Seasonal Cycles in Hematology and Body Mass in Free-Ranging Gray Wolves (Canis lupus) from Northeastern Minnesota, USA

Authors: Mech, L. David, and Buhl, Deborah A.

Source: Journal of Wildlife Diseases, 56(1) : 179-185

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/2018-06-156

DOI: 10.7589/2018-06-156

Journal of Wildlife Diseases, 56(1), 2020, pp. 179–185

Seasonal Cycles in Hematology and Body Mass in Free-Ranging Gray Wolves (*Canis lupus*) from Northeastern Minnesota, USA

L. David Mech[1,2] and Deborah A. Buhl[1] [1]US Geological Survey, Northern Prairie Wildlife Research Center, 8711 37th Street SE, Jamestown, North Dakota 58401, USA; [2]Corresponding author (email: mechx002@umn.edu)

ABSTRACT: Studies of captive gray wolves (*Canis lupus*) showed seasonal cycles in hematologic values and female body mass. We used a remotely controlled recapture collar to determine whether nine female and five male free-ranging wolves handled four to 17 times in NE Minnesota, US showed similar cycles. Hematocrit, hemoglobin, red blood cell count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and body mass increased from summer toward a winter peak and then decreased again toward summer. Several hematologic values differed considerably from those of captive wolves, and the ranges in free-ranging wolves were much greater than those of captives.

Key words: Body mass, *Canis lupus*, hematology, recapture collar, seasonal cycles.

Hematology and body mass are used to assess nutritional condition in free-ranging animals (Browman and Sears 1955; Campbell and Ellis 2007), including gray wolves (*Canis lupus*; Seal et al. 1975; Mech et al. 1984; DelGiudice et al. 1987), and seasonal hematologic and body mass reference values for wolves have been determined (Seal and Mech 1983; Butler et al. 2006). However, because of the difficulty of obtaining serial data from free-ranging wolves, the most definitive study (Seal and Mech 1983) was conducted with well-fed captive wolves and found a seasonal cycle in several hematologic values, as well as in body mass of females. Butler et al. (2006) sampled many free-ranging wolves throughout the year but only assessed two hematologic parameters and did not include data from summer.

Kreeger (2003) recommended obtaining serial hematologic and body mass values from the same individual free-ranging wolves throughout the year for a more accurate assessment of natural wolf physiology. Development of a remote-controlled recapture collar allowed serial sampling of individual free-ranging wolves throughout the year (Mech et al. 1984; Mech and Gese 1992). We present such data on seven hematologic parameters along with seasonal variations in body mass. The research was approved by the US Fish and Wildlife Service Animal Care and Use Committee, Study Plan 27002.03, in 1990 Determining the Effects of Diseases, Parasites, and Genetic Diversity in Limiting Wolf Populations.

We conducted our study in the east-central Superior National Forest in St. Louis and Lake counties, Minnesota, US, during 1989–93. The wolf's prey there was primarily white-tailed deer (*Odocoileus virginianus*), but also moose (*Alces americanus*) and beavers (*Castor canadensis*). Except for winter 1988–89, the wolf density was relatively low (Mech 2009). Temperatures during the study varied from –32 C to +35 C and during the recaptures from –28 C to +23 C.

The wolves were first trapped with modified steel foot traps (Mech 1974) during summer and fall from 28 June 1989 through 1 November 1992, and their masses were estimated for determining drug doses. The animals were then anesthetized by syringe pole with either 10 mg/kg of ketamine (Mylan Inc., Pittsburgh, Pennsylvania, USA) and either 2 mg/kg of xylazine (Bayer Healthcare LLC, Shawnee Mission, Kansas, USA) or 50 mg of promazine (Bristol-Meyers Squibb, Minneapolis, Minnesota, USA) or with 10 mg/kg of tiletamine–zolazepam (Putney Inc., Portland, Maine, USA). They were weighed by spring scale to the nearest pound (later converted to kilograms) and measured (total length and width of one testicle measured by calipers). Pup ages were estimated assuming a late April birth; all nonpups were considered adults. Blood was collected from the saphenous or cephalic veins from four of the wolves, stored in anticoagulant ethylenediaminetetraacetic acid vials, and refrigerated in the field. They were then mailed in refrigerated containers to the US Veterans Administration Hospital, Minneapolis, Minnesota, for hematology assays conducted usually the same day as arrival in the same laboratory and manner as with previous studies (Seal et al. 1975; Seal and Mech 1983; DelGiudice et al. 1991). In this study, we report hematocrit (HCT, % volume), hemoglobin (Hb, g/dL), red blood cells (RBC, $10^6/\mu L$), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dL), mean corpuscular volume (MCV, fL), and white blood cells (WBC, $10^4/\mu L$).

