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HEMATOLOGY, SERUM CHEMISTRY, AND BODY MASS OF FREE-RANGING AND CAPTIVE CANADA LYNX IN MINNESOTA

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ABSTRACT: Baseline blood chemistry data could be particularly valuable if reference values from free-ranging populations of rare or endangered species are not available. The Canada lynx (*Lynx canadensis*) is listed as threatened under the Endangered Species Act in the conterminous United States, even though the species is managed as a furbearer in Alaska and in most provinces of Canada. Body mass, blood chemistry, and hematologic data for free-ranging lynx were collected from 2003 to 2007 and for captive lynx from 1984 to 2007. Up to 2 yr of age, captive lynx were consistently heavier than free-ranging lynx. Body mass of adult free-ranging lynx was similar to body mass of captive adult lynx. Some differences in blood chemistry between free-ranging and captive lynx were statistically significant, but most measured values were within reference ranges for domestic cats. Free-ranging lynx had higher concentrations of aspartate aminotransferase, alanine aminotransferase, and blood urea nitrogen than did captive lynx, and these were outside the reference value ranges for domestic cats. Alkaline phosphatase and phosphorus were higher in juveniles (<12 mo when captured) as compared to adults. Free-ranging lynx maintained body mass between serial captures. Hematologic values, blood chemistry values, and body mass of free-ranging Canada lynx provide support for the hypothesis that Canada lynx in Minnesota, at the southern edge of their range, are in normal physical condition.

Key words: Body mass, Canada lynx, captive, free-ranging, hematology, *Lynx canadensis*, serum chemistry.

INTRODUCTION

When hematologic and serum chemistry data from in situ populations of free-ranging felids are not available, these values are most often compared to values from domestic cats or captive individuals of the same species. The physiologic basis for these comparisons is that blood chemistry of captive individuals is usually similar to blood chemistry of free-ranging individuals (Seal et al., 1975; Fuller et al., 1985). However, the physiologic status of wild-caught animals could differ from the physiologic status of captive animals, for technical reasons such as type of anesthesia, capture method, and time to blood collection, or for ecologic reasons associated with survival under natural conditions. There are few baseline hematologic and serum chemistry values for small, free-ranging felids in the literature, which means that, at present, comparisons must be made to domestic cats and captive animals.

Baseline blood chemistry data are particularly valuable for rare or endangered species because reference values from free-ranging populations may be difficult or impossible to obtain (Beltran et al., 1991). Rare species are often covered by a legal mandate that requires agencies to consider whether management decisions could negatively affect the species. The Canada lynx (*Lynx canadensis*) is listed as threatened under the Endangered Species Act in the conterminous United States, even though the species is managed as a furbearer in most provinces of Canada and in Alaska. Abnormal results from a panel of hematologic and serum chemistry tests could help identify malnutrition, disease, or health problems in specific individuals or across the popula-

tion. Hematologic and serum chemistry results, combined with data on body mass, survival, and reproductive rates could be used to make inferences on the physiologic status of a population and to provide support for management decisions.

To date, there are no published blood chemistry values for free-ranging Canada lynx. Captive Canada lynx had blood chemistry values similar to domestic felids and other wild felid species, but neutrophil counts were higher and lymphocyte counts were lower in captive lynx that were wild-caught as compared to lynx that were born in captivity (Weaver and Johnson, 1995). Elevated blood urea nitrogen (BUN) and creatinine levels were associated with renal dysfunction in two older, wild-caught lynx kept in captivity (Weaver and Johnson, 1995), while elevated BUN in free-ranging bobcats (*L. rufus*) was related to diet (Fuller et al., 1985). Blood chemistry values of the closely related Iberian lynx (*Lynx pardina*) are also similar to other felids, with differences from standard reference values associated with capture method and sex (Beltran et al., 1991; Garcia et al., 2009).

Baseline measurements of blood chemistry, hematology, and body mass were measured on free-ranging Canada lynx in northeastern Minnesota, United States. Differences among male, female, and juvenile Canada lynx, and between free-ranging lynx and captive Canada lynx at the Minnesota Zoological Garden, were tested. These measurements provide baseline values for future blood chemistry studies on all *Lynx* species and can be applied to management and conservation of free-ranging Canada lynx throughout their range.

