

## Hematology and Serum Chemistry of the Island Spotted Skunk on Santa Cruz Island

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**ABSTRACT:** We determined serum biochemistry and hematologic values for island spotted skunks (*Spilogale gracilis amphiala*) on Santa Cruz Island (California, USA). Samples were collected from island spotted skunks chemically restrained with ketamine hydrochloride and acepromazine in August 1999 (dry season) and from skunks manually restrained in August 2000 (dry season) and January 2001 (wet season). One parameter, glucose, significantly differed with season, with higher levels during the wet season. Serum chemistry and hematologic profiles suggest that method of restraint (manual or chemical), as well as other methodologic details, may influence blood characteristics in the island spotted skunk.

**Key words:** Anesthesia, blood, hematology, island spotted skunk, ketamine, serum chemistry, *Spilogale gracilis amphiala*.

The island spotted skunk (*Spilogale gracilis amphiala*), a subspecies of the western spotted skunk, is an insular endemic carnivore that occurs on Santa Cruz and Santa Rosa Islands, the two largest of the eight California Channel Islands (USA). Due to small populations and limited distribution, the island spotted skunk is listed as a subspecies of special concern by the State of California. Prior to our research on spotted skunks on Santa Cruz Island (Crooks, 1994a, b; Crooks and Van Vuren, 1994, 1995, 2000), the status and ecology of the island spotted skunk were unknown. We found that skunks were relatively rare, restricted in distribution, specialized in their resource use, and particularly sensitive to environmental perturbations. Consequently, we suggested that the continued existence of island spotted skunks was precarious and recommended further monitoring of their populations (Crooks, 1994a; Crooks and Van Vuren 1994, 2000).

Blood analyses can be used to evaluate wildlife health and can serve as indicators of nutrition, disease, trauma, habitat quality, and other environmental stressors (Franzmann, 1972; Seal et al., 1975; Brannon, 1985; McCue and O'Farrell, 1987; Beltran et al., 1991; Crooks et al., 2000). Basic serum chemistry and hematologic characteristics for the island spotted skunk have not been previously reported. The only values available for spotted skunks are from six laboratory-confined eastern spotted skunks (*S. putorius interrupta*) wild-caught in Arkansas (Heidt and Hargraves, 1974); the relevance of these values to free-ranging skunks, however, is uncertain. The objectives of this study were to determine serum biochemistry and hematologic values for island spotted skunks on Santa Cruz Island. We present results from the wet and dry season, and from skunks handled by two commonly used methods, chemical or manual restraint.

Santa Cruz Island (34°0'N, 119°45'W) is located 40 km south of Santa Barbara, California. The climate is maritime and Mediterranean with a pronounced dry season (June–November) and wet season (December–April). In August 1999, August 2000, and January 2001, we sampled a total of 36 island spotted skunks throughout the central and western portion of the island. Skunks were live-trapped in single-door box-traps baited with fruit-paste baits (Nick Wyshinski, Berwick, Pennsylvania, USA) and commercial canned and dry cat food. Animals were temporarily marked to allow individual identification within sampling periods; trap location, sex, weight, and other distinguishing characteristics al-

lowed individual identification among sampling periods. Based on date of capture, typical late spring litter production (Howard and Marsh, 1982), and typical age-specific weights (Crabb, 1994; Crooks, 1994a), captured skunks were likely young adults or adults. All captured skunks appeared healthy based on a brief physical exam.

In August 1999, to facilitate processing, captured skunks were chemically restrained with an intramuscular injection of a combination of a general anesthetic (28 mg/kg ketamine hydrochloride) and a sedative (0.3mg/kg acepromazine) to smooth induction and recovery of a Stage III, Plane II anesthesia (Kreeger, 1996). Skunks were generally under anesthesia no longer than 30 min, after which time they were monitored until fully recovered and released. In August 2000 and January 2001, captured skunks were manually restrained during processing without the use of anesthesia. Manually restrained skunks were also processed within 30 min.

Blood samples (1–6 ml) were collected from the jugular vein of captured skunks. For hematologic analyses of August 1999 samples, 3 ml of blood were collected into sterile evacuated glass tubes (Vacutainer, Becton-Dickinson, Rutherford, New Jersey, USA) containing the anticoagulant ethylenediaminetetraacetic acid (EDTA); 2 ml were later withdrawn for other analyses and the remaining 1 ml was used for hematology. For hematologic analyses of August 2000 and January 2001 samples, blood was transferred immediately to sterile 1 ml EDTA tubes. For preparation of serum, blood was transferred immediately to sterile blood collection tubes containing no additive, allowed to clot for a minimum of 2 hr, and then centrifuged at 2,500 (August 1999) or 3,000 (August 2000 and January 2001) rpm for up to 10 min. The same day as centrifugation, serum was extracted from clotted samples with Pasteur pipettes and placed into 1.5 ml Ependorf vials.

