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Blood and Plasma Biochemistry Reference Intervals for Wild Juvenile American Alligators (*Alligator mississippiensis*)

Matthew T. Hamilton,[1,2,4] Caitlin A. Kupar,[1,2] Meghan D. Kelley,[3] John W. Finger Jr.,[1,3] and Tracey D. Tuberville[1] [1]Savannah River Ecology Laboratory, University of Georgia, PO Drawer E, Aiken, South Carolina 29802, USA; [2]Warnell School of Forestry and Natural Resources, University of Georgia, 180 E Green Street, Athens, Georgia 30602, USA; [3]Department of Biological Sciences, Auburn University, 101 Rouse Life Sciences, Auburn, Alabama 36849, USA; [4]Corresponding author (email: hamiltmatt@gmail.com)

ABSTRACT: American alligators (*Alligator mississippiensis*) are one of the most studied crocodilian species in the world, yet blood and plasma biochemistry information is limited for juvenile alligators in their northern range, where individuals may be exposed to extreme abiotic and biotic stressors. We collected blood samples over a 2-yr period from 37 juvenile alligators in May, June, and July to establish reference intervals for 22 blood and plasma analytes. We observed no effect of either sex or blood collection time on any analyte investigated. However, our results indicate a significant correlation between a calculated body condition index and aspartate aminotransferase and creatine kinase. Glucose, total protein, and potassium varied significantly between sampling sessions. In addition, glucose and potassium were highly correlated between the two point-of-care devices used, although they were significantly lower with the i-STAT 1 CG8+ cartridge than with the Vetscan VS2 Avian/Reptile Rotor. The reference intervals presented herein should provide baseline data for evaluating wild juvenile alligators in the northern portion of their range.

Key words: Alligator mississippiensis, American alligator, point-of-care, reference intervals, stress.

Blood and plasma biochemistry values provide an important and minimally invasive tool for evaluating wildlife health in species exposed to environmental stressors, including contaminants, severe weather events, and disease (Hidalgo-Vila et al. 2007). However, reptile biochemistry values can be challenging to interpret due to the influence of sex, age, nutritional status, or environmental conditions (e.g., temperature, season, geographic area; Thrall et al. 2012). American alligators (*Alligator mississippiensis*) are the most studied crocodilian species, yet the majority of reported biochemistry values focus on animals exposed to environmental stressors (Lance and Elsey 1999), individuals from multiple study populations (Mader 2006), captive or farm-raised animals (Huchzermeyer 2003), or alligators located in their southern range (Guillette et al. 1997). Furthermore, no biochemical values are available from healthy, free-ranging, juvenile alligators in their north-central range, where exposure to extreme environmental stressors may influence hematologic parameters.

We sampled 37 (13 female, 20 male, four unknown sex) healthy, wild juvenile American alligators over a 2-yr period (May–July 2014 and 2015) on an 1,120-ha reservoir located on the Savannah River Site near Aiken, South Carolina (Brandt 1991). We hand-captured alligators between 1600 and 0048 hours, and subsequently collected a 1.0 mL blood sample from the occipital sinus using a 25-gauge, 2.54-cm nonheparinized needle within 100±85.51 s (mean±1 SD; n=37) of capture. Following sample collection, we stored each whole blood sample in a 1.3 mL lithium heparin tube on ice for up to 2.5 h until centrifugation for 3 min at 1640 × G. We then aliquoted 200 µL of plasma into a 1.5 mL tube and stored each sample in a freezer (–60 C) until measuring 12 plasma biochemistry parameters (Table 1) using an Avian/Reptile Profile Plus rotor in a Vetscan VS2 analyzer (Abaxis Inc., Union City, California, USA). After sample collection and processing, we examined each alligator for any external injuries or behavioral abnormalities (e.g., lethargy) while determining head length, snout-vent length (SVL), mass, cloacal temperature, and sex (Allsteadt and Lang 1995).

