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Hematology and Plasma Biochemical Values for Free Ranging Cottonmouths (*Agkistrodon piscivorus*) in Central North Carolina, USA

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***Abstract*.—We obtained peripheral blood samples from 40 juvenile and adult Cottonmouths (*Agkistrodon piscivorus*; 21 males, 19 females) to establish baseline hematology and plasma biochemical reference intervals for individual and population health assessment. We collected the snakes near McKinney Lake National Fish Hatchery in central North Carolina, USA during August and September 2011. Hematological and serum biochemical data, packed-cell volumes (PCV), and morphologic characteristics of both erythrocytes and leukocytes in the Cottonmouths we sampled were similar to those of other ophidians. A significant difference between median PCV for male and female snakes (25.0% for males vs. 20.5% for females) may have been confounded by a significant positive correlation between PCV and both snout vent length and mass. There was no apparent relationship between the severity of the frequently observed hemogregarine-like parasitemias and the hematologic parameters examined. Much as in the animals previously collected from this site for exhibition, the two largest and presumably oldest animals collected for this study had an age related increase in azurophilic-monocytes unassociated with increased intensity of hemogregarine-like organism parasitism or erythrocyte viral burden**

Key Words.—Agkistrodon piscivorus; clinical pathology; cottonmouth; hematology; plasma biochemistry

INTRODUCTION

The Cottonmouth (*Agkistrodon piscivorus*) is an ovoviviparous, semi-aquatic venomous snake occupying a variety of habitats associated with permanent or semi-permanent water throughout the southeastern United States (Palmer and Braswell 1995). It is locally abundant in areas from the Florida Keys to Northern Virginia and west into central Texas and eastern Oklahoma (Burkett 1966; Blem and Blem 1995). This species is considered an opportunistic feeder with a varied diet, including fish, amphibians, small mammals, birds and other reptiles (Burkett 1966). Due to its abundance, wide distribution, confusion with harmless non-venomous water snakes, and its notoriety, the Cottonmouth is often displayed as an exhibit animal at zoological institutions and natural history museums around the country.

Established baseline hematological and plasma biochemical reference intervals facilitate the delivery of high quality health care to captive reptiles. Frequently used diagnostic tests to evaluate the health status of captive reptiles include hematology and serum biochemistry panels (Chiodini and Sundberg 1982; Campbell 2006a). To elucidate findings from routine evaluations of the captive Cottonmouths exhibited at the North Carolina Zoological Park, we conducted a field study. Historically, the absolute white blood cell (WBC) and monocyte counts from this collection's Cottonmouths were dramatically higher than what many consider "normal" for most snakes (Parker and McCoy 1977; Mader et al. 1985; Calle et al. 1994; Troiano et al. 1997). Because these findings were consistent and the snakes were in apparent good health, we postulated that the high WBC and monocyte counts could be within baseline parameters for the species and/or related to either the collection site or the age/size of the animal. To examine these questions and improve the usefulness of the complete blood count (CBC) and plasma biochemistry panels as diagnostic tools for captive Cottonmouths, we surveyed the wild, presumably healthy population of origin for the majority of the captive Cottonmouths that have been maintained in the NC Zoo collection.

Materials and Methods

Collection of blood samples.—We collected 21 male and 19 female, juvenile and adult Cottonmouths from the area surrounding the McKinney Lake National Fish Hatchery, located in Richmond County, North Carolina, USA (35° 01′ N, 79° 63′ W) in August and September 2011. The snakes were caught between 1000 and 1500 with an ambient air temperature between 20° C and 32° C. When we encountered individual snakes during searches of the study site, we captured and placed them in plastic 18.9 L (5-gallon) buckets with secure lids and transported to the field station (Fig. 1). We examined each animal and collected blood within 3 h of capture. Notching the first ventral scale just anterior to the anal plate with a handheld electrocautery unit identified each snake sampled (Winne et al. 2006). We determined the sex by cloacal probing and obtained morphometric measurements for each snake by weighing to the nearest gram and measuring to the nearest millimeter for total length, and snout-vent length (SVL). We determined the total length and snout-vent length using a flexible tape measure. For safety purposes, we manually restrained snakes for venipuncture with the head and cranial half of the body in a transparent open-ended acrylic tube. In this position, the ventral caudal vein was accessible. We obtained a blood sample no larger than 0.8% of the body mass with a 3 mL non-heparinized syringe using a 22-gauge or 25-gauge needle. We discarded blood samples with obvious lymph contamination, characterized by transparent fluid entering the syringe first. After venipuncture and data collection, we placed each snake in their original bucket and returned them for release at the site where they were collected. Each snake was sampled only once. Based on the clinical examination in the field, all of the animals we evaluated appeared outwardly to be healthy and in good body condition. We defined Cottonmouth size classes after an examination of the snout-vent length data to identify natural breaks in the data distribution. These breaks aligned well with conservative age estimates based on the data established by Blem and Blem (1995) from Cottonmouth populations in Hopewell, Virginia. We classified snakes as: group I: 0–35.0 cm SVL; group II: 35.1–45.0 cm SVL; group III: 45.1–60.0 cm SVL; or group IV: > 60.0 cm SVL (Blem and Blem 1995).