Samples were maintained at refrigerat-

ed temperatures (ca. 4–6 C) until analyses were conducted 1–4 days following collection. August 1999 samples were analyzed by Antech Diagnostics (Irvine, California), and August 2000 and January 2001 samples were analyzed by Idexx Veterinary Services (West Sacramento, California). Hematologic analyses were conducted on EDTA blood by use of an Abbott Cell-Dyn 3500 analyzer (Abbott Laboratories, Abbott Park, Illinois, USA) and serum biochemical analyses were analyzed on a Hitachi 747-200 analyzer (Roche Diagnostics, Indianapolis, Indiana, USA). Blood smears were made at the time of analysis (August 1999) or at the time of collection (August 2000 and January 2001). Leukocyte differential counts were conducted manually on 100 cells.

Blood samples were analyzed for the following hematologic parameters: white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts. Serum samples were analyzed for albumin: globulin concentration ratio (August 2000 and January 2001 samples only), albumin, alkaline phosphatase (ALP, August 1999 samples only), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium, chloride (August 1999 samples only), cholesterol, creatine kinase (CK), creatinine, globulin, glucose, phosphorus, potassium, sodium, total bilirubin, and total protein concentrations.

We calculated mean, standard errors, and standard deviations for each blood characteristic. We then conducted Student's *t*-tests to compare dry (August 2000) and wet (January 2001) season values for manually restrained skunks; all values were log-transformed and treated as parametric for statistical analyses. Sequential Bonferroni tests were conducted to adjust for inflated Type-1 error rates (Rice, 1989);

TABLE 1. Serum chemistry and hematologic profiles of island spotted skunks anesthetized with ketamine and acepromazine in August 1999 (dry season) and manually-restrained in August 2000 (dry season) and January 2001 (wet season) on Santa Cruz Island (California, USA). For comparison, available mean and ranges from laboratory-confined eastern spotted skunks anesthetized with ethyl ether are also provided (Heidt and Hargraves 1974).

	Chemical restraint			
	Dry season (August 1999)			
	Mean	SE <sup>a</sup>	SD <sup>b</sup>	n <sup>c</sup>
<b>Serum Chemistry</b>				
Albumin:globulin ratio				0
Albumin (g/dl)	2.63	0.03	0.09	12
ALP (IU/L)	13.25	1.61	5.56	12
ALT (IU/L)	121.08	7.41	25.69	12
AST (IU/L)	333.00	38.74	134.19	12
BUN (mg/dl)	25.08	1.28	4.44	12
Calcium (mg/dl)	9.43	0.12	0.43	12
Chloride (mEq/L)	109.92	0.65	2.23	12
Cholesterol (mg/dl)	169.58	7.79	26.98	12
CK (IU/L)	4883.42	1730.52	5994.71	12
Creatinine (mg/dl)	0.24	0.03	0.12	12
Globulin (g/dl)	5.00	0.12	0.40	12
Glucose (mg/dl)	64.75	7.66	26.53	12
Phosphorus (mg/dl)	3.46	0.23	0.81	12
Potassium (mEq/L)	4.18	0.09	0.31	12
Sodium (mEq/L)	146.75	0.84	2.90	12
Total bilirubin (mg/dl)	0.11	0.01	0.03	12
Total protein (g/dl)	7.63	0.12	0.41	12
<b>Hematology</b>				
HCT (%)	33.40	1.45	4.58	10
Hb (g/dl)	10.40	0.27	0.85	10
MCH (pg)	13.87	0.18	0.56	10
MCHC (g/dl)	31.50	0.90	2.84	10
MCV (fl)	44.20	1.44	4.57	10
RBC (10 <sup>6</sup> /μl)	7.54	0.25	0.78	10
WBC (10 <sup>3</sup> /μl)	8.54	0.66	2.10	10
Neutrophil (%)	58.22	4.10	12.29	9
Lymphocytes (%)	33.33	3.81	11.42	9
Monocytes (%)	2.38	0.53	1.51	8
Eosinophil (%)	5.33	1.13	3.39	9
Basophil (%)	1.00			1
Absolute neutrophil segment (per μl)	5041.78	616.67	1850.02	9
Absolute lymphocyte (per μl)	2733.11	317.19	951.57	9
Absolute monocyte (per μl)	185.38	39.88	112.79	8
Absolute eosinophil (per μl)	484.67	139.97	419.90	9
Absolute basophil (per μl)	89.00			1

<sup>a</sup> Standard error.

<sup>b</sup> Standard deviation.

