Hematological and serum chemical characteristics of the Iberian lynx (*Lynx pardina*) in southwestern Spain

J. F. BELTRÁN AND M. DELIBES

Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas, Apartado 1056, E-41080 Sevilla, Spain

AND

F. RECIO AND C. AZA

Hospital de Valme, RASSSA, Carretera de Cádiz, s/n., E-41014 Sevilla, Spain

Received April 2, 1990

BELTRÁN, J. F., DELIBES, M., RECIO, F., and AZA, C. 1991. Hematological and serum chemical characteristics of the Iberian lynx (*Lynx pardina*) in southwestern Spain. Can. J. Zool. **69**: 840–846.

Hematological and serum chemical values were determined for 16 wild Iberian lynxes, *Lynx pardina* (5 adult males, 4 adult females, 4 juvenile males, and 3 juvenile females) captured with box traps and coil-spring traps. The results include reference values, analysis of sex and age differences, and data on the influence of capture method on blood values. Males had higher red blood cell counts ($p = 0.03$) and packed cell volumes ($p = 0.06$) than females, which presented higher mean corpuscular hemoglobin levels ($p = 0.08$) and mean corpuscular hemoglobin concentrations ($p = 0.07$) than males. Juveniles had higher serum levels of alkaline phosphatase ($p = 0.01$), urea ($p = 0.02$), and cholesterol ($p = 0.02$) and lower levels of creatinine ($p = 0.07$) than adults. Four hematological variables (mean platelet volume, platelet size distribution, white blood cell count, and mean corpuscular volume) and two serum variables (concentrations of amylase and calcium) were influenced by capture method. The use of standard procedures to assess base-line blood values in wild carnivores is encouraged.

BELTRÁN, J. F., DELIBES, M., RECIO, F., et AZA, C. 1991. Hematological and serum chemical characteristics of the Iberian lynx (*Lynx pardina*) in southwestern Spain. Can. J. Zool. **69** : 840–846.

Les variables hématologiques et les concentrations de substances chimiques dans le sérum ont été déterminées chez 16 lynx, *Lynx pardina* (9 adultes : 5 mâles et 4 femelles, et 7 juvéniles : 4 mâles et 3 femelles), capturés au moyen de boîtes-pièges et de pièges à ressorts. On trouvera ici des valeurs de référence, des analyses des différences reliées au sexe et à l'âge et des évaluations de l'influence de la méthode de capture sur les variables du sang. Chez les mâles, les érythrocytes sont plus nombreux ($p = 0,03$) et l'hématocrite est plus élevé ($p = 0,06$) que chez les femelles; en revanche, les femelles ont une hémoglobine globulaire moyenne plus élevée ($p = 0,08$) et une concentration globulaire moyenne en hémoglobine plus élevée aussi ($p = 0,07$) que les mâles. Chez les juvéniles, les concentrations sériques d'alcaline phosphatase ($p = 0,01$), d'urée ($p = 0,02$) et de cholestérol ($p = 0,02$) sont plus élevées, alors que les concentrations de créatinine ($p = 0,07$) sont plus faibles. Quatre variables hématologiques (volume moyen des plaquettes, répartition des plaquettes en fonction de leur taille, nombre de globules blancs et volume moyen des globules) et deux variables sériques (concentrations d'amylase et de calcium) diffèrent selon la méthode de capture. L'utilisation de techniques standard pour évaluer les variables de base du sang chez les carnivores en nature est donc une pratique à encourager.

[Traduit par la rédaction]

Introduction

Determination of base-line hematological and serum chemical values for free-ranging individuals is an important starting point in population biology studies (Mech 1980; Mauro 1987). However, with some exceptions, such as ungulates (Seal 1978; Franzmann and Leresche 1978), information on hematology of wild vertebrates in their natural environment is scarce. When endangered species are involved, base-line hematological data from remaining wild populations are critical (*i*) as reference information for captive breeding programs, allowing captive animals' health to be compared with that of wild populations (Bush *et al.* 1987), (*ii*) as a guide to the physiological status of the population, permitting the effects of management plans in the wild to be assessed (Franzmann and Schwartz 1988), and (*iii*) to generate data on population genetic polymorphisms (Newman *et al.* 1985) which have direct implications for the way in which captive animals are bred and possibly reintroduced in the wild.

The blood of carnivores, particularly felids, has been sampled in nature only recently (Currier and Russell 1982; Fuller *et al.* 1985; Kocan *et al.* 1985; Caro *et al.* 1987), and interpretation of blood data is difficult because of the scarcity of both reference values and sample sizes. Reference data have to be taken from captive animals (Wintrobe *et al.* 1981; Currier and Russel 1982; Fowler 1986; Hawkey and Hart 1986; Caro *et al.* 1987) or domestic animals (Wintrobe *et al.* 1981; Bentinck-Smith 1983). In addition, the estimates of blood and serum chemistry values of wild-caught individuals are suspected to be influenced by age, sex, season, nutritional status (Delgiudice *et al.* 1987), reproductive status (Fuller *et al.* 1985), social status (Caro *et al.* 1987, 1989), stress of capture (Brannon 1985*a*, 1985*b*), anaesthesia (Hawkey *et al.* 1980; Seal *et al.* 1987), geographic range and habitat quality (Seal 1987; Seal and Hoskinson 1978; Brannon 1985*b*), and parasitosis (Kocan *et al.* 1985). Inappropriate collection methods and storage, the time elapsed since immobilization (Karns and Crichton 1978; Hawkey *et al.* 1980; Brannon 1985*a*, 1985*b*), and the method of analysis (Bentinck-Smith 1983) also influence results.