Figure 1. Several Cottonmouths (Agkistrodon piscivorus) in a plastic bucket awaiting morphometric measurements and hematological sampling. (Photographed by Larry J. Minter).

Hematology.—We prepared thin blood smears for each snake directly from a non-heparinized syringe immediately after blood collection. These were air dried, heat fixed, and then stained with a Diff-Quik stain (Fisher Scientific, Kalamazoo, Michigan, USA). We determined the packed-cell volume (PCV) by centrifugation and direct measurement of a subsample in a heparinized hematocrit tube. We measured the total solids in the plasma with a refractometer and used this as a correlate of plasma protein. Remaining blood was immediately transferred to a lithium-coated collection tube (BD Microtainer, Becton Dickinson, Franklin Lake, New Jersey, USA) and centrifuged on site within 15 min of collection. We removed the plasma and placed it into a cryotube vial for storage (Fisher Scientific, Kalamazoo, Michigan, USA). We placed plasma samples in a cooler containing ice and transported within 7 h to the North Carolina Zoo for further processing. We manually performed a direct method leukocyte count with the use of a red blood cell (RBC) Unopette with toluidine stain, which was prepared at the field station using whole blood before being transported back to the North Carolina Zoo for processing (Becton Dickinson,

Franklin Lakes, New Jersey, USA). A minimum of 200 white blood cells (WBC) were counted for each sample for determination of differential leukocyte count. The same individual (Purnell) analyzed all blood smears. We classified the leukocytes as heterophils, lymphocytes, monocytes/azurophilic-monocytes, eosinophils, or basophils, based on cell morphology. Monocytoid appearing cells without the azurophilic granules were very rarely observed and due to the extremely low number of cells observed were grouped together with azurophilic-monocytes in the differential cell count. We evaluated erythrocytes for hemoparasites by examining the stained blood smears. We removed one sample from analysis due to apparent lymph contamination. While dilution was not recognized in the field for this sample, excessive dilution of the cellular components was consistent with presumptive lymph dilution. We estimated the intensity of hemoparasitemia and presence of cytoplasmic inclusions separately for each snake and classified the data using the same rubric, group I: no hemogregarine-like parasites or cytoplasmic inclusions found; group II: 1–2 parasites or inclusions found per 10 high powered microscope field (100x objective); group III: 3–4 parasites or inclusions per 10 high powered field; or group IV: ≥ 5 parasites or inclusions per 10 high powered field.

Serum biochemistry.—We performed plasma biochemical assays using the avian–reptilian rotor on the VetScan analyzer (Abaxis, Inc. Whipple City, California, USA). We analyzed plasma specimens for calcium, phosphorus, sodium, potassium, aspartate aminotransferase, creatine kinase, uric acid, glucose, and total protein. The samples from three snakes had insufficient plasma volume for biochemical analysis.

Statistical analysis.—We analyzed the data using JMP, version 9.0 software (SAS Institute Inc., Cary, North Carolina, USA). All of the variables were tested for normality of distribution by applying the Shapiro-Wilk normality test. We assessed overall differences in hematologic and biochemical variables between the sexes using Wilcoxon rank sum tests. We used a Kruskal-Wallis test, followed by a Steel Dwass All Pairs multiple comparison test to evaluate the differences in hematologic and biochemical variables among the size classes. We determined the correlations between PCV and both SVL and weight using Spearman's rank correlation coefficient. Values were reported as median, 10^{th}, and 90^{th} percentiles, maximum and minimum and were considered to be significant at $P \leq 0.05$. We considered values of any parameter > 1.5 times the interquartile range for the cohort as potential outliers, triggering an examination of the field notes, measurements, and assessment of the animal for any indication of a generalized abnormality that would justify exclusion of the animal from the cohort.

