is greatest and comparisons between populations become most appropriate. In most cases, differences between mean urea N:C values of populations will exceed 0.4 mg/mg during mid-late winter, but sample variability also increases. As a result, a sample size of 20 should suffice to compare the level of nutritional restriction of deer populations during this period (Figure 1). However, if mean values are not expected to differ substantially, ≥30 samples may be needed. Because proportions of deer experiencing severe nutritional restriction have been reported to range from 0.00 to 0.44 (Ditchkoff 1994), we suggest collecting approximately 35 samples during sampling periods in mid-late winter to enable the detection of a proportional difference approximately half this size.

DelGiudice (1995) suggested monitoring trends in urea N:C data to detect decreases in nutritional restriction before they become severe. This technique has greater statistical power than comparisons of deer populations during the same time period because differences in urinary urea N:C should be greater temporally than spatially. Because deer populations can be expected to have mean urea N:C levels that vary temporally by >1.0 mg/mg, 10-15 urine samples should be adequate to monitor these gross changes in nutritional restriction according to the unknown-variability model (Figure 1). In addition, a population that is experiencing severe wintering conditions can be expected to have a significant increase in the proportion of individuals with elevated (≥3.5 mg/mg) urea N:C levels. We suggest collecting approximately 35 urine samples/period when comparing proportions, as this will enable the detection of proportional differences ≥0.22 when $\alpha = 0.10$ (Table 1). Sample sizes of 35 maximize statistical power while reducing resampling biases when comparing proportions.

In summary, we have provided results of power analyses to aid in determining the sample sizes required to detect spatial and temporal differences in urea N:C ratios when sample variability is expected to be low or high, or cannot be safely estimated. Although we calculated these estimates using urea N:C data from free-ranging deer in Maine, the models that we used to generate these estimates should suffice for most sampling scenarios. For statistical analysis, using ANOVA to compare mean ratio values requires fewer samples because of greater statistical power. However, data on the proportion of deer with elevated urea N:C ratios (≥3.5 mg/mg) is easier to interpret because mean values can have varying proportions of deer with elevated ratios. We therefore recommend a combination of analyses to examine spatial and temporal trends in the nutritional restriction of wintering white-tailed deer.

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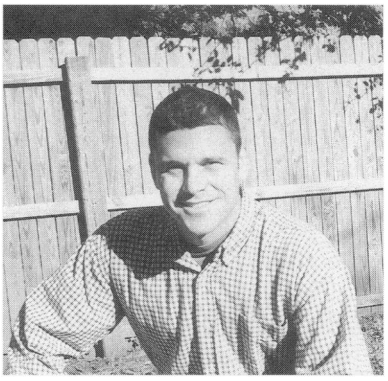

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