The Iberian lynx (*Lynx pardina*) is recognized as a distinct species (Werdelin 1981, 1987; Honacki *et al.* 1982; see also remarks in Tumlison 1987). Its distribution is currently restricted to a few preserved Mediterranean areas in southwestern Spain (Rodriguez and Delibes 1990) and possibly Portugal. This endangered species (International Union for Conservation of Nature and Natural Resources 1986) has probably evolved to prey on European lagomorphs (see Beltrán 1987; Aldama and Delibes 1990), being more similar in size to the Nearctic species of lynxes, the bobcat (*Lynx rufus*) and the Canadian lynx (*Lynx canadensis*), than to the other Palaearctic species, the boreal lynx (*Lynx lynx*). Further information on the ecological requirements of *L. pardina* can be found in Delibes (1980) and Beltrán *et al.* (1991).

Printed in Canada / Imprimé au Canada

TABLE 1. Descriptive statistics for 14 hematologic and 19 blood serum parameters from 9 male (5 adults, 4 juveniles) and 7 females (4 adults, 3 juveniles) free-ranging Iberian lynxes in southwestern Spain

	Mean	SD	Range	*N*
Hematology				
WBC, $\times 10^9$/L	17.2	6.6	8.2–29.6	20
Neutrophils, %	83.4	7.5	67.0–98.0	19
Lymphocytes, %	11.6	6.0	0.0–26.0	19
Monocytes, %	2.7	2.6	0.0–10.0	19
Eosinophils, %	1.0	3.2	0.0–14.0	19
Basophils, %	0.0	0.0	0.0–0.0	19
HB, g/dL	12.6	1.6	9.9–15.6	20
MCV, fL*	49.4	1.7	47.0–53.1	19
EDA, %	30.4	8.8	18.3–40.3	14
PLQ, $\times 10^9$/L*	462.6	191.6	165.0–864.0	13
PCT, %	0.53	0.14	0.37–0.82	9
Serum chemistry				
Glucose, mg/dL	135.6	55.5	55.5–248.0	19
Uric acid, mg/dL	1.0	1.3	0.01–4.9	15
Triglyceride, mg/dL*	38.3	13.8	21.0–64.0	15
GOT, IU/L	214.1	194.7	25.0–730.0	10
GPT, IU/L	100.7	76.5	34.0–307.0	10
LDH, IU/L	1 847.2	1 438.8	119.0–4 220.0	9
CPK, IU/L	20 244.5	40 532.1	109.0–126 400.0	9
Cholinesterase, IU/L	6 490.6	1 167.6	4 671.0–7 929.0	10
γ-GTP, IU/L†	2.5	1.3	1.0–4.0	4
Total bilirubin, mg/dL	0.35	0.12	0.2–0.6	11
Total protein, g/dL	7.7	1.4	5.8–10.8	10
Albumin, g/dL	3.7	0.8	2.5–4.9	5
Globulin, g/dL	4.2	1.0	3.3–6.0	5
Albumin/globulin	0.86	0.08	0.75–0.96	5
Phosphorus, mg/dL	5.5	1.3	3.9–7.2	9
Sodium, mequiv./L†	154.2	11.5	135.0–166.0	5
Potassium, mequiv./L†	4.7	0.18	4.5–4.9	4

NOTE: Two-way ANOVA revealed nonsignificant ($p > 0.1$) differences between sex and age groups. WBC, total leukocyte count; HB, hemoglobin; MCV, mean corpuscular volume; EDA, erythrocyte distribution analysis; PLQ, platelet count; PCT, platelet packed volume; GOT, glutamate-oxaloacetate transaminase; GPT, glutamate-pyruvate transaminase; LDH, lactate dehydrogenase; CPK, creatinine phosphokinase.

*Sex–age interaction was statistically ($p < 0.1$) significant.

†Adults only, no date available for juveniles.

In this paper our objectives are (*i*) to establish reference hematological and serum chemical values for a population of Iberian lynx, (*ii*) to evaluate the variation in blood values on the basis of age, sex, and stress of capture, and (*iii*) to propose improvements in blood collection and analysis procedures, to reduce artifacts in results.

Methods

We analyzed 25 blood samples from nine adult (five males and four females) and seven juvenile (four males and three females, less than two winters old) Iberian lynxes. Age-class criteria were body measurements, capture date, and dentition wear (Beltrán 1988).

Lynxes were trapped in the wild as part of a long-term study carried out at Doñana National Park in southwestern Spain, involving population monitoring of several species of sympatric species of carnivores (Delibes and Beltrán 1986). Two trapping procedures were used: double-door box traps with live bait (Karpowitz and Flinders 1981; Litvaitis 1984) and coil-spring foothold traps (Victor No. 2, Woodstream Co., Chicago, Illinois) with padded jaws (Lembeck 1986). The trapline was checked daily, early in the morning.