MATERIALS AND METHODS

Forty blood samples from free-ranging Canada lynx in northeastern Minnesota (centered on 47.4°N, 91.6°W) were collected from March 2003 to March 2007. Blood samples were collected from three females <1 yr old, 10 males ≥1.5 yr old, and 10 females ≥1.5 yr old. Some lynx were handled more than once during recapture in order to replace radiotelemetry collars (Moen et al., 2008). Lynx were caught in box traps that were baited with road-killed white-tailed deer (*Odocoileus virginianus*) and checked daily (Burdett et al., 2007). After body mass was estimated, lynx were anesthetized with 10 mg/kg ketamine hydrochloride (Ketaject, 100 mg/ml, Phoenix Pharmaceutical, Inc., St. Joseph, Missouri, USA) and 2 mg/kg xylazine (Xylaject, 20 or 100 mg/ml, Phoenix Pharmaceutical, Inc.), which was reversed with 0.11 mg/kg yohimbine (Yobine, 2 mg/ml, Lloyd Laboratories, Inc., Shenandoah, Iowa, USA; Kreeger et al., 2002). Morphologic measurements were taken, and temperature, pulse, and respiration were monitored while the animal was anesthetized. Drugs were administered intramuscularly, although in later years, yohimbine was given both intramuscularly and intravenously.

Blood and serum of free-ranging lynx was analyzed by Marshfield Laboratories (Marshfield, Wisconsin, USA). Samples were processed according to standard protocols and usually analyzed the day after collection (Marshfield Laboratories, 2006). In a few cases, blood analysis was delayed for an additional day and, in one case, for three days due to the remote location of captures. These samples were not deleted from the data set because measured values for blood and serum chemistry were not outliers.

Body mass and blood chemistry values from free-ranging lynx were compared to body mass and blood chemistry values of 13 captive lynx at the Minnesota Zoological Garden (Apple Valley, Minnesota, 44.8°N, 93.2°W); values were obtained in 43 physical examinations from 1984 to 2007. Captive lynx consumed a horsemeat-based diet, which was fed once daily in amounts based upon individual lynx weight and body condition. Bones with some meat attached were fed twice weekly. Captive lynx included six males and seven females that were 2 mo to 14 yr old.

Means and standard errors were calculated for all blood and serum chemistry values for kittens, adult males, and adult females. Captive lynx were classified as normal or abnormal based upon clinical presentation, blood values, other clinical pathology parameters, or any of these factors combined. Comparisons between blood chemistry and hematologic values for adult males and females were made with a factorial analysis of variance (ANOVA) using sex and type (free-ranging or normal captive) and the interaction of sex and type as factors. Finally, differences in alkaline phosphatase

and creatinine in free-ranging juveniles and adults were tested with an unpaired *t*-test and, in free-ranging juveniles and adults, with a factorial ANOVA. Lynx blood chemistry values were compared to reference values for domestic cats (Marshfield Laboratories, 2006).

Statistix 9.0 (Analytical Software, Boca Raton, Florida, USA) and Stata 9.2 (StataCorp, College Station, Texas, USA) were used for statistical analyses with $\alpha=0.05$; *P*-values are reported when appropriate. The animal care protocol for free-ranging animals was approved by the Institutional Animal Care and Use Committee at the University of Minnesota (Codes 0301A39326 and 0602A81086). The Minnesota Zoological Garden follows animal care guidelines established by the Association of Zoos and Aquariums and by the United States Department of Agriculture under the Animal Welfare Act.

RESULTS

All but one free-ranging lynx appeared to be in good condition, based on physical characteristics. A 10.5-kg male lynx captured in 2004, with reduced muscle mass relative to frame size, had probably been caught in a leg-hold trap (R. Moen, pers. obs.). Its right front foot had been partially amputated, on 2 digits, to the level of the metacarpals. Metacarpal bones were exposed at the sites of amputation. This lynx lived at least 3 yr after it was radiocollared and moved approximately 180 km from the capture site to Ontario.