<sup>c</sup> Sample size for each parameter estimate. Agglutination and cell degeneration resulted in omission of some blood samples for hematological analyses, reflected in lower sample sizes.  $n=0$  indicates no parameter estimates available. SE or SD were not calculated for  $n=1$ .

Bonferonni-corrected  $P<0.05$  were considered statistically significant throughout. Due to low or missing samples, statistical comparisons were not conducted for ALP,

chloride, basophil (%), or absolute basophil counts. Only seven of 36 captured skunks were females, and preliminary analyses revealed few differences in pa-

TABLE 1. Extended.

Manual restraint								Eastern spotted skunk Heidt and Hargraves (1974)			
Dry season (August 2000)				Wet season (January 2001)				Mean	Min	Max	n
Mean	SE	SD	n	Mean	SE	SD	n				
0.82	0.03	0.08	6	1.22	0.11	0.48	18				
4.18	0.18	0.43	6	4.79	0.23	0.99	18	1.37	1.09	1.61	6
			0				0				
215.67	106.24	260.23	6	178.72	27.26	115.67	18				
600.17	120.79	295.87	6	611.06	43.34	183.88	18				
42.83	4.98	12.21	6	29.67	2.06	8.76	18	18.50	15.60	20.00	6
5.65	0.47	1.16	6	6.42	0.31	1.30	18	10.40	9.50	10.70	6
			0				0	109.00	101.10	115.20	6
205.17	9.34	22.87	6	210.00	6.81	28.90	18	180.17	163.00	221.00	6
8150.17	5699.79	13961.59	6	3058.06	783.96	3326.06	18				
0.97	0.07	0.16	6	0.77	0.03	0.14	18				
5.15	0.19	0.45	6	4.22	0.20	0.86	18	6.21	5.98	6.49	6
58.83	14.78	36.20	6	135.89	14.46	61.35	18	190.20	127.00	247.00	6
8.07	0.44	1.07	6	6.64	0.35	1.48	18	6.09	4.20	6.90	6
7.75	0.28	0.69	6	7.12	0.25	1.05	18	5.80	5.10	7.10	6
144.17	0.98	2.40	6	145.06	1.66	7.02	18	146.32	143.50	148.50	6
0.27	0.05	0.12	6	0.11	0.03	0.12	18	0.08	0.00	0.12	6
9.33	0.32	0.79	6	9.02	0.20	0.84	18				
46.15	0.93	2.28	6	43.76	0.95	3.70	15	45.07	43.50	47.50	6
14.15	0.40	0.99	6	13.39	0.28	1.07	15	15.15	14.20	15.90	6
13.87	0.15	0.37	6	13.27	0.15	0.58	15				
30.62	0.46	1.13	6	30.63	0.24	0.95	15				
45.50	0.76	1.87	6	43.40	0.50	1.92	15				
10.20	0.32	0.78	6	10.14	0.27	1.05	15	8.93	7.46	11.22	6
8.52	1.16	2.84	6	8.82	0.63	2.44	15	10.03	8.57	11.95	6
69.50	10.77	26.38	6	63.72	4.11	17.43	18				
22.17	8.85	21.68	6	27.94	3.56	15.11	18				
3.67	1.02	2.50	6	3.06	0.47	1.95	17				
5.20	2.20	4.92	5	5.35	0.80	3.32	17				
1.00	0.00	0.00	2	1.40	0.40	0.89	5				
5788.00	1232.73	3019.55	6	5824.27	554.24	2146.57	15				
1874.33	704.95	1726.78	6	2245.13	370.34	1434.30	15				
311.67	87.56	214.47	6	272.36	53.29	199.40	14				
414.20	168.92	377.71	5	493.57	90.10	337.14	14				
92.50	22.50	31.82	2	134.00	51.08	102.17	4				

parameter values between sexes, so results from males and females were pooled for analyses. No statistical comparisons were conducted between chemically and manually restrained skunks because other methodological differences between the August 1999 and the August 2000 and January 2001 samples, such as timing of blood

analyses and procedures at different laboratories, may have acted as confounding factors that influenced results.

Table 1 provides hematologic and serum chemistry profiles for skunks in August 1999 ( $n=11$  males and 1 female), in August 2000 ( $n=3$  males and 3 females), and in January 2001 ( $n=15$  males and 3 fe-

males). When comparing dry season (August 2000) and wet season (January 2001) samples for manually restrained skunks, only glucose concentrations significantly differed between seasons after sequential Bonferonni correction; glucose concentrations were significantly higher in the wet season samples ( $t=3.91$ ,  $P=0.024$ ). Non-significant seasonal trends were evident with other variables; for example, albumin:globulin ratio, BUN, creatinine, globulin, and total bilirubin concentrations tended to be higher during the dry season (Table 1). Other studies have also recorded seasonal variations in blood characteristics (Seal and Mech, 1983; Fuller et al., 1985; McCue and O'Farrell, 1987, 1992; Rietkerk et al., 1994; Crooks et al., 2000).