Captured lynxes were transported to the laboratory in a covered squeeze cage and then anaesthetized with a combination of ketamine hydrochloride (Ketolar, Parke-Davis) and xylazine hydrochloride (Rompum, Bayer) (McCord and Cardoza 1982; Seal 1987). Both products were administered at a dose of 3.75 mg/kg estimated body weight (S. Jonsson, personal communication).

Blood samples (about 0.75 mL/kg body weight; S. Knick, personal communication) were taken from the radial vein 30–40 min after immobilization. Samples were deposited in two tubes: 2.5 mL was put in an EDTA (Tri-K, 5 mg) tube for standard cell counts, and the rest was kept for serum chemistry analysis and blood smears. Samples were refrigerated until analysis, which was usually performed within 24 h.

The blood was analyzed at the Hospital de Valme, using a Coulter S Plus II to determine 18 hematological parameters (Tables 1 and 2): total leukocyte count, (WBC), total erythrocyte count (RBC), hemoglobin (HB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), erythrocyte distribution analysis (EDA), platelet count (PLQ), platelet packed volume (PCT), mean platelet volume (MPV), and platelet distribution analysis (PLQDA). The differential leukocyte count was determined using Giemsa stain. Blood smears were examined with a microscope with immersion objective, to search for intracellular parasites in both red cells (Glenn *et al.* 1983;

TABLE 2. Blood parameters of wild-caught Iberian lynxes with mean values significantly ($p < 0.1$) different between sexes, age-classes (adults and juveniles), and trapping procedures (foothold traps and box traps), tested by ANOVA (two way: age–sex: one-way: trapping method)

	No. of males	No. of females	Males Mean	Males SD	Females Mean	Females SD	Range (total)	p
RBC, $\times 10^{12}$/L	9	10	7.8	1.0	6.8	0.9	5.6–8.8	0.03
PCV, %vol.	9	9	38.7	4.9	34.3	4.1	29.1–43.6	0.06
MCH, pg	6	7	17.3	0.7	18.2	1.4	16.1–21.2	0.07
MCHC, %	9	8	34.1	1.9	36.3	3.5	31.2–42.5	0.07
	No. of adults	**No. of juveniles**	**Adults Mean**	**Adults SD**	**Juveniles Mean**	**Juveniles SD**	**Range (total)**	p
Urea, mg/dL*	8	2	69.2	21.9	115.0	42.4	51.0–145.0	0.02
Creatinine, mg/dL	8	2	2.0	0.5	1.1	0.2	1.0–2.9	0.07
Cholesterol, mg/dL	10	5	127.7	36.4	167.2	23.5	85.0–198.0	0.02
Alkaline phosphatase, IU/L	9	2	53.0	20.7	202.0	16.9	27.8–214.0	0.01
	No. in foothold traps	**No. in box traps**	**Foothold trap Mean**	**Foothold trap SD**	**Box trap Mean**	**Box trap SD**	**Range (total)**	p
MPV, fL	5	2	17.6	2.4	13.1	2.0	11.7–20.6	0.07
PLQDA, fL	4	2	12.9	1.3	10.1	1.1	9.4–14.2	0.07
Amylase, IU/L	6	2	2497.6	235.8	3020.0	296.9	2600–3230	0.04
Calcium, μg/L	6	4	9.9	0.7	10.8	0.4	9.2–11.8	0.06

NOTE: Juvenile Iberian lynxes were all box-trapped. RBC, total erythrocyte count; PCV, packed cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MPV, mean platelet volume; PLQDA, platelet distribution analysis.
*Sex–age interaction is statistically significant.

Blouin *et al.* 1984) and white cells (Lane and Kocan 1983). A Hitachi 705 autoanalyzer (see García-Rodríguez *et al.* 1987) was used to determine 23 serum chemical parameters (Tables 1 and 2).

Statistical analyses were performed with the BMDP package (Dixon *et al.* 1983). We analyzed blood samples from individuals sampled more than once independently because of the time interval between captures (ranging from 30 to 330 days). We used two-way analysis of variance (ANOVA) without repetition to evaluate the variation due to sex and age; the influence of capture procedure was tested with one-way ANOVA. When group variances were different (Levene test), the Welch procedure was applied for comparing means. The threshold significance level for analyses was 0.1 (two-tailed). Means with standard deviation are presented where appropriate, and units are given for the variables.

Results

Hematology

ANOVA revealed statistical differences only between sexes. Males had significantly higher RBC and PCV values than females (Table 2). Female Iberian lynxes had higher MCH and MCHC values than males (Table 2). RBC and body weight were correlated only in adults ($n = 9$, $r = 0.3$, $p = 0.003$), and not when data were pooled with those from juveniles ($n = 18$, $r = 0.05$, $p = 0.83$).

