Journal of Zoo and Wildlife Medicine 38(3): 425–432, 2007

HEMATOLOGIC AND PLASMA BIOCHEMICAL ANALYSIS OF JUVENILE HEAD-STARTED NORTHERN RED-BELLIED COOTERS (*PSEUDEMYS RUBRIVENTRIS*)

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Abstract: The Massachusetts population of the northern red-bellied cooter (*Pseudemys rubriventris*) is listed as federally endangered due to its extremely restricted geographic range and low population. A captive rearing program has been used since 1984 to augment the population. Blood from 30 juvenile specimens from three rearing institutions was collected prior to release, and hematologic and plasma biochemical data were analyzed. Results were generally consistent with previously published data for other species of the family Emydidae. Basophils were the most numerous type of leucocyte. Results for some values varied significantly between institutions, possibly due to unrecognized differences in husbandry at each facility.

Key words: Hematology, plasma biochemistry, *Pseudemys rubriventris*, red-bellied cooter, reptile, turtle.

INTRODUCTION

In 1980, the Massachusetts population of the northern red-bellied cooter (*Pseudemys rubriventris*) was listed as federally endangered due to its extremely restricted geographic range and low population. At that time, fewer than 250 specimens were known to exist in only six ponds in Plymouth County, Massachusetts, USA.[14,18,37,38] As part of the management plan for the species, a head-start program was established in 1984.[18] Nesting areas are monitored throughout the spring egg-laying season, and nests are protected with wire cages to avoid predation. Upon hatching in late summer, a portion of hatchlings are removed from the nests and transferred to a number of local zoos, aquaria, nature centers, and schools, where they are raised under captive conditions for approximately 9 mo. The captive turtles grow quickly. When they are released into the wild the following spring, they are generally two to six times larger than wild cohorts. A positive correlation between body size and survival of head-started turtles from this population has been demonstrated.[18] As a result of the head-start program and other conservation measures, the Massachusetts population of northern red-bellied cooters now contains approximately 2,000–3,000 individuals, and head-started females have begun to reproduce (Dr. Terry Graham, Greenfield Community College, Greenfield, MA 01301, USA, 2005, pers. comm.). This project evaluated selected hematologic and plasma biochemical values of head-started northern red-bellied cooters at three institutions.

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MATERIALS AND METHODS

Blood was collected from 30 9-mo-old, head-started turtles that had been obtained as hatchlings from five protected nests (nests A–E). Blood sampling was conducted as part of the routine health assessment of the turtles prior to release to the wild. Gender of the turtles was not determined, as they had not yet developed sexual dimorphism. The turtles had been reared by three institutions (I1–I3). I1 raised four turtles from nest A; two turtles from nest B; and four turtles from nest C. I2 raised seven turtles from nest D and three turtles from nest E. I3 raised 10 turtles from nest E. General husbandry guidelines had been provided to each institution by the fish and wildlife service of the state of Massachusetts, MassWildlife. The guidelines stated that turtles should be kept in an aquatic environment with a volume of 20 L per turtle, a dry basking area, an infrared or incandescent basking light, an ultraviolet light, filtration or frequent water changes; an 8–12-hr day length, and water temperature of 28–30°C. The recommended diet was a majority of romaine or red-leaf lettuce, supplemented with a floating pelleted turtle diet (Reptomin®, Tetra Terra Fauna, Inc., Blacksburg, Virginia 24060, USA). Despite general guidelines, there may have been differences in husbandry at each institution. Temperature, light spectrum and intensity, caloric intake, and exact dietary rations may have varied between institution, and incomplete recording of such data prevents full determination of these parameters.