The wolves were then fit with Wildlink (Brooklyn Park, Minnesota, USA, now defunct) 520-g capture collars (Mech et al. 1984; Mech and Gese 1992). Two darts positioned on the collar above the cervical and capitol rhomboid muscles dorsally on each side of the spinal column were each filled with a 1.5-mL mixture of 250–500 mg of tiletamine, 250–500 mg of zolazepam, and 37–75 mg of xylazine hydrochloride (Kreeger et al. 1990) and 0.75 mL of propylene glycol as an antifreeze in winter.

Most recaptures were conducted approximately 1 mo apart. Usually, the wolf was first located aerially to ensure it was not close to water or other hazards. From a distance of about 0.5 km, a ground team then transmitted a signal to trigger the dart or darts. Once signals indicated the wolf was immobile, workers located it by telemetry. Induction took 3–11 min, and downtime was 51–162 min. Animals were redosed with 100–200 mg of ketamine hydrochloride as needed (Mech and Gese 1992). Recaptured wolves were processed as for initial captures, including blood sampling during most recaptures. Because recaptured wolves often had full or partly full stomachs, which can hold up to 10 kg of food, workers subtracted the estimated mass of stomach contents to derive an adjusted body mass. Collars were replaced with refurbished collars. Usually within 120 min after induction, wolves were injected intravenously with 15 mg of yohimbine hydrochloride (Alchem USA, Philadelphia, Pennsylvania, USA) to antagonize the xylazine (Kreeger et al. 1987) and then released.

Because hematologic values could be affected during initial captures in traps, we omitted those values from all statistical tests. We used SAS 9.4 (SAS Institute Inc. 2017) for all analyses. Second-order polynomial regression was used to test seasonality of various parameters with the hypothesis that they increased toward winter with nadirs in summer (Seal and Mech 1983). To determine whether significant seasonal trends followed that pattern rather than the reverse, which could also yield significance, we inspected each resulting curve. We also examined differences in hematologic parameters between males and females and between adults and pups. We accounted for correlation among repeated measurements on each wolf by assuming an autocorrelation lag 1 covariance structure (Littell et al. 2006). Statistical significance was considered a matter of degree (Cherry 1998) with alpha$\leq$0.10 having some degree of significance.

Nine female and five male wolves were studied four to 17 times, including original captures, for a total of 101 captures (Table 1). Blood was sampled from nine of the wolves during a total of 47 recaptures. The females ranged from 4 mo old to probably not more than 4 yr old at first capture, and none had bred, on the basis of nipple measurements (Barber-Meyer and Mech 2015); the males ranged from 5 mo to 2–5-yr old (wolf 215) at first capture on the basis of testis size (Mech 2006). Another (wolf 469) was just starting to mature.

We obtained body masses at all captures except one and estimated adjusted masses for 73 captures, including for 58 adults. For one mature adult male, we measured testes during 14 captures (Table 2), and his testis size peaked in December and January (Table 2 and Fig. 1a).

Both actual and adjusted masses of male (34 actual, 26 adjusted) and female (46 actual, 32 adjusted) adults increased significantly (female body mass: P=0.006; female adjusted

TABLE 1. Wolves (*Canis lupus*) captured in the Superior National Forest in NE Minnesota, USA whose serial body mass or hematologic data were studied from 1989 to 1993. Specific ages at capture are given for wolves originally caught as pups, whereas Adult indicates all those that were not pups.

Wolf	Sex	Age	Study period	Captures
137	Female	Adult	28 June 1989–3 October 1989	4
139	Female	Adult	29 June 1989–4 January 1990	5
141	Male	Adult	1 July 1989–15 July 1993	5[a]
163	Female	Adult	24 November 1990–7 March 1991	5
171	Female	4–21 mo	15 August 1991–11 March 1993	12
209	Female	Adult	29 August 1990–8 February 1991	6
215	Male	Adult	10 July 1990–29 November 1991	17
313	Female	Adult	28 July 1991–28 March 1992	8
327	Female	Adult	7 August 1990–27 September 1991	4
335	Female	Adult	17 August 1990–15 January 1991	6
414	Male	5–14 mo	23 September 1991–30 June 1997[a]	9
416	Female	5–12 mo	23 September 1991–9 April 1992	7
447	Male	Adult	1 November 1992–5 March 1993	4
469	Male	6–19 mo	27 October 1992–12 November 1993	9

[a] Last recapture was by modified steel foot trap.

body mass: P=0.002; male body mass: P=0.070; male adjusted body mass: P=0.026) toward winter, with lows in summer (Fig. 1b). Male wolf 215 mass (weighed 17 times from 10 July 1990 to 29 November 1991) increased toward winter, although the seasonal trend in his adjusted mass was not as strong.

