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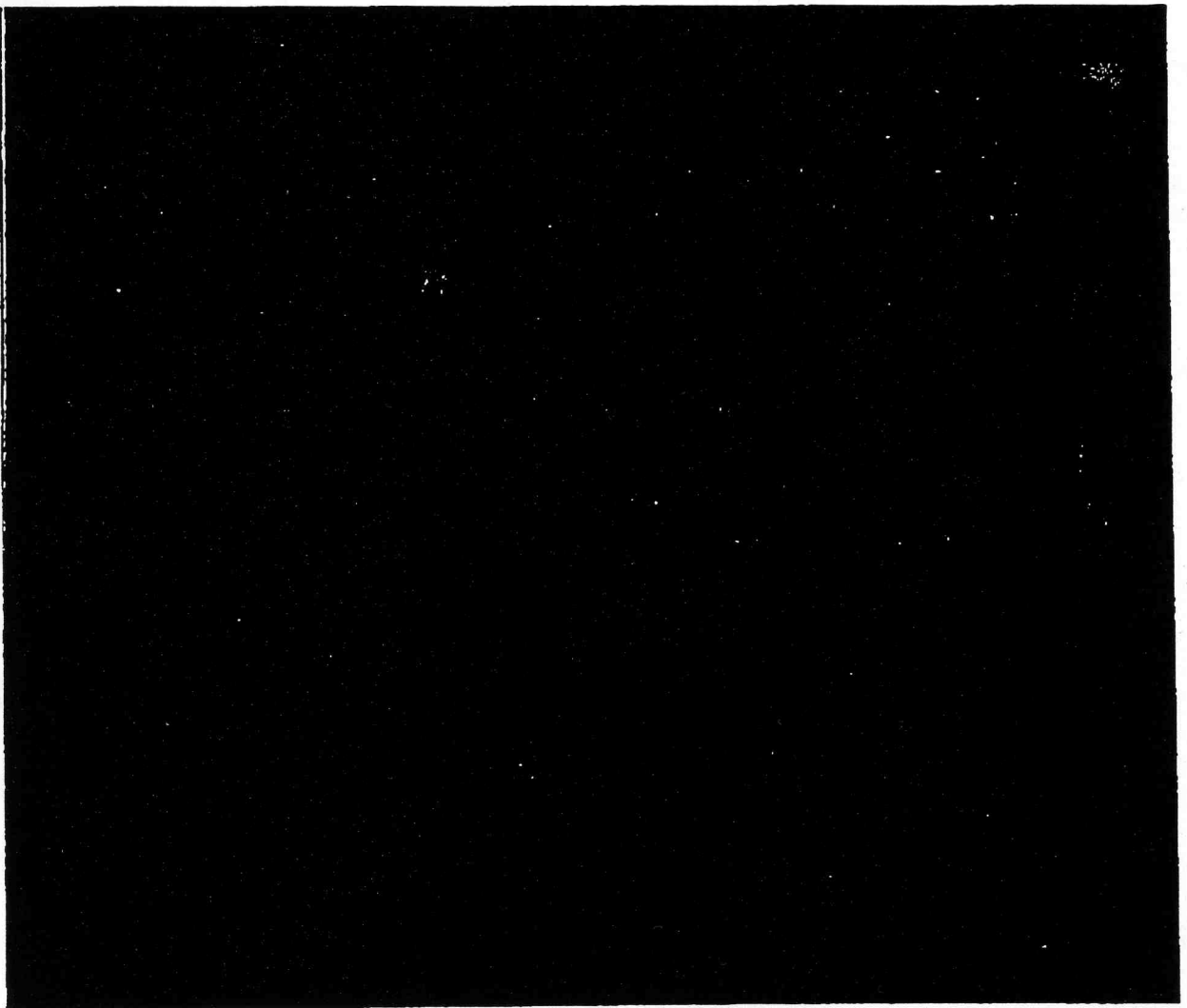
Sampling considerations involved with monitoring the nutritional status of gray wolves Canis lupus via biochemical analysis of snow-urine

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Abstract. Snow-urine analysis is a feasible technique for sensitively, physiologically, and efficiently monitoring the nutritional status of gray wolves Canis lupus. Urinary urea nitrogen : creatinine, sodium : creatinine, and potassium : creatinine ratios can be used to discriminate between fed and fasted wolves; greater ratios occur in fed wolves. These ratios allow estimates of 24-hour excretion and simultaneously correct for dilution by snow. Serial snow-urine collection and analysis best facilitate nutritional monitoring or assessment of wolf pack condition; therefore, factors affecting sampling must be considered. Primary factors discussed include winter snowfall frequency and ambient temperature ranges, intrapack feeding variation, predictions intervals of urea nitrogen:creatinine ratios, and temporal stratification of sampling.