Results

The 40 Cottonmouths we collected ranged between 20 and 2,030 g total body weight (median: male = 330 g, female = 180 g); between 21.6 and 108.0 cm SVL (median: male = 64.7 cm, female = 49.5 cm), and 25.4 to 125.1 cm in total length (median: male = 73.6 cm, female = 57.1 cm; Table 1). When summarized by size classes, snakes assigned to the largest size class had a median total body weight (IV = 490.0 g) that was > 7.5 times that of the animals assigned to the smallest size class (I = 65.0 g) but was only twice the total body length (median: I = 40 cm, IV = 82.5 cm; Table 2).

We examined blood samples from 39 of the 40 snakes we collected for hematological analysis. A single parameter was considered an outlier for six snakes (three for basophil count, and one each for PCV, heterophil count and azurophilic-monocyte count). The field evaluations of these snakes did not establish a reason to exclude any of these animals from the cohort. We collected sufficient plasma from 37 animals for biochemical analysis. We detected a significant difference ($Z = 2.54$, $P = 0.012$) in the median PCV between male and female snakes (25.0% for males vs. 20.5% for females) and between the different size classes, but we did not find a difference in the absolute WBC count or the WBC differential between the sexes or size

Table 1. Overall median, minimum, maximum, and 10–90% quartile range of bodyweight, snout-vent lengths, and total lengths between male and female wild-caught Cottonmouths (Agkistrodon piscivorus) from central North Carolina, USA.

	Male (n = 21)				Female (n = 19)			
Body Measurements	**Median**	**10–90 % Quantiles**	**Max**	**Min**	**Median**	**10–90 % Quantiles**	**Max**	**Min**
Weight (g)	330	110.0–1694.0	2030.0	70.0	180.0	60.0–910.0	1080.0	20.0
Snout–Vent Length (cm)	64.7	38.8–103.1	108.0	33.6	49.5	31.7–76.2	86.3	21.6
Total Length (cm)	73.6	50.2–116.3	125.1	40.6	57.1	38.1–88.9	92.2	25.4

Table 2. Median, minimum, maximum, and 10–90% quartile range of body mass, snout-vent lengths and total lengths by size classes of wild-caught Cottonmouths (Agkistrodon piscivorus) from central North Carolina, USA.

Size Class	Statistic	Weight (g)	Snout-vent Length (cm)	Total Length (cm)
I (n = 6)	Median	65.0	32.7	40.0
	10–90% Quantile	20.0–80.0	21.5–36.2	25.4–43.2
	Max	80.0	36.2	43.2
	Min	20.0	21.5	25.4
II (n = 6)	Median	115.0	40.0	50.5
	10–90% Quantile	60.0-130.0	38.1-43.1	45.7-55.8
	Max	130.0	43.1	55.8
	Min	60.0	38.1	45.7
III (n = 10)	Median	205.0	53.3	62.2
	10–90% Quantile	130.0-483.0	48.2-67.6	53.6-80.2
	Max	500.0	68.5	81.2
	Min	130.0	48.2	53.3
IV (n = 18)	Median	490.0	70.4	82.5
	10–90% Quantile	309.0-1742.0	63.2-104.5	73.1-117.6
	Max	2030.0	107.9	125.0
	Min	210.0	60.9	68.5

classes (Table 3, Table 4). We found, however, that the two largest snakes sampled in this study were the animals with the highest azurophilic-monocyte count, similar to what has been observed in the Cottonmouths procured from this study site for exhibition at the North Carolina Zoo. We detected a significant positive correlation between PCV and both SVL ($\rho = 0.761$, $P < 0.001$) and weight ($\rho = 0.768$, $P < 0.001$). Biochemical parameters of male and female snakes irrespective of size class did not differ significantly (Table 5)

We identified four types of white blood cells: lymphocytes, azurophilic-monocytes, heterophils and basophils (Fig. 2). Seventeen of the 39 (43.6%) snakes we examined hematologically were infected with hemogregarine-like blood parasites: 14 snakes had a relatively light infection (group II) and three snakes had a relatively heavy infection (two group III, one group IV). These parasites were a basophilic staining elongated oval organism with clear zones at each end, which were larger than and slightly displaced the nucleus of erythrocytes (Fig. 2e). We were unable to classify these organisms to genus by examination of blood smears alone. The two largest snakes, which subsequently had the

Table 3. Hematologic parameters for both male and female wild-caught Cottonmouths (*Agkistrodon piscivorus*) from central North Carolina, USA. Significant differences ($P \leq 0.05$) between the sexes are designated with an asterisk (*).

