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HEMATOLOGY AND PLASMA BIOCHEMISTRY VALUES FOR THE GIANT GARTER SNAKE (*THAMNOPHIS GIGAS*) AND VALLEY GARTER SNAKE (*THAMNOPHIS SIRTALIS FITCHI*) IN THE CENTRAL VALLEY OF CALIFORNIA

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ABSTRACT: Hematology and plasma biochemistry parameters are useful in the assessment and management of threatened and endangered species. Although reference ranges are readily available for many mammalian species, reference ranges for snakes are lacking for most species. We determined hematology and plasma biochemistry reference ranges for giant garter snakes (*Thamnophis gigas*) and valley garter snakes (*Thamnophis sirtalis fitchi*) living in four management areas in the Central Valley of California. White blood cell, heterophil, lymphocyte, and azurophil counts in giant garter snakes were approximately twice the values of valley garter snakes. Statistically significant differences in aspartate aminotransferase, globulin, and potassium between the two species did not appear clinically significant. No significant differences were found in the measured parameters between male and female giant garter snakes. Some differences were found among collection sites. These reference ranges provide baseline data for comparisons over time and between collection sites.

Key words: Biochemistry values, hematology, *Thamnophis gigas*, *Thamnophis sirtalis fitchi*.

INTRODUCTION

Hematology and plasma biochemistry values are useful in assessing individual and population health and fitness for wildlife species (Barnes et al., 2008; Deem et al., 2008; Greig et al., 2010), and provide a valuable tool for managing rare or imperiled species. Unfortunately, published studies on the hematology and plasma biochemistry reference ranges for normal snakes are rare (MacMahon and Hamer, 1975; McDaniel et al., 1984; Wojtaszek, 1991; Dutton and Taylor, 2003; Allender et al., 2006). Ranges for garter snakes (*Thamnophis* spp.), in particular, are absent in the published literature. Coates et al. (2009) investigated body condition and growth rate of the giant garter snake (*Thamnophis gigas*) to understand better the relationship between phenotypic traits and fitness. Hematologic and biochemistry assessments may provide a more sensitive indication of population health than morphologic data alone (Calle et. al., 1994; Polo-Cavia et al., 2010).

During the summer of 2008, giant garter snakes and valley garter snakes from four wildlife management areas were sampled to establish hematology and plasma biochemistry parameter reference ranges. This study was part of a comprehensive study to assess the role of contaminants, water quality, and water management in the health and distribution of giant garter snakes across their range. The valley garter snake was chosen to determine the appropriateness of this species as an experimental model for the giant garter snake and as a sentinel for California Central Valley ecosystem health. Information on baseline reference ranges can be used to assess the health of individual snakes and compare the fitness of snake populations inhabiting wetland ecosystems

variably impacted by landscape change and water quality.

MATERIALS AND METHODS

Capture sites

We captured giant garter snakes and valley garter snakes during the peak of the 2008 active season (April–September) from four geographically independent sites within the Central Valley of California (Fig. 1): Natomas Basin (Natomas; 38°36′–38°50′N and 121°28′–121°38′W) in Sacramento and Sutter Counties, Cosumnes River Preserve (Badger Creek; 38°19′–38°20′N and 121°20′–121°21′W) in southern Sacramento County, Grasslands Ecological Area (Los Banos; 37°6′–37°11′N and 120°54′–120°57′W) in Merced County, and Mendota Wildlife Area (Mendota; 36°40′–36°44′N and 120°16′–120°22′W) in Fresno County. Natomas and Badger Creek are located in the Sacramento Valley and represent native wetland and rice agriculture habitat profiles. We captured snakes in minnow traps (Tackle Factory [Cuba Specialty Manufacturing], Fillmore, New York, USA) modified to float (Casazza et al., 2000). We also captured snakes by hand when opportunities arose. At all four sites, we placed traps along the open water–vegetation or open water–bank-side interfaces of aquatic features (irrigation or drainage canals, sloughs, ponds, marshes) with sufficient water depth (≥15cm). Traps were checked for captured snakes at least daily. Captured snakes were measured (mass and snout–vent length), photographed, and marked for permanent identification with the use of passive integrated transponder (PIT) tags; sex was determined with the use of tail confirmation and cloacal probing. Snakes were individually placed in nylon snake bags (MIT/Midwest.com, Independence, Missouri, USA) and transported to the Sacramento Zoo in breathable coolers maintained between 10 and 29 C. All trapping and transporting of snakes was conducted according to the terms and conditions of an approved US Fish and Wildlife Service Recovery Permit ESA10(a)(1)(A)-TE-018177-5. All snakes were released at their original capture location within 48 hr following blood collection.