There was no evidence of a difference between adult males and females for any of the hematologic parameters (HCT: F=0.06,

TABLE 2. Serial body weight and testis measurements for male wolf 215 (≥15 mo old and probably ≥22 mo old) captured in the Superior National Forest in NE Minnesota, USA, 1990–91. Age is known because the wolf was not a pup. Estimated stomach fullness is given because adjusted weight is based on weight minus estimated food mass.[a]

Capture date	Weight (kg)	Stomach (% full)	Adjusted weight (kg)	Testis size (cm)[b]
10 July 1990	38.6	—	—	3.8
11 August 1990	40.5	50	35	3.9
12 September 1990	42.7	—	—	4.9
25 September 1990	43.2	0	43	5.2
11 October 1990	43.2	0	43	5.3
20 October 1990	42.7	0	43	—
13 November 1990	47.3	25	44	6.1
12 December 1990	47.7	50	42	7.4
11 January 1991	41.8	25	39	7.4
15 February 1991	41.8	25	39	6.4
18 May 1991	42.7	50	38	4.8
17 June 1991	45.0	25	42	5.1
15 July 1991	38.6	0	39	5.3
5 September 1991	43.2	50	38	4.9
24 October 1991	44.5	50	40	4.9
24 November 1991	44.5	15	43	—
29 November 1991	50.0	100	40	—

[a] — = no data was collected.

[b] Length plus width.

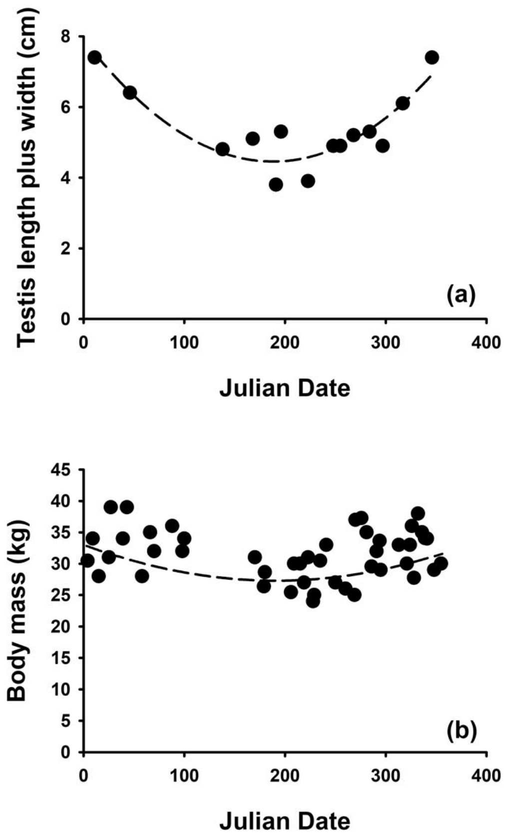

Figure 1. Seasonal trend in (a) testis length plus width of free-ranging adult wolf (*Canis lupus*) 215 from the Superior National Forest of NE Minnesota, USA (n=14) and (b) body mass of adult female free-ranging wolves in the Superior National Forest of NE Minnesota from 46 observations of nine wolves.

df=1,7, P=0.810; Hb: F=0.01, df=1,7, P=0.912; RBC: F=0.10, df=1,7, P=0.760; MCH: F=1.05, df=1,7, P=0.340; MCHC: F=0.18, df=1,7, P=0.684; MCV: F=0.01, df=1,7, P=0.906; WBC: F=0.07, df=1,7, P=0.800), so for additional comparisons these samples were pooled. All pup hematology data (Supplementary Material Table S1) were collected September–February; therefore, when comparing hematologic parameters between adults and pups, only data collected between September and February were included. No significant differences were detected between adults and pups for any of the parameters (HCT: F=0.97, df=1,7, P=0.357; Hb: F=1.01, df=1,7, P=0.348; RBC: F=1.70, df=1,7, P=0.234; MCH: F=0.52, df=1,7, P=0.496; MCHC: F=1.25, df=1,7, P=0.301; MCV: F=0.28, df=1,7, P=0.613; WBC: F=0.34, df=1,7, P=0.576). Values of Hb and RBC were positively correlated with both body mass (n=47; Hb: r=0.30, P=0.039; RBC: r=0.41, P=0.004) and adjusted body mass (n=40; Hb: r=0.39, P=0.014; RBC: r=0.49, P=0.001) for adults and pups pooled. Additionally, HCT was positively correlated with adjusted body mass (r=0.35; P=0.029; n=40).