	Male (n = 21)				Females (n = 18)			
Parameter	**Median**	**10–90% Quantile**	**Max**	**Min**	**Median**	**10–90% Quantile**	**Max**	**Min**
PCV (%)	25.0*	16.8–31.8	32	3	20.5*	14.7–27.7	34	12
Total Solids	5.1	3.9–6.5	7.2	3.4	4.6	3.7–6.0	7.1	3.6
Absolute WBC count (103/µL)	3250	1800–5950	6000	1000	3125	1950–4775	5000	1505
Heterophils (103/µL)	120	34–380	600	27	150	18–380	385	0
Heterophils (%)	4	1–15	17	1	5	1–8	11	0
Lymphocytes (103/µL)	2640	1356–5249	5460	800	2465	1365–3588	4250	1264
Lymphocytes (%)	84	55–94	96	44	79	66–94	98	51
Monocytes (103/µL)	420	102–1154	1500	55	613	137–915	1053	20
Monocytes (%)	12	4–38	42	2	17	5–26	39	1
Eosinophils (103/µL)	0	0–0	0	0	0	0–0	0	0
Eosinophils (%)	0	0–0	0	0	0	0–0	0	0
Basophils (103/µL)	0	0–55	157	0	0	0–100	195	0
Basophils (%)	0	0–2	0	7	0	0–3	6	0

[a] PCV = packed cell volume,
WBC = White blood cell.

highest azurophilic-monocyte count, were not parasitized with hemoparasites (group I; Fig. 3).

Nine of the 39 snakes (23.1%) contained intraerythrocytic inclusions; four snakes had a relatively light aggregation (group II) and five snakes having a relatively heavy aggregation (one group III, four group IV). Four of the nine snakes containing intraerythrocytic inclusions were simultaneously infected with hemogregarine-like blood parasites. The appearance of these inclusions varied. Some cells contained only a clear vacuole, but others had a large reddish/purple granule with or without one or two square shaped blue crystaline inclusions. The nucleus in red cells containing the inclusions was displaced to the end of the red cell when all three inclusions occurred in the same cell (Fig. 2f). Neither of the two largest snakes sampled, which were also the animals with the highest azurophilic-monocyte count, were burdened by erythrocytic viral inclusions (group I; Fig. 4).

Discussion

Hematological and serum biochemical data, PCVs, and morphologic characteristics of both erythrocytes and leukocytes in the Cottonmouths sampled in our study were similar to those seen in other reptiles (Duguy 1970; Frye 1991; Campbell 2006a). Though our conservative approach to identification of outliers indicated six animals with a single hematology parameter greater than 1.5 times beyond the interquartile range, a careful review of the field assessments failed to reveal a basis for exclusion of any of the animals from the cohort. In two of the six cases, had we used a less conservative approach commonly employed to identify outliers (values > 3 standard deviations beyond the mean) the parameters would have been considered an outlier. Though failure to exclude animals that are true outliers could risk kurtosis and skewed results, re-analysis excluding parameters considered potential outliers did not affect the

Table. 4. Hematologic parameters for different size classes (I, II, III, IV) of wild-caught Cottonmouth (*Agkistrodon piscivorus*) from central North Carolina, USA. Significant differences ($P \leq 0.05$) between the size classes are designated with different superscripts (*,†).