Captive lynx kittens were usually heavier than free-ranging lynx kittens >150 days old (Fig. 1). Body mass of some free-ranging lynx was similar to body mass of captive lynx by 2 yr of age. Body mass of adult lynx was similar to body mass of captive lynx (Fig. 2). Female body mass was lower than male body mass. Between captures, body mass of all but one free-ranging individual captured more than once increased, or remained stable (Fig. 2).

Mean drug dosages were 10.6±0.2 mg/kg ketamine, 2.0±0.1 mg/kg xylazine, and 0.11±0.003 mg/kg yohimbine. Mean dosages of ketamine and xlyazine given to female adults was slightly higher than those given male adults (11.6 vs. 10.1 and 2.3 vs. 1.7 mg/kg, $F_{3,55}=3.4$, $P=0.03$, and $F_{3,52}=7.9$, $P=0.0002$) because body mass was overestimated for female adults relative to male adults.

There were differences in blood chemistry between free-ranging and captive lynx (Table 1). Differences in creatinine, phosphorus (P), calcium (Ca), potassium (K), and chloride (Cl) were statistically significant, but measured values were within the reference range for domestic cats (Marshfield Laboratories, 2006) and, thus, may not be biologically significant. In contrast, free-ranging lynx had higher concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) than did both captive lynx and the reference value range for domestic cats (Fig. 3). Similarly, blood urea nitrogen was higher in free-ranging lynx than in captive lynx or domestic cats (Fig. 4). There were no significant differences in blood chemistry between males and females, although the interaction of type (captive vs. free-ranging) and sex was significant for ALT (Table 1). The concentration of ALT in free-ranging males was 166±16 u/l, while in free-ranging females it was 92±16 u/l, compared to 32±22 u/l in captive females and 23±24 u/l in captive males.

Alkaline phosphatase ($t_{35}=8.03$, $P=0.000$, 93.0±9.1 vs. 25.6±2.4 u/l), and phosphorus ($t_{34}=2.30$, $P=0.028$, 7.4±1.2 vs. 5.9±0.2 mg/dl) were higher in juveniles (<12 mo when captured) as compared to adults, while creatinine ($t_{36}=-2.98$, $P=0.005$, 0.6±0.1 vs. 1.2±0.1 mg/dl) was lower in juveniles than in adults. This comparison was limited by sample size ($n=3$ free-ranging juveniles), but the same pattern held in the combined data set of both free-ranging and captive animals, with higher ALP ($F_{1,52}=228.3$, $P=0.00$), higher P ($F_{1,51}=38.1$, $P=0.00$), and lower creatinine ($F_{1,53}=89.3$, $P=0.00$) in juveniles. There were other differences between adults and juveniles for which F-tests in a factorial ANOVA were significant