Mean WBC values of up to 20×10^9/L were observed in several individuals of both sexes and age-classes. Two adult males captured some hundred metres apart showed the highest observed WBC values (25.5 and 25.7×10^9/L). Both had injuries that appeared to be several days old on their flanks and face. Neutrophils (86 and 84%) and monocytes (4 and 3%) were also comparatively high in these two males. In two juvenile females, WBC values showed abrupt changes between successive samples. WBC values for blood samples taken during first captures, on November 22 and December 8, were higher (22.3 and 25×10^9/L, respectively) than for samples taken on December 21 and March 3 (10.1 and 9.2×10^9/L, respectively). Their differential white cell count also shifted between capture dates. When first sampled, counts were very close in both individuals (neutrophils, both 93%; lymphocytes, both 5%; monocytes; 2 and 1%; eosinophils, 0 and 1%). When they were recaptured, however, an increase was noted in the percentage of lymphocytes (15 and 12%, respectively) and eosinophils (14 and 2%, respectively, the former being the upper limit of the observed values (Table 1)). The following October, the first individual was captured a third time and a blood sample was taken. WBC was high (18×10^9/L), the neutrophil (81%) and lymphocyte (15%) percentages were elevated, and eosinophils (1%) were present.

Examination of smears ($n = 18$) revealed no intracellular parasitosis (erythrocytes or leukocytes). No statistically significant sex–age interactions were observed, with exception of PLQ ($p = 0.07$; mean for adult males, 333.2 ± 33.2; mean for adult females, 635.6 ± 212.7; juvenile male, 600.0; mean for juvenile females, 460.5 ± 226.1), and MCV ($p = 0.09$; mean for adult males, 49.2 ± 1.6; mean for adult females, 50.75 ± 1.9; mean for juvenile males, 49.7 ± 1.5; mean for juvenile females, 48.3 ± 1.7).

Serum chemistry

Serum chemistry values were similar between sexes, but significant differences in four serum parameters were found

between age-classes. Juveniles showed higher alkaline phosphatase, urea, and cholesterol levels (Table 2) but lower creatinine levels than adults. Though phosphorus levels were lower in juveniles than in adults, the differences did not reach statistical significance ($p = 0.12$). Sex–age interaction was significant for triglyceride concentration ($p = 0.04$; means: adult males, 39.7 ± 15.0; adult females, 24.0 ± 3.6; juvenile males, 47.0 ± 9.8; juvenile females; 143.0 ± 137.0) and urea concentration ($p = 0.05$; mean for adult males, 76.4 ± 25.8; mean for adult females; 57.33 ± 2.08; juvenile male, 85; juvenile female, 145).

The range observed in creatinine phosphokinase levels is remarkable. The lowest value (109 IU/L) belongs to an adult male, recaptured and sampled twice in 30 days (Table 1). In the first sample, creatinine phosphokinase reached 22080 IU/L. The second sample was used as a control, being from the animal kept for the night following capture in an undisturbed room, so that it was relaxed before anaesthesia and blood sampling. The glucose levels were 175 mg/dL for the first sample and 94 mg/dL for the second; glutamate-oxaloacetic transaminase levels also decreased from 239 to 25 IU/L. Slight changes were observed in glutamate-pyruvate transaminase levels (78 and 66 IU/L, respectively).

Capture method

Juveniles were all captured in box traps, so only data from adults (six males and four females) were used in this analysis. Four blood parameters (platelet volume; platelet size distribution, and amylase and calcium concentrations) appeared statistically different in both groups (Table 2); although, mean WBC and MCV values were higher in coil-spring-trapped (19.9 ± 6.2 × 10^9/L and 50.5 ± 1.5 fL, respectively) than in box-trapped lynxes (13.5 ± 5.3 × 10^9/L, and 48.9 ± 1.3 fL, respectively), they did not reach ($p = 0.13$ and 0.19) the level of significance. This trend is found in differential leukocyte count, neutrophils in particular, for which absolute values were greater in coil-spring-trapped (16.9 ± 6.0 × 10^9/L) than in box-trapped lynxes (10.2 ± 4.3 × 10^9/L) ($p = 0.13$). Immature neutrophils (294 ± 461 × 10^9/L, i.e., 1.7 ± 2.9%, range 0–7%)) were observed only in the coil-spring-trapped group; eosinophils appeared in the blood of a box-trapped adult female. In box-trapped lynxes, RBC and PCV were negatively correlated with MCH ($r = -0.91$, $p = 0.002$; $r = -0.88$, $p = 0.006$, respectively, both $n = 7$) and MCHC ($r = -0.68$, $p = 0.01$; $r = -0.67$, $p = 0.02$, both $n = 11$ respectively.) In the foothold-trapped sample, however, correlations were positive but statistically not significant. In both groups, no correlations were found between RBC and MCV, or between PCV and MCV.

Lynxes captured in coil-spring traps showed a temporary swelling of the digits caught in the trap jaws. In box-trapped adults we observed only slight injuries on the head and forelegs, caused while trying to escape from the trap.