Blood parameters are dynamic values that can vary in complex ways with a variety of factors, including season, sex, age, nutritional state, stress, capture method, and anesthesia (Seal et al., 1972; Wesson et al., 1979; Hawkey et al., 1980; Beltran et al., 1991; Rietkerk et al., 1994). The profiles presented in Table 1 suggest that method of restraint may be one factor influencing serum chemistry and hematologic values in the island spotted skunk. For example, several serum chemistry (e.g., albumin, BUN, creatinine, phosphorus, potassium, total protein concentrations) and hematologic (e.g., RBC, Hb, and HCT) parameters tended to be lower in samples from skunks anesthetized with ketamine hydrochloride and acepromazine (Table 1). In addition to restraint method, however, other differences in methodology between August 1999 and August 2000/January 2001 samples may have altered blood characteristics and thus limit possible inferences about the actual effect of restraint method on serum chemistry and hematologic values.

Although drug effects on serum enzymes are generally poorly defined (Hellgren et al., 1985), several studies reported lower serum chemistry values in chemically restrained animals. For example, through serial sampling of anesthetized ze-

bras (*Equus zebra hartmannae*) and Przewalski horses (*Equus przewalski przewalski*), Kuttner and Wiesner (1987) found that chemistry parameters showed a clear (potassium, calcium, phosphorus, total protein, ALP) to slight (bilirubin, creatinine, chloride, sodium, CK) tendency to decrease during anesthesia. Likewise, Hellgren et al. (1985) found initial declines in serum protein, albumin, ALP, and cholesterol levels in serial samples of collared peccaries (*Tayassu tajacu*) immobilized with ketamine hydrochloride; generally, levels of these parameters gradually recovered with long-term (ca. >1 hr) immobilization. Wesson et al. (1979) recorded lower total protein levels in chemically restrained compared to manually restrained white-tailed deer (*Odocoileus hemionus*).

Reduced levels of RBC, PCV, and Hb are commonly recorded in anesthetized animals (Bond, 1969; Seal et al., 1972; Drevemo and Karstad, 1974; Hawkey et al., 1980; Brannon, 1985; Kuttner and Wiesner, 1987; Cross et al., 1988; Rietkerk and Delima, 1994; Rietkerk et al., 1994). For example, chemically restrained mountain gazelles (*Gazella gazella*) (Rietkerk et al., 1994), white-tailed deer (Wesson et al., 1979), and red deer (*Cervus elaphus*) (Cross et al., 1988) had lower values of RBC, HCT, and/or Hb than did manually restrained animals. Higher levels of RBC, HCT, and Hb in manually restrained animals likely result in part from handling stress; splenic contraction in response to excitement can result in the release of sequestered red blood cells into the circulatory system (Wesson et al., 1979; Hawkey et al., 1980; Brannon, 1985; Feldman et al., 2000).

We are aware of no prior studies of blood characteristics of the western spotted skunk. Heidt and Hargraves (1974) examined 12 serum chemistry and four hematologic parameters of six eastern spotted skunks trapped in Pulaski County, Arkansas in the spring of 1972. Over a 4 mo period, these skunks were maintained in a



laboratory and bled intracardially three times while lightly anesthetized with ethyl ether. For reference purposes, we have presented the mean values and ranges of these parameters in Table 1. Interpretations of these values and comparisons to our results, however, are difficult due to low sample sizes and clear differences in confinement, condition, nutrition, and mode of chemical restraint. In general, values for manually restrained and chemically restrained island spotted skunks were similar to those for the eastern spotted skunks, although some differences were apparent (Table 1). Specifically, Heidt and Hargraves (1974) indicate that most of the serum chemistry and hematologic values they recorded were within the range of values obtained in other studies of wildlife species, with the notable exception of glucose, which was unusually high compared to previous studies. Indeed, the glucose values they recorded were also substantially higher than values from either chemically restrained or manually restrained island spotted skunks, possibly due to differences in nutrition, anesthetic, or general stress or excitement.

Island spotted skunks are vulnerable to local extinction due to small population sizes, restricted distribution, and relatively specialized resource use. Such vulnerability is typical of other insular fauna, including the other endemic carnivore on the California Channel Islands, the island fox, *Urocyon littoralis* (Wayne et al., 1991; Garcelon et al., 1992; Crooks, 1994a; Crooks and Van Vuren, 1994; Crooks et al., 2001). We believe that these basic serum chemical and hematologic data should prove useful for further research on island spotted skunk populations. Because our results suggest that chemical or manual restraint, as well as other methodologic details, may influence serum chemistry and hematologic parameters, care should be taken when comparing blood characteristics using different handling and analytical techniques.

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