We opportunistically analyzed an additional 13 blood analytes (Table 1) from 20 (10

TABLE 1. Biochemistry values and descriptive statistics for plasma (Avian/Reptile Rotor) and whole blood (CG8+) from wild juvenile American alligators (*Alligator mississippiensis*; *n*=37) captured 2014–15 near Aiken, South Carolina, USA. Values outside of the instrument's range were not included in any analyses or reported ranges. Dashes indicate SEs were not calculated for creatine kinase and total protein (values not calculated because they were non-normally distributed).

	n	Mean	SE	Range	Reported mean values[a]
Plasma analytes, 2014 + 2015 Avian/Reptile Rotor					
Aspartate aminotransferase (AST), U/L	36	221.3	7.21	140–314	289
Creatine kinase (CK), U/L	36	511.0[b]	—	177–3,126	2,022
Uric acid (UA), mg/dL	32	0.79	0.05	0.08–1.3	1.6
Glucose (GLU), mg/dL	36	102.6	1.57	82–130	91
Phosphorus (PHOS), mg/dL	36	5.3	0.12	4.2–7.5	2.0–13.4
Calcium (CA^{++}), mg/dL	36	10.8	0.09	9.9–12	10.4–25.1
Total protein (TP), g/dL	36	3.65[b]	—	2.9–4.4	3.6–7.7
Albumin (ALB), g/dL	23	1.1	0.01	1–1.3	1.6
Globulin (GLOB), g/dL	23	2.6	0.05	1.9–3.2	3.5
Potassium (K^+), mmol/L	36	4.9	0.11	3.8–6.1	3.8
Sodium (Na^+), mmol/L	36	140.3	0.69	133–148	146
Whole blood analytes, 2015 $CG8^+$ cartridge					
pH	20	7.46	0.03	7.26–7.75	7.51–7.58[c]
Partial pressure of carbon dioxide ($_pCO_2$), mmHg	20	28.9	1.34	19.8–41.5	29–36.8[c]
Partial pressure of oxygen ($_pO_2$), mmHg	20	24.4	0.98	18–37	60.3–81.3[c]
Base excess (BE), mmol/L	20	–2.75	1.20	(−10)–11	—
Bicarbonate (HCO_3), mmol/L	20	23.8	0.94	17.8–33.6	24.4–36.9[c]
Total carbon dioxide ($_TCO_2$), mmol/L	20	25.4	0.93	19–35	—
Oxygen saturation ($_sO_2$), %	20	84	1.54	70–96	—
Glucose (GLU), mg/dL	20	95	2.59	75–125	91
Hematocrit (Hct), %PCV	20	16	0.48	13–21	22.2[c]
Hemoglobin (Hgb), g/dL	20	5.5	0.16	4.4–7.1	7.8
Ionized calcium (iCa), mmol/L	20	1.54	0.02	1.39–1.78	—
Potassium (K^+), mmol/L	20	4.3	0.16	3.1–5.5	3.8
Sodium (Na^+), mmol/L	20	140	1.04	131–150	146

[a] Mean biochemistry values were summarized from Guillette et al. (1997); Busk et al. (2000); Mader (2006).

[b] Median values were reported for non-normally distributed analyte values.

[c] Arterial samples were used for the quantification of these values.

female, 10 male) of the juvenile alligators captured in 2015. Following sample collection, we aliquoted a 95 µL sample of whole blood to a CG8+ cartridge (Abbot Point-of-Care Inc., Princeton, New Jersey, USA) for analysis on a VetScan i-STAT 1 point-of-care (POC) analyzer (Abaxis). We then followed sample storage and processing as outlined above. To control for sample quality and measurement consistency, the same investigator performed each task in 2014 and 2015.