Blood collection

At the Sacramento Zoo, sex was confirmed with the use of cloacal probing and the presence of calcified hemipenes on a lateral radiograph. A blood sample, less than 1% of body mass, was collected from the ventral tail vein with the use of a sterile 25-gauge needle and 3-ml syringe. Two diagnostic-quality blood smears were made and two microhemocrit capillary tubes (Fisher brand 22-362-574, Fisher Scientific, Pittsburgh, Pennsylvania, USA) were filled with the fresh whole blood. The remaining blood was immediately placed in two heparin microtainer tubes (Becton Dickinson, Franklin Lakes, New Jersey, USA). All blood samples were collected within 48 hr of removal of the snake from the trap. Packed cell volume was determined by centrifuging the microhemocrit tubes for 10 min. Plasma protein was determined with the use of a handheld refractometer (JorVet model J351, Jorgensen Laboratories Inc., Loveland, Colorado, USA). Hemoglobin content was determined with the use of a modified azidemethemoglobulin reaction (B-Hemoglobulin Photometer, HemoCue, Lake Forest, California, USA). Within 3 hr of blood collection, red blood cell count and leukocyte counts were manually determined with the use of the unipette method and a hemocytometer (Campbell and Ellis, 2007). The blood smear was stained (Diff-Stain, IMEB Inc., San Marcos, California, USA) and a leukocyte differential count was determined counting at least 100 leukocytes under 1,000×

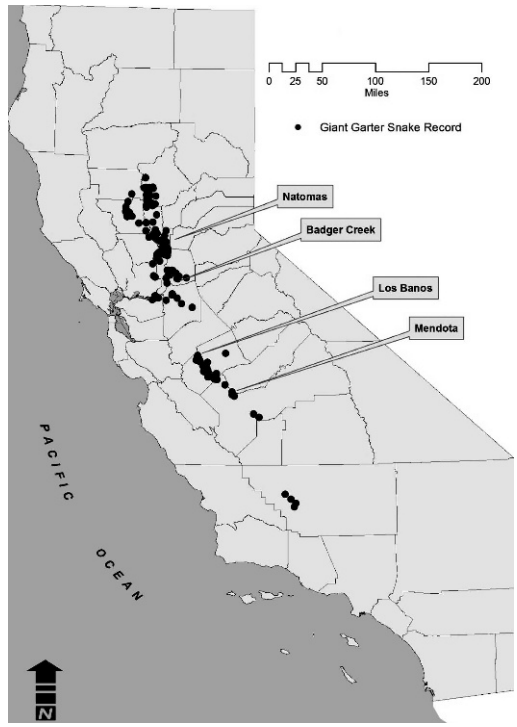

FIGURE 1. Location of study sites surveyed in this study in California, where garter snakes (*Thamnophis* spp.) were captured.

magnification. The second heparinized blood microtainer tube was centrifuged for 10 min and the plasma biochemistry parameters (albumin, aspartate aminotransferase [AST], bile acids, calcium, creatinine kinase [CK], globulin, glucose, potassium, sodium, phosphorous, total protein, uric acid) were determined with the use of an avian/reptile specific rotor (Avian/Reptilian Profile Plus) in the VetScan analyzer (Abaxis North America, Union City, California, USA) within 30 min of blood collection. Because of the small size of some snakes, insufficient blood sample volumes were obtained from some individuals. For these animals, either hematology or biochemistry parameters, but not both, were measured.

