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Hematology and Serum Chemistry Values in the Critically Endangered Red-Crowned Roofed Turtle, *Batakur kachuga* and Three-stripped Roofed Turtle, *B. dhongoka*

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Abstract.—**As part of routine health evaluation, we collected blood samples for hematological and serum biochemical profiling from 10 Red-Crowned Roof Turtles (*Batagur kachuga*) and 10 Three-Striped Roofed Turtles (*B. dhongoka*) older than 2 y held in captivity at the Turtle Breeding and Rehabilitation Centre, Sarnath, India. We housed both species in the same location in species-specific enclosures and turtles were exposed to similar environmental conditions with feeding regimens based on their specific needs. The hematological parameters considered for the study included monocytes, heterophils, eosinophils, and lymphocytes. The serum biochemical parameters included analysis of total protein, albumin, globulin, creatinine, aspartate transaminase, alanine transaminase, urea, uric acid, potassium, sodium, chloride, calcium, and phosphorus. The findings of the hematological and serum biochemical profiling varied significantly between species for monocyte, heterophil count, creatinine, uric acid, and calcium.**

Key Words.—chelonians; rehabilitation; serum biochemistry; subcarapacial venous plexus

Introduction

Freshwater turtles can be considered as ecological engineers that act as natural scavengers and comprise a large fraction of the biomass in many ecosystems (Richardson 2011). Globally, there are approximately 300 species of freshwater chelonians, of which 200 are listed as threatened by the International Union for Conservation of Nature (IUCN; Sarwar et al. 2015). Of these, 75% that are listed as Critically Endangered, Endangered, or Vulnerable are found in Asia (Sarwar et al. 2015). Of the 28 species found in India, 22 are freshwater turtles with at least 14 species occurring along the Ganga Basin (Das 1995). Among these, the Red-Crowned Roofed Turtle (*Batagur kachuga*) and Three-Striped Roofed Turtle (*B. dhongoka*) have significant conservation value; both have been listed as Critically Endangered based on the IUCN Red List (Das et al. 2019; Praschag et al. 2019). The two species are sympatric in the wild and their natural histories are similar (Campbell 2012). Habitat degradation, environmental pollution, consumptive exploitation, agriculture runoff, and large-scale extraction of water resources for agriculture have resulted in declines in their populations (Das 1995). Though a few studies addressing species attributes (Das 1995), status and distribution (Subramaniam Bhupathy, unpubl. report), reproductive output (Sirsi et al. 2017), and phylogeny and taxonomy (Praschag et al. 2007) have been carried out for *Batagur* species, the published literature lacks information on health parameters for either species. Health parameters such as baseline hematology and serum biochemistry data are crucial for assessing the health status of both individuals and populations and can also be used to assess habitat quality (Lassen 2004). We undertook a study to establish baseline reference values of select hematological and serum biochemical parameters in captive *B. kachuga* and *B. dhongoka* that were deemed healthy.

Materials and Methods

We carried out the study on *B. kachuga* and *B. dhongoka* held in captivity at the Turtle Breeding and Rehabilitation Centre, Sarnath, Varanasi, Uttar Pradesh, India. The animals were hatched at a rehabilitation center and were housed separately in two open enclosures. We feed turtles 5–15% of the total body weight on a daily basis. We maintained animals under similar environmental conditions and feeding regimens.

We offered commercial turtle food (Taiyo Turtle Food, Taiyo Group, Tamil Nadu, India) to the animals for the initial 3 mo followed by fish for up to 1 y. Subsequently, we provided a vegetable and fish diet based on total caloric requirements of 35–128 kcal/day/kg. Nominally, the minimum content of the commercial diet included

crude protein (39–46.5%), fat (8.8%), calcium (5.7%), phosphorus (3.0%), methionine (1.03%), and cysteine (0.25%). Additionally, we provided environmental enrichment of the enclosure for both the species that included logs for basking, Duckweed (*Lemna minor*) for cover, supplemental light during winter, shrub shade during summer, and nutritional enrichment with feeding of live fish and commercial turtle food. We also provided mats at exit points to support animals coming out of water for basking and to minimize plastron injuries from abrasive surfaces. We conducted a 20% water change daily, complete cleaning of enclosures monthly, and replaced sand quarterly.

We examined each animal for signs of trauma, weight loss, muscle atrophy, and other abnormalities prior to biological sampling and we selected only healthy individuals in good condition with no obvious sign of disease or injury. We randomly selected 10 each of *B. kachuga* and *B. dhongoka* > 2 y old and restrained them physically. To determine a health profile for these turtles, we drew approximately 0.5–1.5 ml of blood aseptically from the sub-carapacial venous plexus using a sterile syringe and a 23-gauge needle (Hernandez-Divers 2006). We prepared the blood smears from fresh blood samples for hematological study and collected the remaining sample in clot activator vials for biochemical analysis. All the samples with suspected hemolymph contamination were excluded from the study.