Mean (SE) hematologic values varied considerably among individual wolves (Supplementary Material Table S2). For pooled male and female adults, Hb (Fig. 2a; P<0.001), HCT (Fig. 2b; P=0.002), RBC (Fig. 2c; P=0.001), MCH (Fig. 2d; P=0.026), and MCHC (Fig. 2e; P=0.014) increased significantly toward winter, with nadirs in midsummer. A significant relationship was also detected for WBC (P<0.010), but for this parameter, values were largest in summer and lowest in winter (Fig. 2f). Values for MCV were not significantly related to date (P>0.646). The strength of these relationships varied if examining males and females separately with and without pups (Table 3).

Our findings tended to confirm, amplify, and refine those from captive wolves and showed an annual cycle of mass in male and female free-ranging wolves (but see Butler et al. 2006). Testis size in the only mature adult male (wolf 215) followed a similar annual cycle, as with wolves in captivity. This correspondence of results between both captive and wild wolves, despite greatly different feeding regimens, suggested that some intrinsic but unknown mechanism underlies the seasonal body mass cycles.

Similar to captive wolves (Seal and Mech 1983), free-ranging wolves also showed significant annual cycles in several hematologic parameters, with winter peaks and summer lows in HCT, Hb, RBC, MCH, and MCHC but not in MCV. The MCVs in captive wolves (Seal and Mech 1983) also did not appear to be as cyclic as the other hematologic parameters. Values for WBCs in our free-ranging wolves tended to peak in summer, contrary to those in the captives, a finding that we cannot explain.