Blood was collected from 10 turtles at the institution at which they were raised (I1); 20 turtles (I2

and I3) were sampled at a central gathering point after a 2-hr car ride, and a 6–9-hr holding period. Ambient temperature at the time of collection was 23°C for I1, and 26°C for I2 and I3. A representative of each institution was questioned regarding the general history and physical condition of the group, and each turtle was weighed and physically examined. One milliliter of blood was collected from the subcarapacial venipuncture site with the use of a 1-ml syringe and 25-gauge needle.[20] Samples with visible lymph contamination were discarded, and the venipuncture repeated. Blood smears were prepared immediately, and the remainder of the blood was transferred to two heparinized Microtainer® tubes without separator gel (Becton Dickinson and Company, Franklin Lakes, New Jersey 07417, USA). One tube was immediately centrifuged at 3000 rpm for 5 min, and the plasma was harvested and refrigerated at 4°C. Plasma biochemical assays were performed within 18 hr. Heparinized whole blood from the second microtainer tube was immediately refrigerated at 4°C, and hematology evaluation was performed within 2 hr.

Hematocrit (HCT) was measured after centrifugation of whole blood at 12,000 g for 3 min in heparinized capillary tubes. Total white blood cell count (WBC) was calculated with the use of the Eosinophil Unopette® (Becton Dickinson and Company, Franklin Lakes, New Jersey 07417, USA) technique.[7] Differential white blood cell count was performed by counting 100 white blood cells on fixed blood smears stained with Dip Quick® (Jorgensen Laboratories Inc., Loveland, Colorado 80532, USA). Plasma biochemistry values, including aspartate aminotranferase (AST), creatine kinase (CK), total protein (TP), albumin (ALB), globulin (GLOB), blood urea nitrogen (BUN), sodium (Na), potassium (K), uric acid (UA), calcium (Ca), phosphorus (P), and glucose (GLU), were measured with the use of the VetScan® chemistry analyzer Avian/Reptilian Profile Chemistry Rotor (Abaxis Inc., Union City, California 94587, USA).

Subsequent to release of the 30 study turtles, blood was collected from five additional 5-mo-old head-started red-bellied cooters to verify the correct identification of basophils. Blood smears from these turtles were stained with Dip Quick®, as well as Wright-Leishman stain, after a 3-min fixation period to improve staining of basophil granules.

The distribution of turtles from each nest was not equal across all institutions, with nest E being the only nest to have turtles placed at two institutions. Thus, to determine if the physiologic data were best analyzed grouped by institution or by nest, a forward, stepwise regression (SigmaStat 3.1, Systat, Richmond, California 94804, USA) was used to determine which factor was more important in determining differences in the data groupings. The forward stepwise regression was conducted by setting either the institution or nest as the dependent variable, and then determining which of the biochemical variables entered the model ($F = 4.00$ to enter and 3.99 to remove).

Once the institution or nest was determined to be the significant grouping category, the individual parameters were then analyzed with a one-way ANOVA with paired comparisons determined by Tukey's test. Those data failing to meet the assumptions of normality and equal variance were analyzed with a Kruskal–Wallis one-way ANOVA on ranks. White blood cell data were not analyzed statistically because of the high coefficient of variation of manual white blood cell counts, and the relatively low sample size.[30]

RESULTS

The forward stepwise regression demonstrated that four parameters (weight, K, P, and TP) contributed to differences between nests, and accounted for 83.1% of the total variation ($F_{4,25} = 30.76$, $P < 0.001$). In comparison, the parameters of Na, weight, BUN, P, K, and UA contributed to differences between institutions, and accounted for 88.2% of the variation $F_{6,23} = 67.35$, $P < 0.001$). Three parameters (weight, K, and P) were included in each model, accounting for 78.5% and 81.6% of variation in the nest and institution analysis, respectively. However, because the analysis using institution as the primary division resulted in a more inclusive model, it was determined that this was the primary grouping factor against which to assess differences in physiologic parameters.

Sodium was the only factor in which all three institutions differed (Table 1), and was also likely the reason that this parameter accounted for 76% of the variation between institutions. Even with this statistically significant difference, the largest Na value (that of I3) was only 9% greater than that of the smallest value (I1, Table 1). The second variable to enter the stepwise regression was weight. I1, which had turtles from three nests, had a lower average weight than did the other two institutions (Table 1). These first two factors accounted for 85.9% of the variation in determining institution.