We performed all statistical tests in R (Version 3.2.2; R Development Core Team 2015) and GenStat (VSN International, Hemel Hempstead, UK). Because mass was not obtained for some individuals (*n*=11), we performed a linear regression with the natural log (Ln) of SVL (i.e., LnSVL) on Ln mass (Brandt 1991) using individuals whose mass was obtained (*n*=26). We used respective SVLs to estimate mass for all individuals using the regression coefficient (3.00) and constant (–3.82) obtained from this equation. We compared raw mass and estimated mass with a paired *t*-test; however, no difference (*P*=0.814) was observed. Subsequently, we generated body condition indices (BCIs) using Fulton's condition factor with SVL and

estimated mass following Rice et al. (2007). To determine variables influencing analytes, we conducted linear regression with sampling date (encompassing abiotic factors specific to capture night) and sex as factors in analysis, as well as blood collection time (time elapsed between capture and blood collection) and BCIs as covariates in analysis. To understand the effect of blood collection time, we regressed blood collection time on each respective analyte. We also performed paired *t*-tests and tested for correlations between analyte concentrations obtained on the i-STAT versus the VS2. Creatine kinase (CK) and total protein (TP) were log-transformed (i.e., LnCK and LnTP) to meet assumptions of normality. Statistical significance was set at α=0.05.

Mean (±1 SE) cloacal temperature at the time of sample collection was 26.0±0.4 C, head length averaged 7.6±0.2 cm, and mean snout vent length was 26.7±0.8 cm for all alligators. Biochemical values and descriptive statistics are presented in Table 1. We observed no effect of sex or blood collection time on any analyte investigated. However, BCI had a significant effect on both aspartate aminotransferase (AST; linear regression equation: AST=[–12,424×BCI]+27,797; *P*=0.041) and CK (linear regression equation: CK=[–290.30×BCI]+651; *P*=0.005). Elevated plasma AST values can indicate hepatic or muscle injury, septicemia, and exposure to pathogens (Mader 2006). In addition, CK is regarded as a muscle-specific enzyme, and increases in CK levels can indicate muscle exertion (Thrall et al. 2012). The capture and restraint of crocodilians frequently causes the animal to thrash and exert intense muscular activity (Franklin et al. 2003; Olsson and Phalen 2013). Larger alligators are often more difficult to capture and restrain, which might explain the effect of BCI on AST and CK levels. Nevertheless, the concentrations reported here fit within the reference ranges described by Mader (2006).

Sampling date significantly affected glucose (GLU; *P*<0.001), TP (*P*=0.002), and potassium (K^+) levels (*P*=0.012). Concentrations of GLU, TP, and K^+ can vary depending on the nutritional status and diet of a reptile (Thrall et al. 2012), suggesting the differences observed during this study could reflect the temporal variation in prey availability or opportunistic feeding habits of juvenile alligators (Saalfeld et al. 2011). Globulin levels were significantly affected by both sampling date (*P*=0.001) and BCI (*P*=0.041). Globulin recovery is calculated from the TP and albumin (ALB) values produced by the Avian/Reptile Profile Plus rotor and can be used to diagnose dehydration and antigenic stimulation (Abaxis Inc. 2007). Therefore, our globulin results were likely influenced by the effect of sampling date on TP values reported in this study. It is important to note that while bile acids were included in our biochemistry panel, we did not detect any values above the detection limit of the analyzer (<35 μmol/L). We observed no effects of any factor or covariate on ALB, calcium (Ca^{2+}), phosphorus (P), sodium (Na^+), or uric acid levels.

There was no effect of blood collection time on any VS2 analyte. However, we did observe an effect of blood collection time on the following i-STAT analytes: oxygen saturation (sO_2; sO_2=[0.40×blood collection time]+80.74; *P*=0.042), ionized Ca^{2+} (iCa; iCa=[–0.001×blood collection time]+1.58; *P*=0.035), and Na^+ (Na^+=[–0.03×blood collection time]+142.93; *P*=0.009), which were not measured by the VS2. Although sO_2 and iCa are influenced by shifts in acid/base status (Thrall et al. 2012), we did not detect any changes in pH or any other blood gas values. Even though we standardized sample collection and handling in this study, sO_2 and iCa can be influenced by anaerobic activity (Thrall et al. 2012), caused by the capture and restraining protocols used in crocodilian research. In addition, sO_2, iCa, and Na^+ values can be affected by the use of heparin anticoagulant (Thrall et al. 2012; Abbott Point of Care Inc. 2015), which we used during sample preparation and storage. To test for differences in sensitivities between the two POC devices, we compared levels of analytes shared by the i-STAT CG8+ cartridge and VS2 Avian/Reptile Plus rotors. Levels of GLU