Statistical analysis

Reference ranges for hematologic and blood chemistry parameters for the giant garter snake and the valley garter snake were established with the use of parameter values from all apparently healthy free-ranging snakes. Three giant garter snakes and three valley garter snakes were removed from the data set used to calculate reference ranges because they appeared to be unhealthy based on abnormal physical exam and coelomic masses on palpation. Many parameters were not normally distributed based on the Shapiro–Wilk test of normality (Shapiro and Wilk, 1965), and data became sparse once stratified by species and location. Therefore, reference ranges for each species were reported as the 10th and 90th percentiles. Hematologic and blood chemistry parameters, including data from the six apparently nonnormal snakes, were evaluated for differences by capture location for giant and valley garter snakes independently. Only one giant garter snake was captured at Mendota, so comparisons by capture location for giant garter snakes were conducted among only three sites. Similarly, only one male valley garter snake was captured, so differences in parameters by sex were evaluated for giant garter snakes only. The nonparametric Kruskal–Wallis analysis of variance test for within-group differences and the Wilcoxon–Mann–Whitney test was used to evaluate differences between groups. Data were analyzed with the use of the statistical software STATA SE 11.0 (StataCorp, College Station, Texas, USA). Tests were considered statistically significance if $P<0.05$.

RESULTS

We captured and obtained blood samples from 49 giant garter snakes and 39 valley garter snakes. A variable number of snakes were captured at each site. Table 1 lists the demographic characteristics of the snakes captured for this study. Hematology and plasma biochemistry reference values for male and female giant garter snakes and valley garter snakes are presented in Tables 2 and 3. Table 4 shows reference values for snakes with parameters that differed significantly by location.

We found a significant difference in white blood cell count, heterophil count, lymphocyte count, and azurophil count between species ($P<0.01$), with values in giant garter snakes approximately twice

TABLE 1. Distributions of male and female snakes and mass weight (in grams) for free-ranging giant garter snakes (*Thamnophis gigas*) and valley garter snakes (*Thamnophis sirtalis fitchi*) captured at four sites in the Central Valley of California, summer 2008.

	Badger Creek	Los Banos	Mendota	Natomas	Total
Giant garter snake					
Median mass (g)	222.5	260	340	305	260
Mass min–max (g)	148–370	140–565	NA	148–465	148–565
Number males	3	2	0	3	8
Number females	17	5	1	18	41
Total number	20	7	1	21	49
Valley garter snake					
Median mass (g)	225	215	190	200	210
Mass min–max (g)	157–380	125–315	150–400	112–441	112–441
Number males	0	1	0	0	1
Number females	5	10	8	15	38
Total number	5	11	8	15	39

TABLE 2. Hematology and plasma biochemistry reference values for apparently healthy free-ranging giant garter snakes (*Thamnophis gigas*) captured in the Central Valley of California.

Parameter	*n*	Median	10th percentile	90th percentile	Range
White blood cell count ($\times 10^3$/μl)	46	11.5	6.8	16.4	2.5–18.6
Red blood cell count ($\times 10^6$/μl)	46	0.8	0.5	1.1	0.2–1.4
Hemoglobin (gm/dl)	46	10	7.7	11.8	6.9–13.6
Pack cell volume (%)	46	31	22	38	17–45
Heterophils ($\times 10^3$/μl)	45	0.99	0.51	1.97	0.35–2.18
Lymphocytes ($\times 10^3$/μl)	46	7.9	3.98	12.28	1.27–14.97
Basophils ($\times 10^3$/μl)	45	0.33	0.13	0.64	0.09–0.83
Azurophils ($\times 10^3$/μl)	44	1.75	0.5	2.79	0.37–4.4
Plasma protein (gm/dl)	45	5	4.5	5.8	4.2–6.7
Aspartate aminotransferase (IU/l)	44	22	10	45	8–74
Bile acids (μmol/l)	45	35	35	35	0–35
Creatinine kinase (IU/l)	42	439	74	1070	20–1,666
Uric acid (mg/dl)	44	5.7	2.9	8.2	1.2–13.1
Glucose (mg/dl)	43	81	58	115	44–154
Calcium (mg/dl)	45	15.2	13.8	16	12.9–20.0
Phosphorus (mg/dl)	43	3.8	3.3	5.6	1.9–6.3
Total protein (gm/dl)	44	5	4.4	5.7	3.9–6.1
Albumin (gm/dl)	45	1.2	1.1	1.6	1.0–1.7
Globulin (gm/dl)	43	3.6	3.1	4.3	2.7–4.7
Potassium (mmol/dl)	44	5.2	3.9	7.9	2.7–8.8
Sodium (mmol/dl)	44	159	150	166	147–170

the values for valley garter snakes. Comparing biochemical parameters, AST levels in giant garter snakes (median 22 IU/l) were significantly higher than in valley garter snakes (median 16 IU/l, $P=0.021$); globulin levels in giant garter snakes (median 3.6 gm/l) were significantly lower than in valley garter snakes (median 4.0 gm/l, $P=0.047$);

TABLE 3. Hematology and plasma biochemistry reference values for apparently healthy free-ranging valley garter snakes (*Thamnophis sirtalis fitchi*) captured in the Central Valley of California, USA.