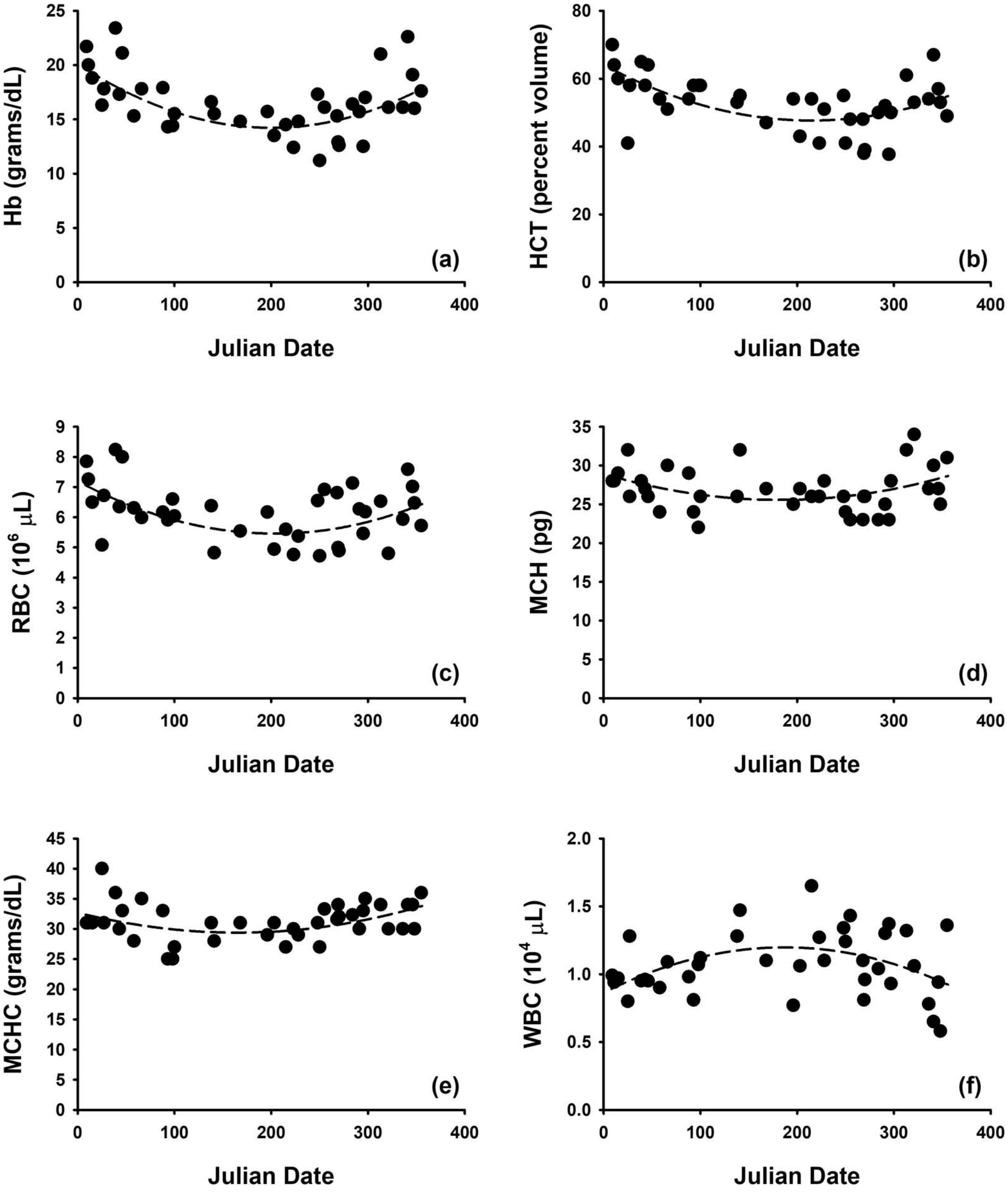

Figure 2. Seasonal trend in pooled data from nine adult male and female free-ranging wolves (*Canis lupus*) in NE Minnesota, USA (n=39) for (a) hemoglobin (Hb), (b) hematocrit (HCT), (c) red blood cells (RBC), (d) mean corpuscular hemoglobin (MCH), (e) mean corpuscular hemoglobin concentration (MCHC), and (f) white blood cells (WBC).

Overall means of HCT and MCV from free-ranging adult wolves were higher than the highest values of captive wolves (Seal and Mech 1983) throughout the year, and the MCHC mean value was lower than the lowest value for those captives. Mean values of HCT, MCH, and MCV from captive wolves (Kreeger 2003) were all lower than those of free-ranging Superior National Forest wolves; Hb was about the same; and RBC, MCHC,

TABLE 3. Probability values that wolf seasonal hematologic values between 1987 and 1993 in NE Minnesota, USA did not follow a second-order polynomial trend (Fig. 2) for females and males separately with and without pup data included. Low probabilities indicate that they did show such a trend. All significant trends peaked in winter except for WBC, which peaked in summer.

				Analytes[a]						
Sex	Includes pups?	Wolves	No. of captures	HCT (% volume)	Hb (g/dL)	RBC (10^6/μL)	MCH (pg)	MCHC (g/dL)	MCV (fL)	WBC (10^4/μL)
Females	Yes	7	31	0.277	0.037	0.168	0.055	0.009	0.534	0.012
	No	7	25	0.033	0.002	0.013	0.027	0.020	0.892	0.033
Males	Yes	2	16	0.189	0.005	0.073	0.534	0.052	0.779	0.351
	No	2	14	0.002	0.001	0.022	0.828	0.216	0.986	0.144

[a] HCT = hematocrit; Hb = hemoglobin; RBC = red blood cells; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; WBC = white blood cells.

and WBC were higher (Supplementary Material Table S2). Pup MCH in our wild wolves was higher than in captive wolves (DelGiudice et al. 1991).

The ranges of values for HCT, Hb, MCHC, MCV, and RBC from free-ranging wolves were all greater than those for captive wolves (Seal and Mech 1983). Of the free-ranging wolf values whose ranges were greater than the ranges of the captive wolves, the means of HCT, MCH, and MCV were greater (no overlap in SDs or SEs) than the means of captives summarized by Kreeger (2003; Supplementary Material Table S2).

The lack of significant differences in hematologic values between male and female free-ranging wolves was generally similar for captives. However, with the study of captive wolves, enough data were available to parse out monthly differences, and male values were consistently, but not significantly, higher during July–November (Seal and Mech 1983). The lack of significant differences in HCT and Hb between our free-ranging adults and pups contrasted with findings from Quebec (Messier 1987).

In summary, we documented an annual cycle in body mass and hematology for free-ranging wolves and traced changes in annual body mass and testis size for a free-ranging wolf. Our hematologic values demonstrated differences from captive wolves as well as the considerable variation among free-ranging wolf populations. These differences and variations confirmed that more data are needed from other populations and that hematologic values must be interpreted cautiously when used to assess wolf condition (DelGiudice et al. 1991).