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Nutritional indices of wolves and snow-urine analysis

Nutrition is critical to our understanding of gray wolf ecology. Direct and sensitive monitoring of the nutritional status of wolves should significantly contribute to our knowledge of their biological requirements, demography, behavior, and prey relationships.

Free-ranging wolves live a feast-or-famine existence. Study of captive wolves showed that concentrations changes of several haematological, serum, and urinary characteristics permit discrimination between fed and fasted wolves (DelGiudice et al., 1987). Urinary urea nitrogen (U), potassium (K), and sodium (Na) from single samples were compared as ratios to creatinine (C) to estimate 24-hour excretion of the metabolites (DelGiudice et al., 1987). Significantly ($P < 0.05$) lower ratios occurred in fasted wolves compared to fed wolves (Table 1). Urea is the end-product of protein metabolism and therefore, is a nutritional index of considerable interest. Urinary U:C patterns closely paralleled those of serum urea nitrogen (SUN). Differences were not observed for calcium (Ca):creatinine (DelGiudice et al., 1987).

Collection of blood and urine samples for analysis requires capture and chemical immobilization a process that can be time-consuming and expensive. Additionally, recaptures and statistically-valid sample size may be difficult to obtain (DelGiudice et al., 1992). However, during winter, wolf urine may be collected along trails in snow (Peters and Mech, 1975; Rothman and Mech, 1979) and assayed for the afore mentioned chemistries, overcoming most of the above concerns (Mech et al., 1987). Comparing metabolite data as ratios to C corrects for dilution by the snow.

Differences between fed and fasted captive wolves for U:C, Na:C, and K:C ratios calculated from snow-urine analyses were similar to those in bladder urine samples, with greater ($P < 0.05$) values in fed animals

Table 1. Comparison of $\bar{x}$ (± SE) values of urine chemistries for captive gray wolves fed venison ad libitum with (1-10 days) captive wolves, and fre-ranging wolves recently feeding on deer. [a,b]

Urine chemistries [c,d]	Captive wolves				Free-ranging wolves	
	Bladder urine[e]		Snow-urine			
	Fed	Fasted	Fed	Fasted	Around deer kills[f]	Along trails[g]
U : C	43.9 (4.2)	6.3 (0.5)	44.4 (3.5)	4.9 (0.2)	72.5 (11.1)	43.7 (6.9)
Na : C x 100	26.8 (9.9)	6.3 (2.1)	82.6 (28.1)	2.1 (1.1)	71.1 (17.1)	78.3 (14.1)
K : C x 100	85.8 (12.2)	25.2 (2.4)	173.2 (20.6)	15.7 (3.5)	166.0 (24.9)	160.1 (18.1)
Ca : C x 1,000	9.2 (3.6)	8.4 (6.7)	21.6 (3.1)	3.4 (0.8)	47.6 (9.2)	30.7 (12.8)

[a] Modified from DelGiudice et al. (1987) and Mech et al. (1987).
[b] Sample sizes for different assays varied from 8 to 19.
[c] U:C = urea nitrogen:creatinine, Na:C = sodium:creatinine, K:C potassium:creatinine, and Ca:C = calcium:creatinine.
[d] Significant ($P < 0.05$) differences of U:C, Na:C, and K:C occured in bladder urine and snow-urine from captive fed wolves vs. fasted wolves; Ca:C was different only in snow-urines. Urea nitrogen:C, Na:C, and K:C in snow-urines of fre-ranging wolves were greater ($P < 0.05$) than those of captive fasted wolves. Urea nitrogen:C in snow-urines around deer kills were greater ($P < 0.05$) than U:C in those along trails. Calcium:C of samples around kills were greater ($P < 0.05$) than those of fasted wolves.
[e] Data pooled from fed and fasted wolves of Groups A and B (DelGiudice et al., 1987).
[f] Samples collected within 100 m of white-tailed deer killed by wolves. The sample of 234 snow-urines used U and C analyses in Mech et al. (1987) was subsampled (N = 13) for these analyses.
[g] Samples were collected along wolf trails leading away from kills.