Mean, median, standard deviation, minimum, and maximum values for hematologic and plasma biochemical data are presented in Table 1. Minimum and maximum values are presented based on current recommendations of the American Society for Veterinary Clinical Pathology for studies involving less than

40 individuals.[11] Basophils were the most numerous type of leucocyte for I1 and I2, and the second most numerous leucocyte for I3. Heterophils were the most numerous type of leucocyte for I3, and third most numerous for I1 and I2. Lymphocytes were the second most numerous leucocyte for I1 and I2, and third most numerous for I3. Relatively few eosinophils were encountered, and monocytes were only observed in 17% of the turtles.

Basophils were generally 12–15 μm in diameter and were characterized by a pale, blue, central to slightly eccentric nucleus that was often obscured by the presence of numerous, deeply basophilic cytoplasmic granules. On Dip Quick®-stained smears, basophil cytoplasm often appeared somewhat vacuolated or moth-eaten due to degranulation of their water-soluble granules under conditions of inadequate fixation. Basophil granules were better preserved and more distinct in well-fixed Wright–Leishman-stained smears. Heterophils were generally 13 μm in diameter, and were characterized by eccentric, circular to slightly irregular nuclei; relatively low nuclear to cytoplasmic ratio; and numerous spindle-shaped, pink to orange cytoplasmic granules. Lymphocytes were generally 8–10 μm in diameter, and were characterized by a relatively high nuclear to cytoplasmic ratio, with basophilic, circular, central nuclei and scant to moderate blue cytoplasm. Eosinophils were generally 14–18 μm in diameter, and were characterized by basophilic, bilobed to irregular eccentric nuclei; relatively low nuclear to cytoplasmic ratio; and numerous round, pink to orange cytoplasmic granules. Monocytes were generally 13 μm in diameter and were irregular to circular in shape, with large, basophilic, bilobed to irregular nuclei; moderate, lightly basophilic cytoplasm; and occasional clear cytoplasmic vacuoles. Thrombocytes were not counted, but were noted in all samples. Thrombocytes were approximately 16 × 6 μm, elliptical, with central ovoid basophilic nuclei, lightly basophilic cytoplasm, and were often noted in small clusters. Erythrocytes were generally 20 × 10 μm, elliptical, with basophilic central nuclei, and light grey to pink cytoplasm. No hemoparasites were noted.

Turtles reared at I1 had lower weight and hematocrit, and higher P and K values compared to those reared at I2 and I3. Turtles from I3 had a greater BUN than turtles from I1 and I2. GLOB was greatest for I2, but the overall power of this test ($a = 0.05$) was low (0.547). GLU was lower for I2 turtles than for I1 and I3 turtles. AST, CK, TP, ALB, UA, and Ca did not differ between institutions.

DISCUSSION

The data presented herein provide hematologic and plasma biochemical values for 30 juvenile, head-started northern red-bellied cooters. Although many values were found to be statistically similar among all turtles, a number of parameters varied significantly between institutions. For example, the weight of the turtles from I1 was much lower than that of turtles from I2 and I3. Although it is possible that this illustrates genetic variation of growth rate, this is unlikely, because individuals from three different nests were held by I1, and they all grew similarly. It is more likely that the parameters that varied among institutions were influenced by variation in precise diet, caloric intake, temperature, water chemistry, etc. Although husbandry guidelines are provided to each institution, it is likely that the day-to-day care of the turtles varied among institutions. The variation in turtle size noted in this study is consistent with that seen over the past 20 yr among various head-start facilities in Massachusetts, and is not considered atypical (Dr. Terry Graham, Greenfield Community College, Greenfield, Massachusetts, USA, pers. comm.; David Taylor, Massachusetts red-bellied cooter head-starting program coordinator, P.O. Box 263, Byfield, Massachusetts 01922, USA, unpubl. data).[18]