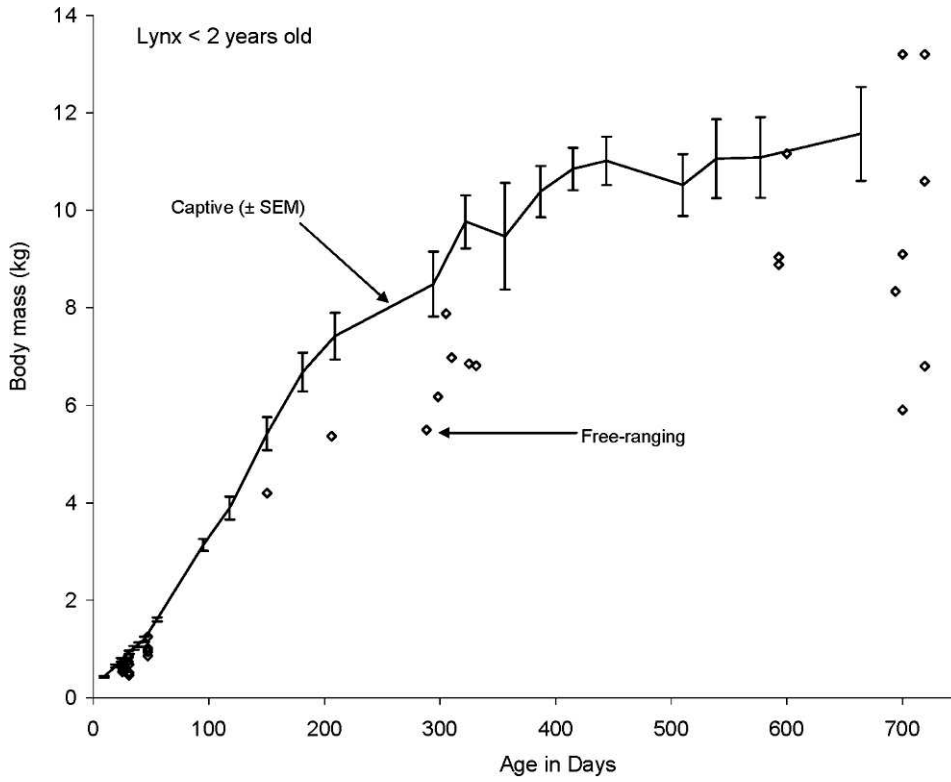

FIGURE 1. Body mass of captive and free-ranging Canada lynx <2 yr old. Captive lynx body mass (mean±SEM) is for three kittens at the Minnesota Zoological Garden (two or three measurements on each day). Body mass of free-ranging lynx from northeastern Minnesota was obtained during den visits, when lynx were caught in box traps on the radio-telemetry project, or when a carcass was recovered.

(glucose, bilirubin, total protein, Ca, sodium, and Cl), but Bonferroni comparisons indicated the differences were not solely due to the juvenile-adult contrast.

Because fewer parameters were analyzed on captive lynx, there were fewer hematologic values to compare between free-ranging and captive lynx. There were statistical differences that probably were not biologically significant because hematologic values were within, or close to, reference ranges for domestic cats (Table 2). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were all at, or just above, the high end of the domestic cat reference range for free-ranging lynx. The percent of segmented neutrophils was higher in free-ranging lynx as compared to captive lynx, while the percent of lymphocytes was lower in free-ranging lynx than in captive lynx (Table 2).

DISCUSSION

Changes in physiologic status can be particularly important for monitoring in a species such as Canada lynx because of the population cycles of their main prey, the snowshoe hare (*Lepus americanus*). Across their range, over 80% of the winter diet of lynx is snowshoe hare (Mowat et al., 2000), including in Minnesota (Burdett, 2008; Hanson and Moen, 2008). Some changes in blood chemistry and hematology can be associated with a decrease in body mass that is a part of the response of lynx to reduced caloric intake when hare populations are low. Hemoglobin, red blood cell counts, and hematocrits were higher in fasted wolves