Discussion

Hematology

Although the hematological values from Iberian lynxes that we registered (Table 1) are within the range of values reported for other felids, we detected some particularities regarding RBC and WBC and some related variables. Mean RBC values for Iberian lynx are similar to those for a number of felid species (6.1–10.25 × 10^9/L; Currier and Russell 1982; Fuller *et al.* 1985; Kocan *et al.* 1985; Hawkey and Hart 1986; Bush *et al.* 1987; Caro *et al.* 1987; Seal *et al.* 1987). However, sexual differences in RBC values have not been previously reported among Felidae, but have been described in other mammalian species that show sexual dimorphism (Guyton 1981); male Iberian lynxes are 25% heavier than adult females (Beltrán 1988). In grizzly bears, Brannon (1985*a*) found differences in RBC values between the sexes in one of his study areas, and a significant correlation between RBC and body weight in the other. Our data support both findings.

Besides providing a biological basis for higher RBC and PCV values in species dimorphic for size, there are other, nonexclusive explanations for this finding. First, Iberian lynx males may have been more stressed during capture than females. Mean cell volume should then have been higher in samples from males because of immature erythrocytes, which would have been mobilized by higher MCV and MCHC (Guyton 1981; Brannon 1985*a*). However, this does not seem to be the case, as MCV was similar in both sexes, and no significant correlation between RBC and MCV was observed. Second, males may have shown a different response to the effects of anaesthesia (Berrie 1972; Brannon 1985*a*); Seal *et al.* (1987) reported increased PCV, among four other blood parameters, as a secondary effect of xylazine, although at a dose three times greater than the one we used. At present we cannot evaluate the importance of this explanation. Third, the males may have been in better condition than the females in our study area during the sample period. For example, in fasted gray wolves, Delgiudice *et al.* (1987) pointed out that HB and PCV levels were lower in females; Caro *et al.* (1989) reported health differences between resident and transient male cheetahs. Blood sampling of Iberian lynxes was done in autumn and winter, when rabbit availability was lowest (Beltrán 1988); moreover, female Iberian lynxes, if nonresident, may have found preying on other species more difficult than males did, because of their smaller body size (Beltrán *et al.* 1985; Litvaitis *et al.* 1984). On the other hand, Kocan *et al.* (1985) found a higher RBC, although not statistically significant, in bobcats infected with intraerythrocytic piroplasms than in healthy bobcats. Although no intracellular parasites were detected, Iberian lynxes at Doñana are severely infected by ectoparasites (ticks and flies; J. F. Beltrán *et al.*, unpublished observations), but we have no data on sex differences in the level of ectoparasitism. Finally, it is relevant to note that Hawkey *et al.* (1980) found statistical variation in some hematological parameters, including RBC and PCV, depending on the time elapsed between anaesthesia and blood sampling. When serial blood sampling becomes impractical, these authors recommend standardizing the time interval as far as possible. In our study, the influence of this factor is difficult to assess, but it is probably randomly distributed among the blood samples.

Interpretation of the higher MCH and MCHC values observed in Iberian lynx females than in males is controversial. They could be attributed to a compensating mechanism for lower RBC and PCV, permitting maintenance of the hemoglobin content (Brannon 1985*a*). However, results of correlation analyses also suggest some influence of trapping method on these parameters (see below). Although Hawkey *et al.* (1980) found no variation in MCH, MCHC, and MCV during serial blood sampling, Brannon (1985*a*), analyzing two blood samples 1 h apart, found that MCH, MCHC, and MCV decreased during this interval.

The mean volume of red cells in Iberian lynx is similar to that observed in bobcats by Fuller *et al.* (1985) (49.35 ± 10.49 fL). Mean MCV values in Iberian lynx support the observation by Hawkey and Hart (1986) that there is a direct relationship

between red cell volume and average body weight of the species. As expected, these values are lower, in general, than those reported for larger felids (cheetah, 51.2–60.6 (Caro *et al.* 1987); tiger, from 53.3 (Bush *et al.* 1987) to 56.9 (Seal *et al.* 1987).

The mean WBC value observed in this study is comparatively higher than previously reported in wild felids (6.4–15.8 $\times$ 10^9/L; Currier and Russell 1982; Fuller *et al.* 1985; Kocan *et al.* 1985; Hawkey and Hart 1986; Bush *et al.* 1987; Caro *et al.* 1987; Seal *et al.* 1987). Nevertheless, Smith and Rongstad (1980) observed values in wild coyotes (*Canis latrans*) ranging from 15.5 to 24.0 $\times$ 10^9/L, close to those we found in this study. Elevated WBC should be expected as a response to infections; in addition, stress and strenuous exercise could also remove the white cells attached to the vein walls during high blood flow (Guyton 1981; Smith and Rongstad 1980; see below for influence of capture method). Eosinophilia is known to be a response to parasitic diseases (Guyton 1981; Kocan *et al.* 1985; Hawkey and Hart 1986; Caro *et al.* 1987). Juveniles attaining independence, low availability of food, bad weather, the rutting season, and changes in social status (Beltrán 1988) are all likely to increase the probability of Iberian lynxes acquiring infectious diseases during winter, and therefore to affect WBC and differential count. Further research should include the collection of more precise data, to evaluate the impact of such diseases on remaining Iberian lynx populations.