(Table 1). Greater. ($P < 0.05$) U:C, Na:C, and K:C ratios were also noted in urine deposited in snow by free-ranging wolves around white-tailed deer *Odocoileus virginianus* kills and along trails leaving kills compared to those calculated from snow-urines of captive fasted wolves (Table 1). Interpretation of Ca:C ratio was ambiguous, although they seem to show some promise (Table 1).

Application of snow-urine analysis - sampling considerations

Snow-urine analysis may best be used in combination with radio telemetry to closely monitor and compare the nutritional status of specific pack. Three advantages of nutritionally monitoring radio-tagged wolf packs include : specific pack identification; the ability to locate,

track, and sample packs in the snow at any time; and the potential for determining pack history (Peters and Mech, 1975).

Designing a sampling scheme depends on the study objectives, but the following factors should be considered for collecting snow-urine from wolves to determine condition: (1) snowfall frequency, (2) ambient temperature, (3) intrapack feeding variation, (4) urination frequency, and temporal stratification of sampling.

A primary consideration of sample design is weather. Sampling frequency is partly dependent on snowfall frequency. Sample collections after recent snowfall, although not absolutely necessary, permit a more accurate attribution of snow-urine data to a specific time.

Furthermore, the potential for contamination of snow-urine samples from ground debris may be related to snow depth and time since the last snowfall; the shallower

ging wolves

Along trails[g]
43.7 (6.9)
78.3 (14.1)
160.1 (18.1)
30.7 (12.8)

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the snow becomes, the more difficult it may be to collect samples free of debris.

Ambient temperature is also important to consider. Above freezing temperatures might increase the potential for rapid bacterial multiplication and urease breakdown of urea before collection (Codes, 1980). Analysis of human urine in snow, collected at 1, 2, 3, and 5 days post-deposition in northern Minnesota, while ambient temperatures ranged from -15°C to 3.3°C, showed no apparent urea breakdown; U:C ratios remained stable (DelGiudice et al., unpubl. data). However, potential effects of above freezing temperatures on the stability of wolf urinary U:C ratios remain to be examined.

Intrapack feeding variation is probably minimal where pack size averages 2-8 members and large ungulates are the principal prey, so this factor may not be an important sampling consideration. However, where pack size is greater or where wolves rely on smaller prey, subordinate wolves may be at a disadvantage at kills and feed less often (Murie, 1944), resulting in considerable within pack condition variation.

Accurate interpretation of metabolite data for wolves of various ages or ranks can be facilitated by documenting the type of urination collected and determining the pack social structure and territory location. Raised-leg urinations (RLU) by the alpha pair, used primarily for scent-marking, occur more frequently along wolf trails than squat urinations (SQU), which are more indicative of subordinate individuals; it is easy to discriminate between the 2 types (Peters and Mech, 1975). Thus one can monitor the nutritional status of the breeding pair alone or the entire pack.

Frequency of urinations by fed versus fasted wolves should be considered, and may be critical to a sampling scheme. During winter wolves probably derive most of their excretory water for clearance of nitrogen and electrolytes from food, and since protein intake stimulates renal blood

flow and glomerular filtration rate in canines (Pitts, 1944), urinary output is greater in fed animals. Significantly decreased urinary output has been documented in other monogastrics when fasted (Young and Scrimshaw, 1971; Harlow and Seal, 1981). Whether this translates into decreased urination frequency or decreased volume per urination is not known. However, in a 3-winter study of scent marking by free-ranging and captive wolves, Peters and Mech (1975) estimated the most RLU's were probably comprised of only about 5 ml of urine. If this is accurate, the frequency of this urination type may not markedly decrease during fasting. On the average in that study, 3.4 RLU's and 0.3 SQU's occurred every km along roads and trails, and the frequency of SQU's varied with pack size (Peters and Mech, 1975).