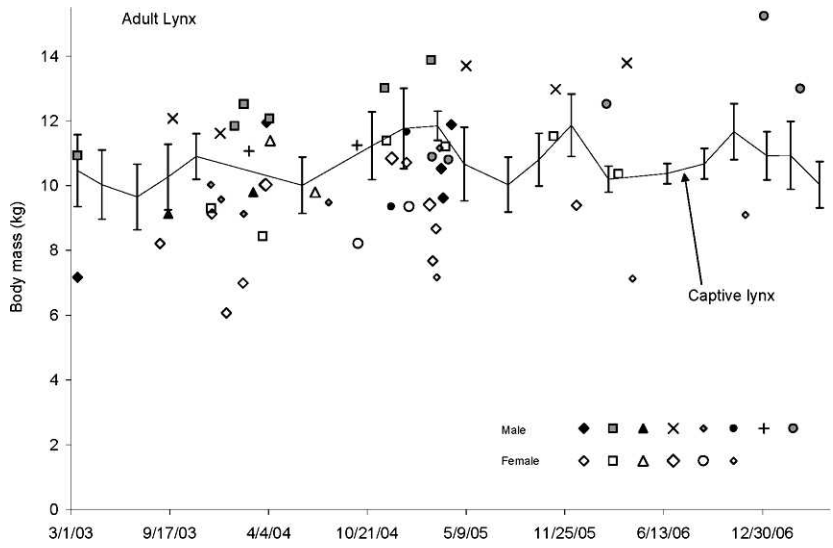

FIGURE 2. Body mass of captive lynx, 3 to 6 yr old, at the Minnesota Zoological Garden, and body mass of free-ranging lynx >2 yr old from northeastern Minnesota. Captive lynx body mass (mean±SEM) is from four lynx, 3 to 6 yr old. Free-ranging lynx were weighed when lynx were caught in box traps on the radio-telemetry project, or when a carcass was recovered. Date of capture or carcass recovery is plotted for free-ranging lynx.

(*Canis lupus*) than in fed wolves, while white blood cell counts and blood urea nitrogen were lower (Delgiudice et al., 1987). If calorie intake is low enough, lynx die of starvation, survive on alternate prey, or move to new areas (Ward and Krebs, 1985).

Some differences in blood chemistry between captive and free-ranging lynx may have been due to handling protocols. Captive lynx were fasted and had water withheld for about 12 hr before anesthetization, but otherwise had greater access to water than did free-ranging lynx. Free-ranging lynx obtained water in the form of snow, or from ingested food, in winter when most of the trapping occurred. Free-ranging lynx that were captured in box traps may have had limited access to snow while inside the trap. Together, these factors could have caused detectable differences in concentrations of K, P, Ca, and Cl in the blood of free-ranging lynx, even though measurements were still within the reference range for domestic cats and were not clinically significant.

Other differences had a probable biologic basis, or remain unexplained. The higher concentration of ALP and Ca in juveniles is likely related to bone growth (Beltran et al., 1991; Weaver and Johnson, 1995; Garcia et al., 2009). Creatinine concentrations are generally lower in juveniles because of lower muscle mass (Barr et al., 2005). The lack of difference in blood chemistry and hematology between males and females is consistent with measurements on Iberian lynx (Beltran et al., 1991; Garcia et al., 2009).

The higher BUN in free-ranging lynx could be due to several causes, including mild dehydration. Wild-caught animals did not have access to water while in a trap, although water restriction would not have been much longer than the 12 hr water was withheld from captive animals. Blood urea nitrogen may increase if free-ranging animals consume a diet with higher protein content than the diet of captive animals (Fuller et al., 1985). However, the meat and bone supplement diet of lynx at the Minnesota Zoological Garden would be similar in protein to the diet of free-ranging lynx feeding on

TABLE 1. Blood chemistry values for free-ranging Canada lynx in Minnesota and captive lynx held at the Minnesota Zoological Garden. Mean, SE, and range for serum chemistry measured on free-ranging and captive adult lynx. Significant ($P<0.05$) P-values for F-test with factorial ANOVA of type (captive or free-ranging), sex, and the type×sex interaction are bolded. Cat reference values are from Marshfield Laboratories (2006).