We centrifuged the samples at 149–208 g (Relative Centrifugal Force) for 15 min to extract serum and we refrigerated and analyzed the serum within 6 h of collection. We analyzed the hematological parameters that included manual differential leucocyte count (monocyte, heterophils, eosinophils, lymphocytes) using Leishman-stained slides prepared from fresh blood samples. We analyzed serum samples at room temperature by using Eppendorf ECOM-F 6124 semi-automatic biochemical analyzer (Mannheim Bio-medicals Limited, Mumbai, India). We used Erba biochemical reagent kits (Transasia Bio-medicals Ltd., Daman, India) for total protein (TP), albumin, globulin, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), urea, uric acid, potassium, sodium, chloride, calcium, and phosphorus. The methodology and the reagents used for each parameter were as per the recommendations of the manufacturer of the analyzer system. Hematological and serum biochemical data were not normally distributed; therefore, we used non-parametric Mann-Whitney U tests ($\alpha = 0.05$) to compare analytes between species. We calculated within-species means, medians, and standard deviations for each variable.

Results

The median value for differential white blood cell count such as Heterophils (19.5) and lymphocytes (70) were highest in *B. kachuga* and monocytes (6) and eosinophils (7) were highest in *B. dhongoka*. Among the differential white blood cell count, the values of monocytes were significantly higher in *B. dhongoka* than in *B. kachuga* ($U = 23$, df = 18, $P = 0.031$) though heterophils were significantly higher in *B. kachuga* ($U = 17$, df = 18, $P = 0.010$). Eosinophils and lymphocytes did not vary significantly between the two species. Although the values of biochemical constituents varied between *B. kachuga* and *B. dhongoka*, the values for creatinine ($U = 14$, df = 18, $P = 0.010$), uric acid ($U = 18.5$, df = 18, $P = 0.021$) and calcium ($U = 23$, df = 18, $P = 0.041$) were significantly higher in *B. kachuga* than in *B. dhongoka*.

Discussion

The published information on the hematological and biochemical profile for *Batagur* species is limited to a single quantitative study on *B. kachuga* that reported total erythrocyte and total leucocyte counts (Bardi et al. 2018). We compared the values obtained in our study for *B. kachuga* and *B.dhongoka* with other turtle species, including the Eastern Box Turtle (*Terrapene carolina*), Ornate Box Turtle (*Terrapene ornata*), Yellow-bellied Slider Turtle (*Trachemys scripta*), Painted Turtle (*Chrysemys picta*), Southeast Asian Box Turtle (*Cuora amboinensis*), and Pascagoula Map Turtle (*Graptemys gibbonsi*; Mitruka and Rawnsley 1981; Jacobson 1988; Crawshaw and Holz 1996; Stein 1996; Perpinan et al. 2008). Monocyte, heterophil, and eosinophil counts that we obtained were similar to those reported for *C. amboinensis* and *G. gibbonsi* (Perpinan

Table 1. Hematological values of 10 Red-crowned Roofed Turtles (*Batagur kachuga*) and 10 Three-striped Roofed Turtles (*Batagur dhongoka*) at the Turtle Breeding and Rehabilitation Centre, Sarnath, Varanasi, Uttar Pradesh, India. The test statistics are for Mann-Whitney U tests. The abbreviation SD = standard deviation.

	Batagur kachuga			*Batagur dhongoka*			Test statistics	
Parameters (%)	Range	Median	Mean ± SD	Range	Median	Mean ± SD	U	*P*
Monocytes	4–6	4	4.60 ± 0.84	4–8	6	5.90 ± 1.45	23	0.031
Heterophils	15–22	19.5	18.9 ± 2.38	15–18	16	16.2 ± 1.14	17	0.010
Eosinophils	4–10	6	6.30 ± 1.83	6–10	7	7.00 ± 1.25	36	0.274
Lymphocytes	66–76	70	70.4 ± 2.91	66–72	69.5	69.6 ± 2.17	42.5	0.572

Table 2. Serum biochemistry values of 10 Red-crowned Roofed Turtles (*Batagur kachuga*) and 10 Three-striped Roofed Turtles (*Batagur dhongoka*) at the Turtle Breeding and Rehabilitation Centre, Sarnath, Varanasi, Uttar Pradesh, India. The test statistics are for Mann-Whitney U tests. Abbreviations are ALT = Alanine transaminase, AST = Aspartate transaminase, and SD = standard deviation.