Significant differences in hematologic and plasma biochemical data for chelonians have been noted with different laboratory methodologies, different venipuncture sites, and seasonally.[1,2,6,9,10,12,13,22,24] For example, significant differences in white blood cell counts of loggerhead sea turtles (*Caretta caretta*) were noted using the Natt–Herrick staining technique vs. the Eosinophil Unopette technique.[2] Lymph contamination may produce significant differences in some hematologic and plasma biochemical parameters in samples drawn from the dorsal coccygeal vein, subcarapacial venipuncture site , or postoccipital venous plexus of chelonia.[6,13,20] Preliminary attempts at jugular venipuncture of several specimens in this study indicated that sedation would be required for consistent access, and an alternative site was chosen. The subcarapacial site was easily accessed, and blood was reliably obtained. In rare cases, gross lymph contamination was noted, and the sample was discarded. Hence, it is possible that undetectable lymph contamination may have influenced hematologic and plasma biochemical results from this site. For example, the results for total protein and potassium in this study are more similar to lymph-contaminated samples than uncontaminated samples for red-eared sliders, *Trachemys scripta elegans*.[6] Lymph contamination

Table 1. Mean, median, standard deviation (SD), minimum, and maximum values of weight, hematology, and plasma biochemistry parameters of 30 juvenile, captive, head-started red-bellied cooters from three institutions.

	Institution 1 (n = 10)				
	Mean	Median	SD	Minimum	Maximum
Weight (g)[a]	153[c]	148	20	121	183
Hematocrit (%)[b]	19	19[c]	4.1	14	26
WBC (cells/μl)	15,545	14,076	7,297	5,714	26,253
Heterophils (cells/μl)	1,434	1,356	902	514	3,150
Lymphocytes (cells/μl)	4,554	4,344	2,823	1,028	8,500
Eosinophils (cells/μl)	1,038	1,083	503	0	1,801
Basophils (cells/μl)	8,524	6,785	5,035	3,485	17,852
Monocytes (cells/μl)[f]	65.6	0	144	0	410
AST (IU/L)[b]	69	64	15	51	93
CK (IU/L)[a]	817	858	315	321	1,351
GLU					
(mmol/L)[b]	4.1	4.1[c]	0.7	3.1	5.0
[mg/dl][b]	74	74[c]	12	57	90
TP					
(g/L)[b]	28	26	5	23	38
[g/dl][b]	2.8	2.6	0.5	2.3	3.8
ALB					
(g/L)[a]	14	14	2	12	19
[g/dl][a]	1.4	1.4	0.2	1.2	1.9
GLOB					
(g/L)[a]	13.7[c,d]	12.5	3.0	11	19
[g/dl][a]	1.37[c,d]	1.25	0.3	1.1	1.9
BUN					
(mmol/L)[a]	2.7[c]	2.6	1.0	1.4	4.2
[mg/dl][a]	7.8[c]	7.5	3	4	12
Ca					
(mmol/L)[a]	2.5	2.5	0.15	2.2	2.7
[mg/dl][a]	9.8	9.9	0.6	8.8	10.7
P					
(mmol/L)[b]	1.9	2.0[c]	0.3	1.6	2.6
[mg/dl][b]	6.0	6.2[c]	1.0	4.7	7.8
Ca:P[b]	1.7	1.7[c]	0.3	1.3	2.0
K (mmol/L)[a]	4.7[c]	4.9	0.4	4	5.2
Na (mmol/L)[b]	135	135[c]	1.7	132	138
UA					
(mmol/L)[a]	0.06	0.06	0.02	0.03	0.08
[mg/dl][a]	1.0	1.0	0.3	0.5	1.3

[a] Normally distributed data, analysis by Tukey's test one-way ANOVA.

[b] Not normally distributed data, analysis by Kruskal–Wallis one way ANOVA on ranks.

[c,d,e] Different superscripts in the same line indicate significant difference between institutions.

[f] Number of individuals with monocyte count > 0, I1 = 2, I2 = 0, I3 = 3. Zero values included in calculation of mean and median value.

could also explain the lower hematocrit seen in I1. It is emphasized that comparison of future values to those reported herein must consider possible differences of laboratory methods, venipuncture sites, and time of year. Future studies of red-belly turtle hematology and plasma biochemistry should consider obtaining blood from both the subcarapacial site and the jugular vein to better assess the possibility of lymph contamination.