		Mean (SE)		ANOVA P-values			Range		
Component[a]	Units	Wild	Zoo	Type	Sex	T×S[b]	Wild	Zoo	Cat
Glucose	mg/dl	101 (7)	153 (12)	0.34	0.69	0.36	25–264	94–164	56–153
AST	u/l	105 (6)	35 (2)	**<0.01**	0.50	0.26	45–320	16–65	14–54
ALT	u/l	125 (15)	37 (4)	**<0.01**	0.10	**0.04**	35–406	20–54	26–128
ALP	u/l	26 (2)	22 (2)	0.66	0.46	0.70	13–88	12–39	14–102
Bilirubin	mg/dl	0.2 (0.0)	0.2 (0.0)	0.36	0.55	0.28	0.1–1.1	0.1–0.4	0.0–0.2
Cholesterol	mg/dl	135 (4)	120 (7)	0.26	0.30	0.79	87–259	115–199	71–218
Total protein	g/dl	7.1 (0.1)	6.8 (0.1)	0.21	0.22	0.36	6.3–10.7	5.8–7.5	5.9–8.4
Albumin	g/dl	4.0 (0.1)	3.8 (0.1)	0.76	0.72	0.35	1.7–4.8	3.6–4.5	2.3–3.9
BUN	mg/dl	46 (2)	32 (2)	**<0.01**	0.22	0.89	20–66	26–41	18–36
Creatinine	mg/dl	1.2 (0.1)	2.6 (0.2)	**<0.01**	0.24	0.57	0.7–2.0	1.8–3.5	0.6–2.0
Phosphorous	mg/dl	5.9 (0.2)	5.1 (0.2)	**<0.01**	0.17	0.77	3.5–9.7	3.6–5.8	2.7–8.1
Calcium	mg/dl	9.2 (0.1)	9.6 (0.1)	**0.01**	0.26	0.17	8.2–10.5	8.9–10.5	8.7–11.7
Sodium	mmol/l	152 (1)	153 (1)	0.10	0.90	0.93	119–158	150–155	146–160
Potassium	mmol/l	3.9 (0.0)	4.3 (0.1)	**0.01**	0.80	0.41	3.4–4.6	3.7–5.2	3.3–5.4
Chloride	mmol/l	115 (1)	119 (0)	**<0.01**	0.08	0.95	81–121	117–121	110–123
GGT	u/l	2.5 (1.0)	2.2 (0.9)	0.38	0.52	0.73	1.5–36.0	0.0–3.0	0.0–5.0
Globulin	g/dl	3.1 (0.1)	3.0 (0.1)	0.24	0.20	0.17	2.3–9.0	2.2–3.3	2.6–5.1
Thyroxine	ug/dl	1.7 (0.1)	—	—	—	—	0.2–3.1	—	1.9–4.8

[a] AST = aspartate transaminase; ALT = alanine transaminase; ALP = alkaline phosphatase; BUN = blood urea nitrogen; GGT = gamma-glutamyl transpeptidase.

[b] Interaction of type×sex.