Serum chemistry

The serum values we observed were in the range previously reported for other wild felids (Fuller *et al.* 1985; Caro *et al.* 1987; Hawkey and Hart 1986). Alkaline phosphatase concentrations are known to be associated with age, i.e., linked to bone development, in raptors (Mauro 1987; M. Ferrer, personal communication), ungulates (Blakenship and Varner 1982), and carnivores (Seal *et al.* 1975; Smith and Rongstad 1980; Bentinck-Smith 1983; Brannon 1985*b*). The two latter authors also include phosphorus and calcium levels as chemical parameters affected by age. Creatinine concentration and the albumin/globulin ratio have been associated with age and body weight in some carnivores, in correlation with muscle mass (see Brannon 1985*b*).

Capture procedure

Although neither capture method causes permanent injuries, coil-spring trapping is presumably more stressful for the captured animal than box trapping, therefore a difference in blood values could be expected between coil-spring-trapped and box-trapped individuals. Nevertheless, it is known that capture success with the latter method is influenced by such factors as food availability and individual behaviour (depending on age, social status, nutritional status, health, etc.); for example, a young or hungry lynx could be vulnerable to box trapping, while a resident adult male lynx would be very difficult to capture with this procedure. We cannot evaluate the bias introduced by these factors in our results.

Several findings suggest a higher stress level in coil-spring-trapped lynxes than in box-trapped ones. Although not statistically significant, mean MCV and WBC values were higher in coil-spring-trapped lynxes than in box-trapped individuals. Karns and Crichton (1978) reported changes in MCV associated with handling or stress; Caro *et al.* (1987) also found macrocytic red cells in free-living female cheetahs but not in captive ones. A higher MCV in the coil-spring-trapped sample could be a physiological response to higher exercise and stress levels, permitting more oxygen to be carried, through the incorporation into the blood of immature erythrocytes (Guyton 1981), which have greater cell volume. Similarly, immature white cells also appeared in the coil-spring-trapped sample. Besides the contribution of the marginal pool, tissue damage and inflammation response may cause changes in the differential count. Monocytes are the first to respond to such perturbations (within 1 h), followed by neutrophils (within 6–12 h) (Guyton 1981). Smith and Rongstad (1980) considered that the high WBC they registered in wild coyotes compared with pen-raised individuals represented "stress neutrophilia" (Hawkey and Hart 1986; Kreeger *et al.* 1990); our samples confirm these observations. Differences in MPV and PLQDA between trapping methods would reflect a similar delayed mechanism of response to tissue damage.

The differences in serum chemistry parameters between trapping procedures did not show a clear trend; stress and nutritional condition probably influence the observed values in a complex way. However, they also suggest that box trapping would be less stressful for free-ranging carnivores than using coil-spring traps with padded jaws. It could be expected, therefore, that using coil-spring traps without padding would increase these effects, and Kreeger *et al.* (1990) have confirmed it.

Acknowledgements

We are grateful to Inés Camacho and Rocío Pérez-Ayala for their help and support during the early phase of this study. Pedro García analysed smears for intracellular parasites. We thank the staff at the Department of Hematology, the Laboratory of Biochemistry of the Hospital de Valme, and the Doñana Biological Station for their cooperation and help. Tim Caro, Steven Knick, and two anonymous reviewers made suggestions that improved successive versions of the manuscript. The senior author held predoctoral fellowships from the Consejo Superior de Investigaciones Científicas (CSIC) and Junta de Andalucía, and a postdoctoral fellowship from CSIC. This research was supported by CSIC, the Comisión Asesora de Investigación Científica y Técnica, and the Dirección General de Investigación Científica y Técnica (project PB87-0405). The Asociación para Defensa de la Naturaleza (ADENA–World Wildlife Fund) made a valuable contribution to this study.

ALDAMA, J., and DELIBES, M. 1990. Some preliminary results about the energy utilization from a rabbit diet by the Spanish lynx. Doñana Acta Vertebr. **17**: 116–121.

BELTRÁN, J. F. 1987. Base bibliográfica de especies amanazadas: el lince ibérico. Agencia de Medio Ambiente-Juanta de Adalucía, Sevilla.

——— 1988. Ecología y conducta espacio-temporal del lince ibérico (*Lynx pardina* Temminck, 1824) en el Parque Nacional de Doñana. (English summary.) Ph.D. thesis, University of Sevilla, Spain.

BELTRÁN, J. F., ALDAMA, J., and DELIBES, M. 1991. Ecology of the Iberian lynx in Doñana National Park. *In* Transactions of the 18th Congress of the International Union of Game Biologists, Kraków, Poland, August 1987. In press.

BELTRÁN, J. F., SAN JOSÉ, C., DELIBES, M., and BRAZA, F. 1985. An analysis of the Iberian lynx predation upon Fallow deer in the Coto Doñana, SW Spain. *In* Transactions of the 17th Congress of the Union of Game Biologists, Brussels, September 1985. *Edited by* S. A. de Crombrugghe. Ministry of Agriculture of Belgium, Brussels. pp. 961–967.

BENTINCK-SMITH, J. 1983. A roster of normal values for dogs and cats. *In* Current veterinary therapy. VIII. Small animal practice. *Edited by* R. W. Kirk. W. B. Saunders Co., Philadelphia. pp. 1206–1215.

BERRIE, P. M. 1972. Sex differences in response to phencyclidine hydrochloride in lynx. J. Wildl. Manage. **36**: 994–996.