	I (n = 6)				II (n = 6)			
	Median	**10–90% Quantile**	**Max**	**Min**	**Median**	**10–90% Quantile**	**Max**	**Min**
PCV (%)	20.0†	15.0–22.0	22	15	19.0†	3–23	23	3
Total Solids	4.5	4.0–5.6	5.6	4.0	4.3	3.9–5.3	5.3	3.9
Absolute WBC count (103/µL)	2600	2250–3000	3000	2250	3625	2500–5750	5750	2500
Heterophils (103/µL)	175	50–292	292	50	135	50–287	287	50
Heterophils (%)	7	2–13	13	2	3	2–6	6	2
Lymphocytes (103/µL)	1762	1327–2550	2550	1327	3223	2250–4887	4887	2250
Lymphocytes (%)	70.5	51–85	85	51	85	82–95	95	82
Monocytes (103/µL)	524	210–1053	1053	210	356	97–600	600	97
Monocytes (%)	22	7–39	39	7	8	3–13	13	3
Eosinophils (103/µL)	0	0–0	0	0	0	0–0	0	0
Eosinophils (%)	0	0–0	0	0	0	0–0	0	0
Basophils (103/µL)	39	0–157	157	0	12	0–195	195	0
Basophils (%)	2	0–7	7	0	1	0–6	6	0

	III (n = 10)				IV (n = 18)			
PCV (%)	22.0*,†	12.8–33.2	34	12	26*	19.0–32.0	32	18
Total Solids	4.3	3.4–5.8	5.9	3.4	5.4	4.6–7.1	7.2	4.4
Absolute WBC count (103/µL)	3500	2050–4200	4250	2000	3250	1404–6000	6000	1000
Heterophils (103/µL)	130	21–259	262	20	144	26–428	600	0
Heterophils (%)	4	1–7	7	1	4	1–16	17	1
Lymphocytes (103/µL)	2679	1816–3350	3357	1800	2242	1171–5364	5460	800
Lymphocytes (%)	82	69–98	98	69	78	52–91	91	44
Monocytes (103/µL)	435	24–897	900	20	420	136–1266	1500	120
Monocytes (%)	13	1–25	25	1	14	7–42	42	6
Eosinophils (103/µL)	0	0–0	0	0	0	0–0	0	0
Eosinophils (%)	0	0–0	0	0	0	0–0	0	0
Basophils (103/µL)	0	0–70	72	0	0	0–45	47	0
Basophils (%)	0	0–2	2	0	0	0–1	2	0

[a] PCV = packed cell volume, WBC = White blood cell.

Table 5. Plasma biochemical parameters for both male and female wild-caught Cottonmouths (Agkistrodon piscivorus) from central North Carolina, USA

	Male (n = 20)				Female (n = 17)			
	Median	10–90% Quantile	Max	Min	Median	10–90% Quantile	Max	Min
Aspartate aminotransferase (U/L)	25.0	13.1–178.6	315	12	33.0	15.4–340.8	724.0	13.0
Creatine kinase (U/L)	382.5	124.0–879.7	1301.0	124.0	382.0	167.2–1256.8	1704.0	132.0
Uric acid (mg/dL)	3.7	2.2–12.2	16.5	2.1	3.8	2.2–11.0	16.6	2.1
Glucose (mg/dL)	70.0	49.8–119.0	132.0	47.0	72.0	40.0–93.8	97.0	36.0
Calcium (mg/dL)	15.2	13.7–17.9	18.6	13.6	15.2	14.1–17.36	20.0	14.1
Phosphorus (mg/dL)	4.6	2.8–6.4	7.6	2.6	4.4	2.9–5.6	6.9	2.8
Total Protein (g/dL)	5.4	4.3–6.3	7.7	3.8	4.6	4.0–6.9	7.5	3.9
Potassium (mmol/L)	7.0	5.6–9.1	9.9	5.4	7.3	5.4–9.9	11.8	5.1
Sodium (mmol/L)	165	158–180.0	180.0	157.0	162.0	153.6–176.8	180.0	152.0