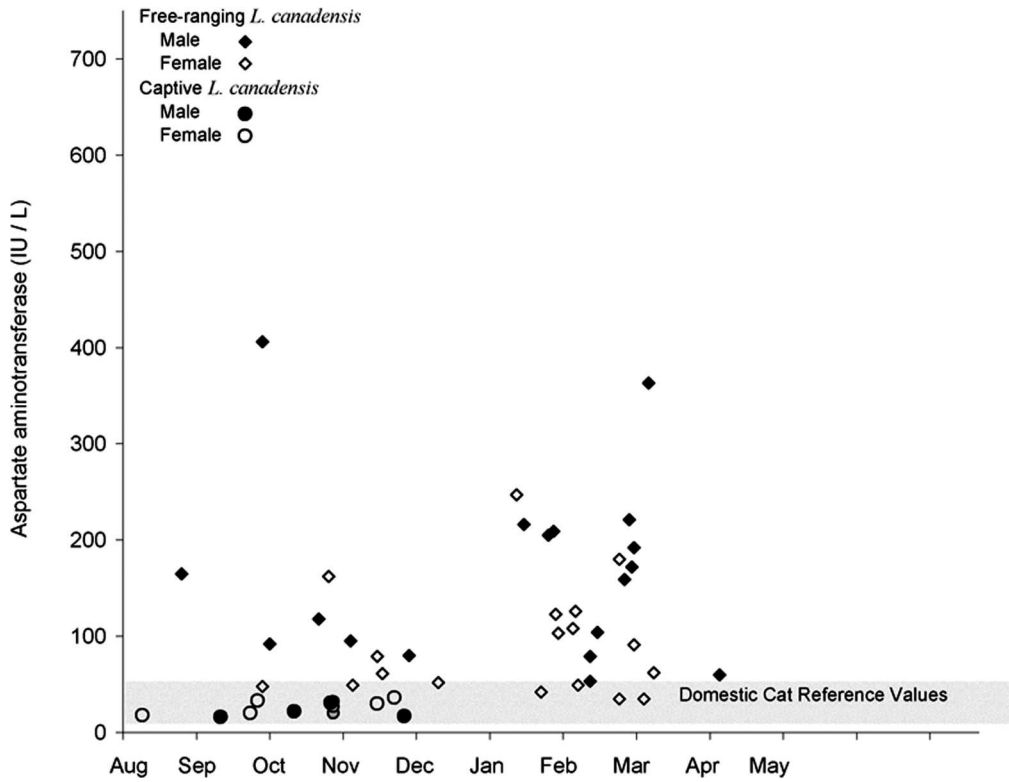

FIGURE 3. Serum AST levels in free-ranging Canada lynx and for captive Canada lynx at the Minnesota Zoological Garden. The grey-shaded area is the range for domestic cats (Marshfield Laboratories, 2006).

snowshoe hares. The BUN value was not different between captive and free-ranging Iberian lynx when captive lynx were fed a diet similar to the diet of free-ranging lynx (Garcia et al., 2009). Renal dysfunction can be a cause of high BUN in specific animals (Weaver and Johnson, 1995). There were no very high BUN values in captive or free-ranging lynx in Minnesota (Fig. 4), other than in one captive lynx with renal dysfunction. Creatinine concentrations, although higher in captive lynx than in free-ranging lynx, were within the normal range of domestic cats in both cases, indicating renal dysfunction is unlikely in free-ranging lynx.

Higher AST in free-ranging lynx occurred when captive and free-ranging lynx were compared in the same study (Garcia et al., 2009), or when studies with captive animals were compared to studies with free-ranging animals (Beltran et al., 1991, Weaver and Johnson, 1995). Restraint of captive European wildcats (*Felis sylvestris*) led to higher AST levels (Marco et al., 2000). Increased AST has been associated with stress, which may have a physical or a psychologic component in felids and in many other mammals including rats (*Rattus norvegicus*), Arabian sand gazelles (*Gazella subgutturosa*), bowhead whales (*Balaena mysticetus*), tammar wallabies (*Macropus eugenii*), and red fox (*Vulpes vulpes*) (Kreeger et al., 1990; Vassart et al., 1994, Heidel et al., 1996; Chen et al., 1998; McKenzie et al., 2004). A relative increase in neutrophils and a decrease in lymphocytes, which was detected in free-ranging and captive lynx, also was attributable to adrenocortical activation (Marco et al., 2000). In our case, the increased AST is likely a response to a perceived