BLAKENSHIP, L. H., and VARNER, L. W. 1982. Blood parameters as indicators of deer and habitat condition. *In* Transactions of the 14th Congress of the International Union of Game Biologists, Dublin, October 1979. *Edited by* F. O'Gorman and J. Rochford. Irish Wildlife Publications, Dublin. pp. 475–488.

BLOUIN, E. F., KOCAN, A. A., GLENN, B. L., KOCAN, K. M., and HAIR, J. A. 1984. Transmission of *Cytauxzoon felis* Kier, 1979 from bobcats, *Felis rufus* (Schreber), to domestic cats by *Dermacentor variabilis* (Say). J. Wildl. Dis. **20**: 241–242.

BRANNON, R. D. 1985*a*. Hematological characteristics of grizzly bears (*Ursus arctos*) in central and northeastern Alaska. Can. J. Zool. **63**: 58–62.

——— 1985*b*. Serum chemistry of central and northern Alaska grizzly bears. J. Wildl. Manage. **49**: 893–900.

BUSH, M., PHILLIPS, L. G., and MONTALI, R. J. 1987. Clinical management of captive tigers. *In* Tigers of the world. *Edited by* R. L. Tilson and U. S. Seal. Noyes Publications, Park Ridge, NJ. pp. 171–204.

CARO, T. M., HOLT, M. E., FITZGIBBON, C. D., BUSH, M., HAWKEY, C. M., and KOCK, R. A. 1987. Health of adult free-living cheetahs. J. Zool. (Lond.), **212**: 573–584.

CARO, T. M., FITZGIBBON, C. D., and HOLT, M. E. 1989. Physiological costs of behavioural strategies for male cheetahs. Anim. Behav. **38**: 309–317.

CURRIER, M. J. P., and RUSSELL, K. R. 1982. Hematology and blood chemistry of the mountain lion (*Felis concolor*). J. Wildl. Dis. **18**: 99–104.

DELGIUDICE, G. D., SEAL, U. S., and MECH, L. D. 1987. Effects of feeding and fasting on wolf blood and urine characteristics. J. Wildl. Manage. **51**: 1–10.

DELIBES, M. 1980. Feeding ecology of the Spanish lynx in the Coto Doñana. Acta Theriol. **25**: 309–324.

DELIBES, M., and BELTRÁN, J. F. 1986. Radio-tracking of six species of carnivores in the Doñana National Park, SW Spain. Mesogée, **46**: 113–120.

DIXON, W. J., BROWN, M. J., ENGELMAN, L., FRANA, J. W., HILL, M. A., JENNRICH, R. I., and TOPOREK, J. D. 1983. BMDP statistical software. University of California Press, Berkeley.

FOWLER, M. E. 1986. Zoo and wild animal medicine. W. B. Saunders Co, Philadelphia.

FRANZMANN, A. W., and LERESCHE, R. E. 1978. Alaskan moose blood studies with emphasis on condition evaluation. J. Wildl. Manage. **42**: 334–351.

FRANZMANN, A. W., and SCHWARTZ, C. C. 1988. Evaluating condition of Alaska black bears with blood profiles. J. Wildl. Manage. **52**: 63–70.

FULLER, T. K., KERR, K. D., and KARNS, P. D. 1985. Hematology and serum chemistry of bobcats in north central Minnesota. J. Wildl. Dis. **21**: 29–32.

GARCIA-RODRIGUEZ, T., FERRER, M., RECIO, F., and CASTROVIEJO, J. 1987. Circadian rhythms of determined blood chemistry values in buzzards and eagle owls. Comp. Biochem. Physiol. A, **88**: 663–669.

GLENN, B. L., KOCAN, A. A., and BLOUIN, E. F. 1983. Cytauxzoonosis in bobcats. J. Am. Vet. Med. Assoc. **183**: 1155–1158.

GUYTON, A. C. 1981. Textbook of medical physiology. W. B. Saunders Co, Philadelphia.

HAWKEY, C., and HART, M. G. 1986. Haematological reference values for adult pumas, lions, tigers, leopards, jaguars and cheetahs. Res. Vet. Sci. **41**: 268–269.

HAWKEY, C., FRANKEL, T., JONES, D., ASHTON, D., NEVILL, G., HART, M., ALDERSON, C., and BIRCHER, P. 1980. Preliminary report of a study of changes in red blood cells of zoo animals during sedation. *In* The comparative pathology of zoo animals. *Edited by* R. J. Montali and G. Migaki. Smithsonian Institution Press, Washington, DC. pp. 625–632.

HONACKI, J. M., KINMAN, K. E., and KOEPPL, J. W. 1982. Mammals species of the world: a taxonomic and geographic reference. Allen Press and The Association of Systematic Collections, Lawrence, KS.

INTERNATIONAL UNION FOR CONSERVATION OF NATURE AND NATURAL RESOURCES. 1986. IUCN red list of threatened animals. International Union for Conservation of Nature and Natural Resources, Gland, Switzerland.