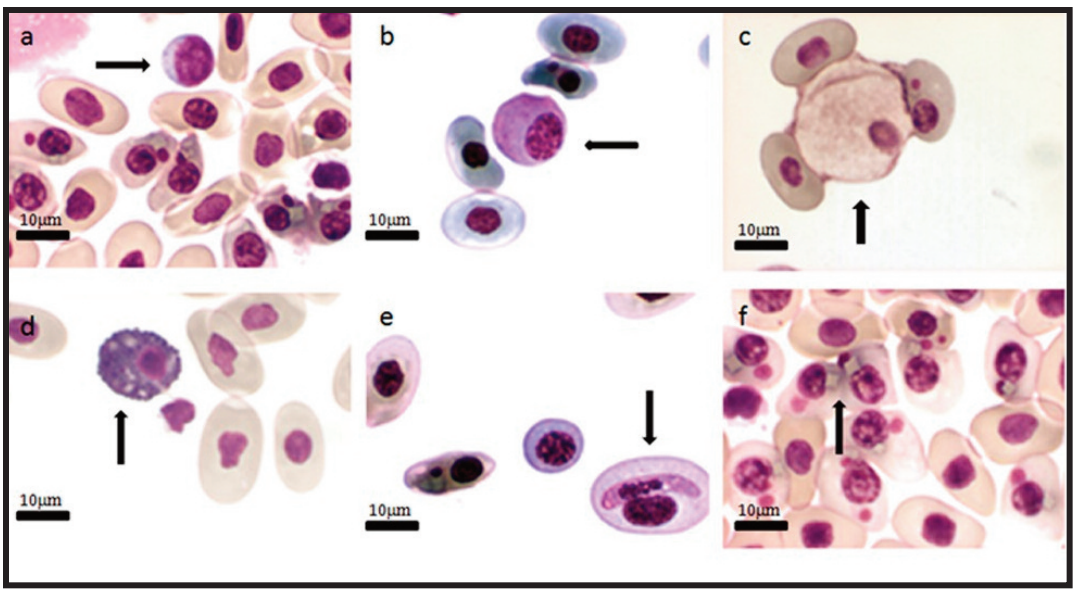

Figure 2. Light micrographs of blood cells of wild-caught Cottonmouths (*Agkistrodon piscivorus*). (a) Multiple erythrocytes surrounding a single lymphcyte (thin arrow); (b) Erythrocytes and a azurophilic-monocyte (thin arrow); (c) Heterophil (thin arrow) surrounded by three erythrocytes; (d) Basophil (thin arrow); (e) Hemogregarine infected erythrocyte (thin arrow), elongated oval organism slightly displacing the nucleus of the erythrocyte; (f) Multiple erythrocytes containing pirhemocyton intraerythrocytic inclusions, which varied from clear vacuoles to a large reddish/purple granule and still others containing square shaped blue colored crystal appearing inclusions (thin arrow).

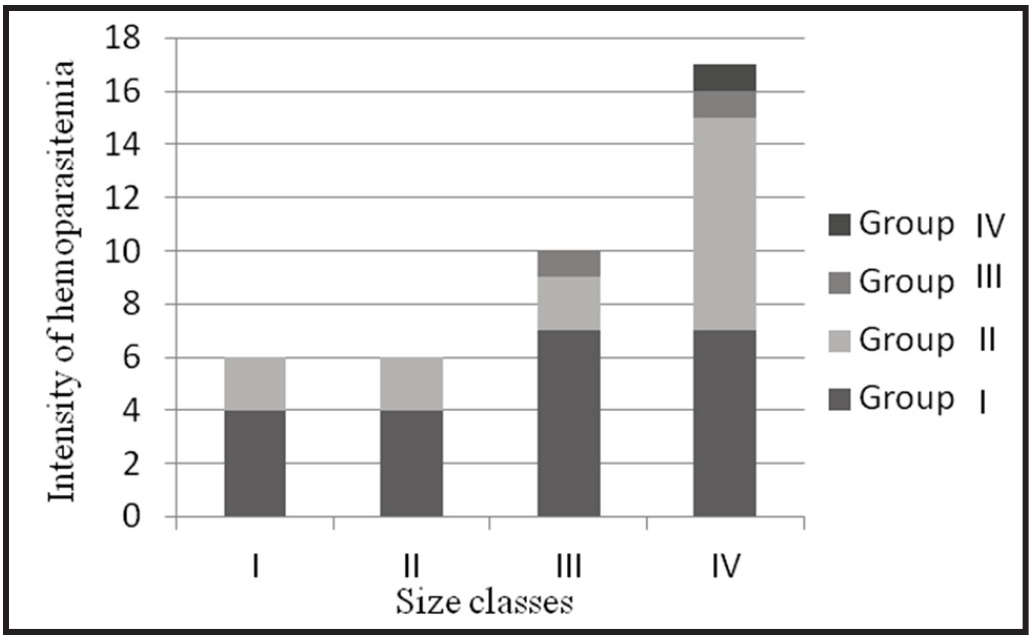

Figure 3. Intensity of hemoparasitemia between different age classes of wild-caught Cottonmouths (*Agkistrodon piscivorus*) from central North Carolina, USA. Classification of intensity: group I, no Hemogregarine-like parasites; group II, 1–2 parasites found per 10 high powered microscope field (100x objective); group III, 3–4 parasites per 10 high powered field; or group IV, ≥ 5 parasites per 10 high powered field.