Parameter	*n*	Median	10th percentile	90th percentile	Range
White blood cell count ($\times 10^3$/μl)	35	6.6	3.7	11.9	3.1–13.7
Red blood cell count ($\times 10^6$/μl)	36	0.9	0.6	1.1	0.5–1.3
Hemoglobin (gm/dl)	36	9.4	6.3	11.3	5.2–13.2
Pack cell volume (%)	37	29.5	21	36	18.5–42
Heterophils ($\times 10^3$/μl)	37	0.59	0.37	1.65	0.23–3.70
Lymphocytes ($\times 10^3$/μl)	37	4.27	2.23	9.21	1.66–15.10
Basophils ($\times 10^3$/μl)	37	0.29	0.10	0.71	0.65–0.86
Azurophils ($\times 10^3$/μl)	36	0.73	0.36	1.54	0.19–1.94
Plasma protein (gm/dl)	35	5.5	4.4	7.3	4.0–8.3
Aspartate aminotransferase (IU/l)	34	16	9	30	8–48
Bile acids (μmol/l)	34	35	35	35	35–47
Creatinine kinase (IU/l)	35	387	100	1138	17–1428
Uric acid (mg/dl)	33	5.1	3.3	10.9	1.7–16.0
Glucose (mg/dl)	33	89	63	120	53–167
Calcium (mg/dl)	33	15.7	13.2	16	11.7–16.6
Phosphorus (mg/dl)	34	3.6	2.3	6.7	1.8–7.6
Total protein (mg/dl)	35	5.2	4.3	6.4	4.1–7.9
Albumin (mg/dl)	36	1.2	1	1.7	1.0–2.1
Globulin (gm/dl)	36	4.0	0	5.2	0–7.0
Potassium (mmol/dl)	36	4.4	2.5	5.6	1.8–7.0
Sodium (mmol/dl)	35	157	152	162	136–166

TABLE 4. Median hematology and plasma biochemistry values for parameters that differed significantly among giant garter snakes (*Thamnophis gigas*) captured at different sites within the Central Valley of California. ($P<0.05$).

Parameter	Badger Creek ($n=20$)	Los Banos ($n=7$)	Mendota ($n=1$)	Natomas ($n=21$)
Heterophil ($\times10^3$/μl)	0.93	2.04	0.88	1.06
Basophil ($\times10^3$/μl)	0.47	0.37	0.38	0.23
Azurophil ($\times10^3$/μl)	1.92	3.70	2.26	1.27
Calcium (mg/dl)	14.5	16.0	16.0	16.0
Phosphorus (mg/dl)	3.6	4.5	5.6	4.8
Albumin (gm/dl)	1.2	1.3	1.5	1.4
Globulin (gm/dl)	3.9	3.9	3.6	3.3
Potassium (mmol/l)	5.4	4.1	5.2	5.5
Sodium (mmol/l)	154	161	153	163

potassium levels in giant garter snakes (median 5.2 meq/l) were significantly higher than in valley garter snakes (median 4.4 meq/l, $P=0.001$). We found no significant differences in reference values between male and female giant garter snakes.

Hematologic and plasma biochemistry values in giant garter snakes differed significantly among capture locations for heterophil, basophil, and azurophil counts, calcium, phosphorus, albumin, globulin, potassium, and sodium (Table 4). Among valley garter snakes, hemoglobin, packed cell volume, plasma protein, calcium, phosphorus, total protein, albumin, and globulin levels differed significantly across sites (Table 5).