KARNS, P. D., and CHRICHTON, F. J. 1978. Effects of handling and physical restraint on blood parameters in woodland caribou. J. Wildl. Manage. **42**: 904–908

KARPOWITZ, J. F., and FLINDERS, J. T. 1981. Bobcat research in Utah: a progress report. *In* Proceedings of a Conference on Bobcat Research. *Edited by* L. Giroux Blum and P. C. Escherich. Sci. Tech. Ser. 6. National Wildlife Federation, Washington, DC. pp. 70–73.

KOCAN, A. A., BLOUIN, E. F., and GLENN, B. L. 1985. Hematologic and serum chemical values for free-ranging bobcats, *Felis rufus* (Schreber), with natural infections of *Cytauxzoon felis* Kier, 1979. J. Wildl. Dis. **21**: 190–192.

KREEGER, T. J., WHITE, P. J. SEAL, U. S., and TESTER, J. R. 1990. Pathological responses of red foxes to foothold traps. J. Wildl. Manage. **54**: 147–160.

LANE, J. R., and KOCAN, A. A. 1983. *Hepatozoon* sp. infection in bobcats. J. Am. Vet. Med. Assoc. **183**: 1323–1324.

LEMBECK, M. 1986. Long term behaviour and population dynamics of an unharvested bobcat population in San Diego County. *In* Cats of the world: biology, conservation and management. *Edited by* S. D. Miller and D. D. Everett. National Wildlife Federation, Washington, DC. pp. 305–310.

LITVAITIS, J. A. 1984. Bobcat movement in relation to prey density. Ph.D. thesis, University of Maine at Orono.

LITVAITIS, J. A., STEVENS, C. L., and MAUTZ, W. W. 1984. Age, sex and weight of bobcats in relation to winter diet. J. Wildl. Manage. **48**: 632–635.

MAURO, L. 1987. Hematology and blood chemistry. *In* Raptor management techniques manual. *Edited by* B. A. Giron, B. A. Millsap, K. W. Cline, and D. M. Bird. Sci. Tech. Ser. 10. National Wildlife Federation, Washington, DC. pp. 269–276.

MCCORD, C. M., and CARDOZA, J. E. 1982. Bobcat and lynx. *In* Wild mammals of North America. *Edited by* J. A. Chapman and G. A. Feldhamer. The Johns Hopkins University Press, Baltimore. pp. 728–766.

MECH, L. D. 1980. Making the most of radio-tracking: a summary of wolf studies in northeastern Minnesota. *In* A handbook on biotelementary and radio tracking. *Edited by* C. J. Amlaner and D. W. Macdonald. Pergamon Press, London. pp. 85–95.

NEWMAN, A., BUSH, M., WILDT, D. E., VAN DAM D., FRANKENHUIS, M. T., SIMMONS, L., PHILLIPS, L., and O'BRIEN, S. J. 1985. Biochemical genetic variation in eight endangered or threatened felid species. J. Mammal. **66**: 256–267.

RODRIGUEZ, A., and DELIBES, M. 1990. Distribución y problemas de conservación del Lince Ibérico en España. Ministerio de Agricultura, Pesca y Alimentación, Madrid, Spain.

SEAL, U. S. 1978. Assessment of habitat condition by measurement of biochemical and endocrine indicators of the nutritional, reproductive, and disease status of free-ranging animal populations. *In* Proceedings of a Symposium on Classification, Inventory, and Analysis of Fish and Wildlife Habitat, Phoenix, Arizona, 1977. U.S. Fish and Wildlife Service, Washington, DC. pp. 305–329.

——— 1987. Chemical immobilization of furbearers. *In* Wild furbearer management and conservation in North America. *Edited by* M. Novak, J. A. Baker, M. E. Obbard, and B. Malloch. Ontario Ministry of Natural Resources, Toronto. pp. 191–215.

SEAL, U. S., and HOSKINSON, R. L. 1978. Metabolic indicators of habitat condition and capture stress in pronghorns. J. Wildl. Manage. **42**: 755–763.

SEAL, U. S., MECH, L. D., and BALLENBEGHE, V. V. 1975. Blood analyses of wolf pups and their ecological and metabolic interpretation. J. Mammal. **56**: 64–75.

SEAL, U. S., ARMSTRONG, D. L., and SIMMONS, L. G. 1987. Yohimbine hydrochloride reversal of ketamine hydrochloride and xylazine hydrochloride immobilization of bengal tigers and effects on hematology and serum chemistries. J. Wildl. Dis. **23**: 296–300.

SMITH, G. J., and RONGSTAD, D. J. 1980. Serological an hematological values of wild coyotes in Wisconsin. J.Wildl. Dis. **16**: 491–497.

TUMLISON, R. 1987. *Felis lynx*. Mamm. Species No. 269. pp. 1–8.

WERDELIN, L. 1981. The evolution of lynxes. Ann. Zool. Fenn. **18**: 37–71.

——— 1987. Supernumerary teeth in *Lynx lynx* and the irreversibility of evolution. J. Zool. (Lond.), **211**: 259–266.

WINTROBE, M. M., LEE, G. R., BOGGS, D. R., BITHELL, T. C., FOERSTER, J., ATHENS, J. W., and LUKENS, J. N. 1981. Clinical hematology. Lea and Febiger, Philadelphia.