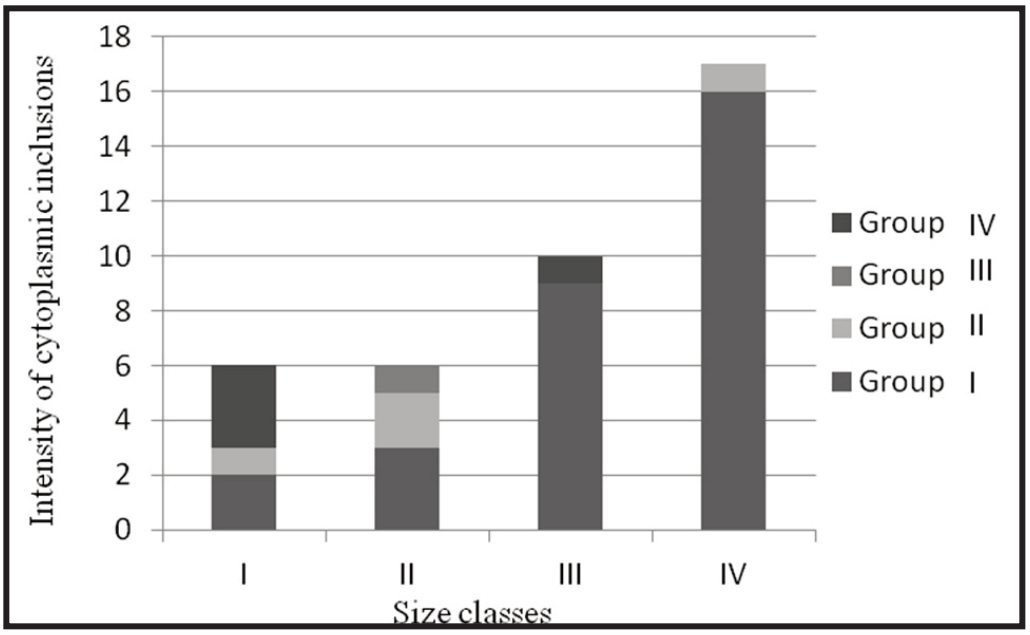

Figure 4. Intensity of intraerythrocytic inclusions between different age classes of wild-caught Cottonmouths (*Agkistrodon piscivorus*) from central North Carolina, USA. Classification of intensity: group I, no cytoplasmic inclusions; group II, 1–2 inclusion found per 10 high powered microscope field (100x objective); group III, 3–4 inclusions per 10 high powered field; or group IV, ≥ 5 inclusions per 10 high powered field.

results in a way that would alter clinical interpretation, and we chose to retain these animals in the cohort to avoid potentially artificially narrowing the baseline range.

We found significant differences in the PCV between the sexes, with higher PCVs in males compared to females, which has been reported in Eastern Massasauga Rattlesnakes (*Sistrurus catenatus catenatus*), Anacondas (*Eunectes murinus*), and Grass Snakes (*Natrix natrix natrix*; Wojtaszek 1991; Calle et al. 1994; Allender et al. 2006). The PCVs of the female Cottonmouths sampled in our study were similar to those reported for gravid female Cottonmouths (Birchard et al. 1984). While gender variation in PCV has been reported in several species of reptiles, the differences observed in our study may be explained by the significant positive correlation between the PCVs and the SVL, masses, and presumably the age of the Cottonmouths sampled in our study (Frye 1991). Male Cottonmouths sampled during this study were overall larger than female snakes sampled, supporting the potential interpretation that the apparent sex difference in PCV might not be a sex associated difference per se, but rather a difference in size and/or age.