DISCUSSION

This study establishes hematology and plasma biochemistry reference ranges for giant garter snakes and valley garter snakes free ranging in the Central Valley of California during the summer of 2008. Clinically significant differences were found between the species in that the total white blood cell counts, heterophil counts, lymphocyte counts, and azurophil counts among giant garter snakes were significantly higher compared to valley garter snakes. As these changes were not site dependent, we conclude that it is a significant species difference that must be considered when evaluating hemograms from different species of garter snakes. Although the cause underlying these biologically significant differences is not known, species-specific variability in these parameters supports the need for using species-specific reference ranges. As giant garter snake populations are declining throughout their range and valley garter snakes are not declining, the changes in the hemograms may be reflecting increased environmental pressures on the giant garter snakes as compared to those

TABLE 5. Median hematology and plasma biochemistry values for parameters that differed significantly among valley garter snakes (*Thamnophis sirtalis fitchi*) captured at different sites within the Central Valley of California ($P<0.05$).

Parameter	Badger Creek ($n=5$)	Los Banos ($n=11$)	Mendota ($n=8$)	Natomas ($n=15$)
Hemoglobin (gm/dl)	10.9	8.1	8.1	9.7
Packed cell volume (%)	33	30	25	30
Plasma protein (gm/l)	6.6	5.5	4.4	5.9
Calcium (mg/dl)	16.0	15.6	13.8	16.0
Phosphorus (mg/dl)	2.6	4.1	3.5	4.5
Total protein (gm/dl)	6.4	5.0	4.6	5.6
Albumin (gm/dl)	1.4	1.2	1.0	1.3
Globulin (gm/dl)	5.2	3.8	3.4	4.3

faced by the valley garter snakes. In comparison to published hemograms in other snake species, the heterophil, lymphocyte, and azurophil counts of the giant garter snakes appear elevated. This may be an indication of chronic inflammation. Observed differences between giant garter snake and valley garter snake plasma biochemistry parameters did not appear to be clinically significant.

The reference ranges established in this study were derived from apparently healthy snakes that had been captured in traps and transported from the trap to a central processing site. Snakes could not be bled at the capture site because of the difficulty of obtaining noninvasive blood samples and the need for other assessments of the snakes. Sample bias may have resulted from using trapped snakes, and parameter values may have changed from the time the snake entered the trap until a blood sample was obtained. A stress leukogram has not been conclusively shown in garter snakes, but stress-related changes in the hemogram would be expected (Evans, 2000; Campbell, 2006). Although we did not measure stress, the trapping, transportation, and blood sampling experienced by the garter snakes would be considered moderately stressful in other species. The presumed stress experienced by these snakes was consistent across sample sites and similar to what would be expected in other capture studies. Although not examined in this study, hematology and plasma biochemistry values for these snakes would likely also vary depending upon the time of year that the snakes were sampled, as has been found in Brazilian boas (*Boa constrictor amarali*; Machado et al., 2006). The lack of apparent significant differences between male and female snakes is likely due to the small number of males sampled (9 of 49 giant garter snakes and 1 of 39 valley garter snakes). The proportionally small number of males captured for this study may be due to behavioral differences that confounded capture success among the sexes, or this may reflect substantially skewed sex demographics in these populations.

In comparing hematologic values across capture sites, it is interesting to note that heterophil, basophil, and azurophil counts differed for giant garter snakes, whereas hemoglobin, packed cell volume, and plasma protein differed for valley garter snakes. Among plasma biochemistry parameters, calcium, phosphorous, albumin, and globulin values differed across capture sites for both giant and valley garter snakes compared to the reference range. We plan to perform additional studies to correlate these changes with environmental parameters, exposure to heavy metals and pesticides, as well as prey availability and quality. The Natomas and Badger Creek collection sites are specifically managed for giant garter snake conservation, whereas the Mendota and Los Banos sites are managed for waterfowl conservation. As a result of these management differences, the Natomas and Badger Creek sites have water available year-round, whereas Mendota and Los Banos experience periods of greatly reduced or no water in the garter snake habitat. Because of differences in the ecology of these two species of garter snake, these environmental differences may affect each species differently. Future research efforts are planned to correlate environmental and prey differences at each site with the hematology and biochemical differences in the snakes.

Hematologic and biochemistry assessments may provide a more sensitive indication of population health than morphologic data alone (Calle et al., 1994; Polo-Cavia et al., 2010). The reference ranges reported here should be used to assess baseline health of garter snake populations in the Central Valley of California and compare these to populations in other localities.

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