Hematocrit values for *P. rubriventris* are similar

Table 1. Extended.

Institution 2 (n = 10)					Institution 3 (n = 10)				
Mean	Median	SD	Minimum	Maximum	Mean	Median	SD	Minimum	Maximum
536[d]	559	81	346	624	576[d]	615	159	324	838
27	29[d]	3.5	20	31	26	29[d]	7	15	34
10,835	11,395	5,436	2,750	18,066	12,903	11,351	5,323	8,761	27,121
2,057	2,278	756	742	3,121	4,911	3,736	756	3,047	15,730
3,163	2,165	3,021	581	9,379	3,015	2,387	2,187	388	2,387
460	398	349	0	1,139	644	578	349	225	1,358
5,132	4,048	3,640	1,127	12,826	4,282	4,496	1,611	1,659	6,729
0	0	0	0	0	50	0	87	0	248
63	61	5	59	72	66	66	2.8	62	70
845	723	270	604	1,397	778	754	152	608	1,045
3.1	3.0[d]	0.7	2.5	5.1	4.1	4.1	0.5	3.2	5.0
58	55[d]	13	45	91	74	75[c]	9	58	90
32	31	7	25	47	27	25	3	25	34
3.2	3.1	0.7	2.5	4.7	2.7	2.5	0.3	2.5	3.4
14	14	2	12	18	14	12	3	11	18
1.4	1.4	0.2	1.2	1.8	1.4	1.2	0.3	1.1	1.8
18.2[c]	18.5	7.0	10	29	13.1[d]	12.0	3.0	11	18
1.82	1.85	0.7	1	2.9	1.31[d]	1.2	0.3	1.1	1.8
3.5[c]	3.8	1.1	2.1	5.9	12.1[d]	11.3	4.2	5.6	18.5
10.5[c]	11.5	3	6	17	34.6[d]	32.5	12	16	53
2.5	2.5	0.18	2.3	2.9	2.4	2.4	0.08	2.3	2.6
10.0	9.8	0.7	9.2	11.5	9.6	9.6	0.3	9.1	10.3
1.2	1.1[d]	0.3	0.7	1.7	1.2	1.1[d]	0.1	1.0	1.4
3.5	3.2[d]	0.9	2	5.2	3.5	3.4[d]	0.4	3	4.1
3.0	3.0[d]	0.7	2.2	4.7	2.8	2.8[d]	0.3	2.2	3.4
3.8[d]	3.8	0.2	3.5	4.1	4.2[d]	4.2	0.6	3.3	5.2
140	140[d]	3.5	135	146	148	149[e]	3.0	143	151
0.05	0.05	0.02	0.02	0.06	0.05	0.05	0.01	0.03	0.07
0.8	0.8	0.3	0.3	1.1	0.9	0.9	0.2	0.5	1.2

to those reported for several other freshwater, aquatic chelonia of the family *Emydidae*, including bog turtles (*Glyptemys* [*Clemmys*] *muhlenbergii*), painted turtles (*Chrysemys picta*), and *T. s. elegans*.[4,12,21,27,28,32,36] Hematocrit values for I1 were lower than for I2 and I3. This difference could be due to any of the variables discussed above, or may represent normal variation in the less physically mature individuals from I1. Hematocrit values tend to be lower in less mature individuals of several vertebrate species.[10,16,17] Total erythrocyte counts have been reported to be lower in juveniles than adults of one Emydid, the box turtle (*Terrapene carolina*).[3] Erythrocyte size of *P. rubriventris* is similar to that previously published for the closely related *T. scripta*.[12,36]

Total leukocyte counts for *P. rubriventris* are similar to New Guinea snapping turtles (*Elseya no-*

vaeguineae) and *T. s. elegans*, but are higher than values reported for *G. muhlenbergii*, European pond turtles (*Emys orbicularis*), and Caspian turtles (*Mauremys caspica*).[1,4,10,21,31,36] However, variability of leukocyte counting methodologies among studies makes comparisons difficult. Manual leukocyte counts using the eosinophil Unopette method may have coefficients of variability up to 12.7%.[30] As a result, statistical analysis and clinical interpretation of manual leukocyte counts remain problematic, particularly for small study populations.