The low frequency of erythrocytic viral inclusions varying in both size and staining characteristics are similar to previously described *Toddia* sp. protozoal hemoparasites in *Agkistrodon piscivorous*, but later shown to be consistent with iridoviral inclusions by ultrastructural studies in Northern Water Snakes (Marquardt and Yeager 1967; Smith et al. 1994). No clinically observed detrimental effects were observed in the snakes that had erythrocytic viral inclusions when judged in comparison to snakes without the inclusions. Our finding that lymphocytes were the most numerous leukocytes noted in Cottonmouths is consistent with reports in other snakes and reptiles (MacMahon and Hamer 1975; Alleman et al. 1999; Lamirande et al. 1999; Salakij et al. 2002; Allender et al. 2006). While it has been reported that female reptiles tend to have a higher lymphocyte count than males (Duguy 1970; Campbell 2006a), no significant difference between sexes was discernible in this study. We did not document eosinophils in any of the snakes captured during this study, but the reported number of circulating eosinophils in healthy snakes varies among species (Duguy 1970; Montali 1988; Dotson et al. 1995; Alleman et al. 1999; Campbell 2006a). Some authors have suggested that ophidians do not have eosinophils and others have proposed that what is being described in the literature as eosinophils is most consistent with a second type of heterophil (Montali 1988; Dotson et al. 1995; Alleman et al. 1999). While the significance of eosinophils in reptiles is unknown, it is suggested they may be associated with parasitism and immune system stimulation (Mead and Borysenko 1984; Strik et al. 2007). If eosinophils are associated with a response to parasitism, there did not appear to be a correlation of the presence of either hemogregarine-like parasites or erythrocytic viral inclusions with eosinophil count in this study. More than half of the snakes sampled were infected with either hemogregarine-like parasites or erythrocytic viral inclusions though no eosinophils were documented in their blood. It is possible these snakes were free of other endoparasites, but it would seem unlikely for wild caught snakes. We did not sample for endoparasites. Azurophilic-monocytes were the second most frequently observed leukocyte in snakes captured during this study. The cytochemical and ultrastructural similarities and the extremely low numbers of monocytic cells without the azurophilic granules observed precluded confident segregation of these cells from monocytes, and for that reason, we grouped monocytes with and without azurophilic granules together in for the differential cell count (Montali 1988; Dotson et al. 1995; Campbell and Ellis 2007).

No snakes exhibited clinical signs of disease, and there was no apparent relationship between the severity of erythrocytic hemogregarine-like parasitemia and hematologic parameters examined. Hemogregarine-like parasites are commonly encountered in reptiles, and often considered an incidental finding (Campbell 2006b); however, animals with severe infections have been reported to exhibit hemolytic anemia and leukocyte derangements characterized by a monocytosis and lymphocytosis (Nadler and Miller 1985; Wozniak et al. 1996; Wozniak et al.

1998; Campbell 2006b; Bonadiman et al. 2010). We did not observe these relationships in our study.

Based on the clinical experiences with captive snakes at the North Carolina Zoo, we expected the larger and presumably older snakes to have an increase in their baseline azurophilic-monocyte count. We also expected increased duration of exposure to parasite vectors for older snakes to correlate with a more intense parasitism by hemogregarine-like parasites. Our study did not support either relationship. It was noted, however, that the two largest snakes sampled were the animals with the highest azurophilic-monocyte counts, similar to what has been observed in the Cottonmouths procured from this study site for exhibition at the North Carolina Zoo. Monocytosis is often associated with granulomatous inflammation caused by chronic parasitic or bacterial infections (Dotson et al. 1995; Jacobson et al. 1997; Campbell and Ellis 2007). While rare in reptiles, myeloproliferative diseases have also been reported (Frey and Carney 1972; Goldberg and Holshuh 1991). Neither of these animals appeared clinically ill, nor were they heavily parasitized by hemoparasites or burdened by erythrocytic viral inclusions. Regrettably, we performed no gastrointestinal endoparasite sampling during this study. Our failure to detect a heavy erythrocytic parasitism in these larger snakes could have been due to intense natural selection exerted by removing individuals more susceptible to the heavy parasitism from the population for exhibition, though the sampling pressure at this site has not been particularly intensive. It is possible older animals develop an immune response to the hemoparasites that controls their proliferation. Perhaps, most likely, the monocytosis may be a response to an entirely different subclinical condition not examined in our study, and not related to the intensity of hemogregarine or erythrocyte viral inclusion burdens. Despite our inability to elucidate the cause of the monocytosis observed in the largest specimens from the collection site examined, this study evaluated hematological and plasma biochemical values of free-ranging Cottonmouths from North Carolina that can provide both veterinarians and research biologists with baseline reference intervals. These data will be useful for comprehensive medical assessment of this species in captivity, and for monitoring their health status in the wild.

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