Parameters (Unit)	*Batagur kachuga*			*Batagur dhongoka*			Test statistics	
	Range	Median	Mean ± SD	Range	Median	Mean ± SD	U	P
AST (IU/L)	28.7–68.2	43.25	47.1 ± 15.91	34.1–84.1	55.2	57.2 ± 18.4	35	0.260
ALT (IU/L)	20–50	36.25	35.0 ± 10.19	20.0–56.2	40.0	37.6 ± 12.9	42	0.540
Albumin (g/dL)	2.0–4.1	3.20	3.37 ± 0.66	2.3–4.1	3.2	3.3 ± 0.58	45.5	0.732
Globulin (g/dL)	2.1–4.3	3.37	3.27 ± 0.65	2.1–3.7	3.2	3.1 ± 0.57	39	0.430
Creatinine (mg/dL)	0.26–0.97	0.575	0.61 ± 0.27	0.73–1.2	0.96	0.94 ± 0.13	14	0.010
Total protein (g/dL)	4.8–8.3	6.83	6.68 ± 1.02	5.1–7.7	6.5	6.3 ± 0.90	41	0.510
Urea (mg/dL)	17.2–36.2	22.1	23.2 ± 5.90	17.2–46.0	33.4	30.9 ± 9.6	29.5	0.124
Uric Acid (mg/dL)	4.7–8.7	5.96	6.12 ± 1.20	2.9–6.8	4.4	4.7 ± 1.2	18.5	0.021
Potassium (mEq/L)	3.66–6.87	5.25	5.24 ± 0.97	2.9–9.0	5.3	5.2 ± 1.7	47.5	0.853
Sodium (mEq/L)	118.5–140.7	122.9	125.0 ± 6.63	118.7–140.0	122.0	126.6 ± 7.9	45.5	0.737
Chloride (mEq/L)	88.1–102.0	89.6	91.5 ± 4.51	88.0–109.8	94.3	96.3 ± 6.9	33.5	0.210
Calcium (mg/dL)	5.9–9.1	8.05	7.89 ± 1.01	7.8–9.7	8.7	8.7 ± 0.63	23	0.041
Phosphorus (mg/dL)	2.1–3.8	2.73	2.74 ± 0.52	2.1–4.1	2.6	2.9 ± 0.71	45	0.721

et al. 2008), while the values differed from *T. carolina* with fewer monocytes and more heterophils found in our study (Mitruka and Rawnsley 1981; Frye 1994; Stein 1996). Lymphocytes were higher as compared to values reported for *T. carolina*, *C. amboinensis*, and *G. gibbonsi* (Mitruka and Rawnsley 1981; Frye 1994; Stein 1996; Perpinan et al. 2008).

The range of serum biochemical parameters for AST, albumin, globulin, creatinine, total protein, uric acid, sodium, potassium, chloride, calcium, and phosphorus in *B. kachuga* and *B. dhongoka* in our study were found to be similar to those recorded in *T. carolina, T. ornata* and *T. scripta* (Mitruka and Rawnsley 1981; Jacobson 1988; Frye 1994; Crawshaw and Holz 1996; Stein 1996). The values for ALT were lower in *T. carolina* (Mitruka and Rawnsley 1981; Frye 1994; Stein 1996). The values we found for AST, creatinine, total protein, sodium, calcium, and phosphorus were also similar to those recorded for *C. picta* except that albumin, globulin, uric acid, and chloride found higher in *C. picta* than in *B. kachuga* and *B. dhongoka* (https://www.isis.org).

The specific reasons for variations in the values obtained in the study could not be conclusively determined; however, such variations may be attributed to extrinsic factors such as temperature, environmental conditions, husbandry, diet, and living conditions, or intrinsic factors (e.g., species and sex) that are known to alter these parameters (Campbell 2012). We documented a comparative profile of select hematological and biochemical parameters of two sympatric species that were reared in captivity and housed under similar conditions. These are the first data of blood chemistry for these species, although we recognize that these data are based on limited population testing and do not account for variations within sub-populations defined by age, sex, subspecies, or extrinsic factors such as instrumentation and blood collection techniques. Further studies with larger sample sizes from free-living populations would be highly advantageous to make the results most reflective of the healthy population.

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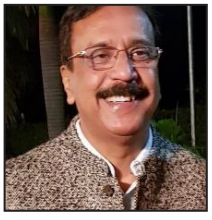

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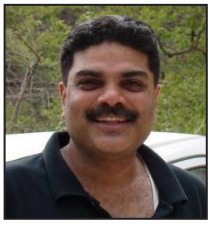

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