The high percentage of basophils seen in *P. rubriventris* is consistent with previous reports indicating that these cells are common in freshwater chelonia, including *G. muhlenbergii*, snapping turtles (*Chelydra serpentina*), *C. picta*, *E. orbicularis* (*europoea*), Reeve's turtles (*Chinemys reevesi*), and *T. scripta*.[4,25,26,29,35,36] Differential WBC of *C. serpentina*, *C. picta*, and *T. scripta* demonstrate 50 to 65% basophils.[25,26,35,36] Unfortunately, these studies did not provide detailed description of the health of the turtles. Similarly, our study did not definitively rule out illness as a cause of the generally high basophil percentage. Some turtles (e.g. I1) also had moderately high WBC, lymphocyte, and eosinophil counts. Although the turtles were believed to be healthy based on history, physical examination, and comparison to historical data of conspecifics, we cannot exclude the possibility of subclinical illness. High basophil percentages should not be considered to be the rule for all freshwater chelonia, as basophils have been reported to be less numerous in several species including *E. novaeguineae*, and the Indian soft-shelled turtle (*Lissemys punctata punctata*).[1,23]

Plasma biochemistry values of *P. rubriventris* are clinically similar to those reported for other freshwater chelonia, including *G. muhlenbergii*; *C. picta* (*marginata*) *belli*; *T. s. elegans*; wood turtles (*Glyptemys* [*Clemmys*] *insculpta*); Blanding's turtles (*Emydoidea blandingi*); *C. serpentina*; Cuban terrapins (*Trachemys decussata* [*Pseudemys rugosa*]; *E. novaeguineae*; map turtles (*Graptemys geographica*); mud turtles (*Kinosternon subrubrum*); musk turtles (*Kinosternon* [*Sternotherus*] *odoratus*); and *E. orbicularis*.[4,5,6,9,15,28,33,34,40]

Although there was statistically significant variation in potassium and glucose among institutions, the reported ranges of these dynamic parameters for each institution are clinically similar. Sodium was significantly greater in the turtles that were transported and held prior to sampling (I2 and I3), and BUN was also greatest in one of these groups (I3). These parameters may have been influenced by husbandry variability, transport stress, or hydration status. It is possible that the higher plasma phosphorus values and lower calcium-to-phosphorus ratios seen for I1 turtles represent normal variation in these less physically mature individuals. Serum and plasma phosphorus levels tend to be higher in less mature individuals of several vertebrate species.[8,17,19,39,41] Although data on plasma phosphorus levels of juvenile reptiles are sparse, relatively high phosphorus levels have been documented for juvenile vs. adult green iguanas (*Iguana iguana*).[8] I1 turtles had no gross shell abnormalities, and had clinically acceptable plasma calcium, plasma phosphorus, and calcium-to-phosphorus ratios. As such, it is unlikely that their plasma phosphorus levels were higher than I2 and I3 due to nutritional secondary hyperparathyroidism.

Despite the institutional variation in some parameters, these data will be useful for future comparison. Further research should focus on characterizing hematologic and plasma biochemical values in larger numbers of head-started red-bellied cooters, and changes of these parameters in ill individuals. Study of the influence of husbandry variables on these parameters, and documentation of these parameters for free-ranging juvenile and adult specimens, are also warranted.

Acknowledgments: The authors would like to thank Dr. Tom French of MassWildlife for permission to collect samples, Dr. Terry Graham and Dave Taylor for developing and coordinating the head-start program, and the staff of the Berkshire Museum, New England Aquarium, and Green Briar Nature Center for care of the turtles. Pam Conboy of Abaxis, Inc. provided biochemical rotors. Dr. Joerg Mayer and the technical staff of the exotic animal service of Cummings School of Veterinary Medicine, Tufts University, performed biochemical assays, and Zachary Innis and William Domey performed data entry. We thank Dr. Kenneth Latimer for serving as a third-party reviewer of several blood smears.

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Received for publication 27 February 2006