This study was funded by the US Fish and Wildlife Service and the US Veterans Administration Hospital, Minneapolis, Minnesota, where the blood samples were analyzed. We thank U. S. Seal (deceased) for the inspiration for the study. We thank M. E. Nelson and several volunteer wildlife technicians as well as several pilots who assisted with the remote wolf recaptures. T. J. Kreeger and G. D. DelGiudice and two anonymous reviewers offered suggestions for improving a draft manuscript. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2018-06-156. All data used in analyses are available through US Geological Survey ScienceBase at https://doi.org/10.5066/P9VSFC7J.

LITERATURE CITED

Barber-Meyer SM, Mech LD. 2015. Evaluation of a formula that categorizes female gray wolf breeding status by nipple size. *Northeast Nat* 22:652–657.

Browman LG, Sears HS. 1955. Erythrocyte values and alimentary canal pH values in the mule deer. *J Mammal* 36:474–476.

Butler MJ, Ballard WB, Whitlaw HA. 2006. Physical characteristics, hematology, and serum chemistry of free-ranging gray wolves, *Canis lupus*, in southcentral Alaska. *Can Field-Nat* 120:205–212.

Campbell TW, Ellis CS. 2007. *Avian and exotic animal hematology and cytology*, 3rd Ed. Blackwell, Ames, Iowa, 286 pp.

Cherry S. 1998. Statistical tests in publications of The Wildlife Society. *Wildl Soc Bull* 26:947–953.

DelGiudice GD, Mech LD, Seal US. 1991. Grey wolf density and its association with weights and hematology of pups from 1970 to 1988. *J Wildl Dis* 27:630–636.

DelGiudice GD, Seal US, Mech LD. 1987. Effects of feeding and fasting on wolf blood and urine parameters. *J Wildl Manage* 51:1–10.

Kreeger TJ. 2003. The internal wolf: Physiology, pathology, and pharmacology. In: *Wolves, behavior, ecology, and conservation*, Mech LD, Boitani L, editors. University of Chicago Press, Chicago, Illinois, pp. 192–217.

Kreeger TJ, Fagella AM, Seal US, Mech LD, Callahan M, Hall B. 1987. Cardiovascular and behavioral responses of gray wolves to ketamine-xylazine immobilization and antagonism by yohimbine. *J Wildl Dis* 23:463–470.

Kreeger TJ, Kuechle VB, Mech LD, Tester JR, Seal US. 1990. Physiological monitoring of gray wolves (*Canis lupus*) by radio telemetry. *J Mammal* 71:259–261.

Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O. 2006. *SAS for mixed models*, 2nd Ed. SAS Institute Inc., Cary, North Carolina, 813 pp.

Mech LD. 1974. Current techniques in the study of elusive wilderness carnivores. In: *Proceedings of the 11th international congress of game biologists*, National Swedish Environment Protection Board, Stockholm, Sweden, 3–7 September, 1973, pp. 315–322.

Mech LD. 2006. Age-related body mass and reproductive measurements of gray wolves in Minnesota. *J Mammal* 87:80–84.

Mech LD. 2009. Long-term research on wolves in the Superior National Forest. In: *Recovery of gray wolves in the Great Lakes region of the United States: An endangered species success story*, Wydeven AP, Van Deelen TR, Heske EJ, editors. Springer, New York, New York, pp. 15–34.

Mech LD, Chapman RC, Cochran WW, Simmons L, Seal US. 1984. A radio-triggered anesthetic-dart collar for recapturing large mammals. *Wildl Soc Bull* 12:69–74.

Mech LD, Gese EM. 1992. Field testing the Wildlink capture collar on wolves. *Wildl Soc Bull* 20:221–223.

Messier F. 1987. Physical condition and blood physiology of wolves in relation to moose density. *Can J Zool* 65:91–95.

SAS Institute Inc. 2017. *SAS OnlineDoc® 9.4*. SAS Institute Inc., Cary, North Carolina. https://support.sas.com/documentation/94/. Accessed December 2018.

Seal US, Mech LD. 1983. Blood indicators of seasonal metabolic patterns in captive adult wolves. *J Wildl Manage* 47:704–715.

Seal US, Mech LD, VanBallenberghe V. 1975. Blood analyses of wolf pups and their ecological and metabolic interpretation. *J Mammal* 56:64–75.

Submitted for publication 20 June 2018.
Accepted 6 July 2019.