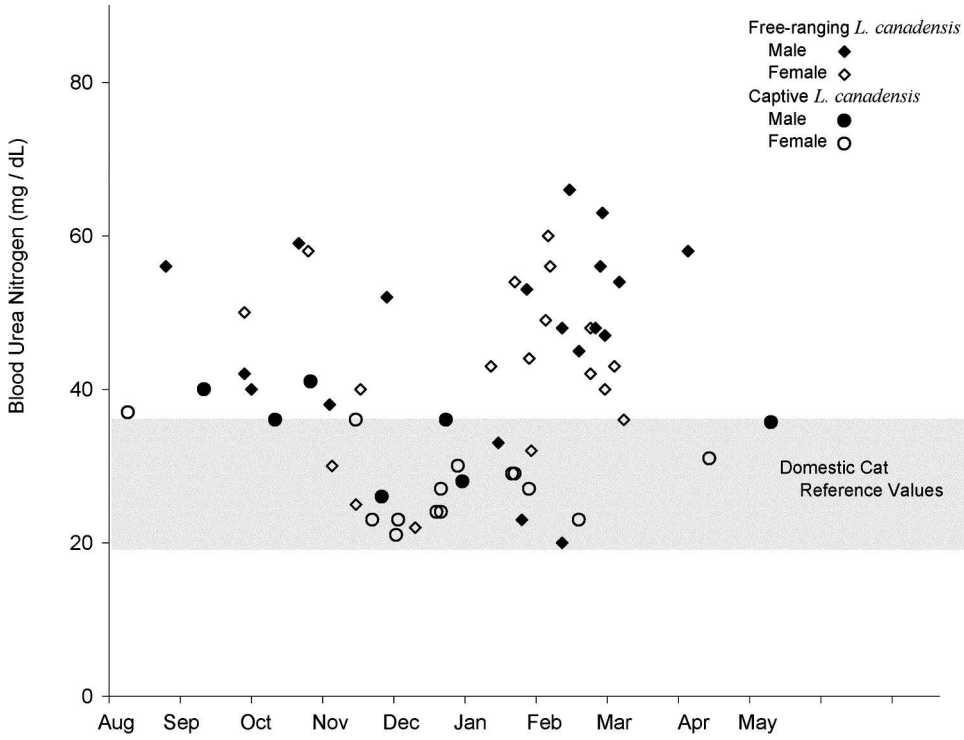

FIGURE 4. Blood urea nitrogen (BUN) levels in free-ranging Canada lynx and in captive Canada lynx at the Minnesota Zoological Garden. The grey-shaded area is the range for domestic cats (Marshfield Laboratories, 2006).

threat rather than a result of physical stress, or is a long-term psychologic response associated with the trapping done for this radio-telemetry project. Several lynx were caught multiple times, which indicates that little, or no, residual avoidance behavior caused by stress seems likely to be associated with the handling process.

In summary, body mass, blood chemistry, and hematologic data provide support for the hypothesis that Canada lynx in Minnesota, at the southern edge of their range, are in normal physical condition. Other indicators also support this hypothesis. Radio-collared adult females in Minnesota were pregnant every year for which reproductive status could be monitored (Moen et al., 2008). Home ranges of female lynx in Minnesota and Maine were about half the size of home ranges recorded elsewhere for female lynx (Burdett et al., 2008), indicating that prey could be obtained in a small area. For comparative purposes, it is desirable to obtain blood chemistry data from the central portion of the lynx range, especially during the periodic prey scarcity encountered by lynx at the nadir of the hare population cycle.

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TABLE 2. Hematologic values for free-ranging Canada lynx in Minnesota and normal captive lynx held at the Minnesota Zoological Garden. Mean, SE, and range for serum chemistry measured on free-ranging and captive adult lynx. Significant ($P<0.05$) P-values for F-test with factorial ANOVA of type (captive or free-ranging), sex, and the type×sex interaction are bolded. Cat reference values are from Marshfield Laboratories (2006).

		Mean (SEM)		ANOVA P-values			Range		
Component[a]	Units	Wild	Zoo	Type	Sex	T*S	Wild	Zoo	Cat
Red blood cell count	$\times 10^6$/ul	7.4 (0.1)	—	—	—	—	6.0–9.3	—	5.8–11.0
Hemoglobin	g/dl	14 (0)	9.5 (0.5)	**0.00**	0.81	0.46	11–17	9.5–12.5	9–16
Hematocrit	%	39 (1)	30 (1)	**0.00**	**0.01**	0.45	32–50	30–43	28–47
MCV	g/dl	52 (0)	—	—	—	—	47–59	—	38–50
MCH	pg	18 (0)	—	—	—	—	17–20	—	12–17
MCHC	u/l	35 (0)	—	—	—	—	30–38	—	—
White blood cell count	$\times 10^3$/ul	9.3 (0.5)	10 (1)	0.39	0.60	0.41	4.1–15.1	6–14	3.7–20.5
Segmented neutrophils	%	84 (1)	71 (2)	**0.00**	0.50	0.60	60–92	58–87	—
Band neutrophils	%	1.7 (0.4)	5.4 (2.1)	0.68	0.77	0.77	1–3	0–4	—
Lymphocytes	%	13 (1)	19 (2)	**0.00**	0.11	0.39	6–31	6–35	—
Monocytes	%	2.2 (0.3)	2.9 (0.4)	0.97	0.34	0.13	1–5	0–6	—
Eosinophils	%	2.3 (0.4)	2.6 (0.4)	0.27	0.44	0.93	1–6	0–9	—

[a] MCV = mean corpuscular volume; MCHC = mean corpuscular hemoglobin concentration; MCH = mean corpuscular hemoglobin.

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