Finally, snow-urine collection should be temporally stratified to accurately sample actual distribution of fed and fasted periods. Notable decreases in urinary U:C occur in captive wolves 18 hours after consuming venison *ad libitum*, and after 2-3 days of fasting, U:C ratios diminish to lower, stable levels for at least an additional 7-8 days. Urinary data from captive wolves (DelGiudice et al., 1987) show a 95% upper prediction interval for U:C ratios of $P(Y \leq 13.8)$ to be indicative of fasted wolves. The effects of fasts longer than 10 days on wolf U:C have not been examined, but low SUN concentrations in dogs *Canis familiaris* remained stable from day 7 to completion of a 21-day fast (Brady et al., 1977). A similar 95% prediction interval for U:C ratios determined from wolves fasted 18 hours, then fed venison *ad libitum* for 2 days is $P(Y \geq 15.4)$ (calculated from DelGiudice et al., 1987). Thus, 3 categories for classifying the state of wolves from urinary U:C ratios are fasted, fed, and a narrow interval of uncertainty in between.

Because of the dynamics of urinary U:C, snow-urine analysis should permit determination of whether wolves have been fasted or fed within approximately 48 hours

prior to urination. Desired confidence limits of feeding incidence by a pack will dictate the needed frequency of locating the wolves and collecting snow-urine samples during the winter. Since it is not known for certain what effects fasts, longer than 10 days have on U:C ratios of wolves, the interval between sampling should probably be less than this.

Urinary U:C ratios of fed and fasted wolves are so distinctly different (Table 1) that if our sampling design is temporally stratified and if all pack members feed at kills and are in similar condition, the required sample size is minimized. Only 1 snow-urine sample is needed from each location sampled. If there is any question about intrapack nutritional variation, than at least 1 RLU and 1 SQU should be collected each time. Because only small sample sizes are required, conceivably one could collect samples every 2 days throughout the winter from accessible packs. This should provide very accurate feeding estimates and refine assessment of wolf condition over time.

Snow-urine analysis may be a very viable technique for extensive surveys of wolf populations, even without the aid of radio telemetry. However, many more sampling factors would have to be considered due to the greater number of unknown variables involved.

Advantages of snow-urine analysis

Snow-urine analysis has several advantages as a means of assessing wolf nutrition and condition during winter. It is relatively inexpensive and less time-consuming compared to traditional methods of collecting physiological data, so it can be more easily incorporated into multifaceted ecological studies of wolves. It permits continuous monitoring of the study animals with minimal human impact, and allows a sensitive and quantitative assessment of condition, free of potential handling or drug effects. Furthermore, inexperienced personnel may be easily trained for sample collection. Finally, snow-urine analysis should be applicable to all northern mammalian predator and prey species for assessing nutritional condition.

Acknowledgements

Literature cited

Brady, L.J., M.K. Armstrong, K.L. Muiruri, D.R. Romsos, W.G. Bergen, and G.A. Leveille. 1977. J. Nutr. 107: 1053-1060.

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

DelGiudice, G.D., U.S. Seal, and L.D. Mech. 1987. J. Wildl. Manage. 51: 1-10.

————, U.S. Seal, and L.D. Mech. 1992. Nutritional indices of wild mammals: a new direction for wildlife and habitat management systems. Proc. XVIIIth IUGB Congress of Jagiellonian Univ., Krakow, Poland.

Harlow, H.J., and U.S. Seal. 1981. Can. J. Zool. 59: 2123-2128.

Mech, L.D., U.S. Seal, and G.D. DelGiudice. 1987. J. Wildl. Manage. 51: 10-16.

Murie, A. 1944. The wolves of Mount McKinley. U.S. Natl. Park Serv. Fauna Ser. No.5, Wahington, D.C. 238pp.

Peters, R.P., and L.D. Mech. 1975. Am. Sci. 63: 628-637.

Pitts, R.F. 1944. Am. J. Physiol. 142: 355-365.

Rothman. R.J., and L.D. Mech. 1979. Anim. Behav. 27: 750-760.

Young, V.R., and N.S. Scrimshaw. 1971. Sci. Am. 225: 14-21.