(r=0.99), Na^+ (r=0.77), and K^+ (r=0.97) were highly correlated between the two POC devices, although GLU ($P<0.001$) and K+ ($P<0.001$) values differed significantly between devices, whereas Na^+ levels were not (P=0.61). Both GLU (Holtkamp et al. 1975) and potassium (Asirvatham et al. 2013) concentrations are influenced by sample type (blood, serum, plasma) and sample collection and processing time (Thrall et al. 2012). In addition, the VS2 (Abaxis Inc. 2007) and i-STAT (VetScan i-STAT 1: Operator's Manual 2009) use different measurement and calculation techniques to quantify analyte concentrations, which may have contributed to the observed differences in GLU and K^+ values.

Biochemical reference intervals are an important diagnostic tool when assessing blood and plasma analytes from individuals exposed to a variety of stressors. Although we report variation across sampling date, BCI, and sampling time for several analytes, our biochemistry values are within the range of those reported previously (Guillette et al. 1997; Lance and Elsey 1999; Busk et al. 2000; Huchzermeyer 2003; Mader 2006; Hamilton et al. 2016). The slight deviations in whole blood analytes from our previous study are likely related to size (mean head length for alligators in this study was half that of our previous study) and/or environmental differences (captive vs. wild) experienced by animals in the two different studies. Moreover, although the alligators from our previous study were captive, they were originally obtained from Louisiana, which may also account for the slight deviations (see Hamilton et al. 2016).

In conclusion, we are the first to characterize biochemistry and blood gas parameters for wild juvenile alligators in their north-central range. These blood and plasma values provide a baseline for investigating effects of abiotic and biotic factors on juvenile alligators across their large distribution.

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LITERATURE CITED

Abaxis Inc. 2007. VetScan® Avian Reptilian Profile Plus. http://www.abaxis.com/sites/default/files/resource-packages/VetScan%20Avian-Reptilian%20Profile%20Plus%20PI.pdf. Accessed June 2015.

Abbott Point of Care Inc. 2015. Cartridge test information sheet: Ionized calcium/iCa. https://www.abbottpointofcare.com/support/technical-documentation/cartridge-test-information-sheets. Accessed December 2015.

Allsteadt J, Lang JW. 1995. Sexual dimorphism in the genital morphology of young American alligators, *Alligator mississippiensis*. *Herpetologica* 51:314–325.

Asirvatham JR, Moses V, Bjornson L. 2013. Errors in potassium measurement: A laboratory perspective for the clinician. *N Am J Med Sci* 5:255–259.

Brandt LA. 1991. Growth of juvenile alligators in Par Pond, Savannah River Site, South Carolina. *Copeia* 1991:1123–1129.

Busk M, Overgaard J, Hicks JW, Bennett AF, Wang T. 2000. Effects of feeding on arterial blood gases in the American alligator *Alligator mississippiensis*. *J Exp Biol* 203:3117–3124.

Franklin CE, Davis BM, Peucker SKJ, Stephenson H, Mayer R, Whittier J, Lever J, Grigg GC. 2003. Comparison of stress induced by manual restraint and immobilisation in the estuarine crocodile, *Crocodylus porosus*. *J Exp Zool* 298:86–92.

Guillette LJ, Woodward AR, Crain DA, Masson GR, Palmer BD, Cox MC, You-Xiang Q, Orlando EF. 1997. The reproductive cycle of the female American alligator (*Alligator mississippiensis*). *Gen Comp Endocrinol* 108:87–101.

Hamilton MT, Finger JW Jr, Winzeler ME, Tuberville TD. 2016. Evaluating the effect of sample type on American alligator (*Alligator mississippiensis*) analyte values in a point-of-care blood analyser. *Conserv Physiol* 4:1–7.

Hidalgo-Vila J, Díaz-Paniagua C, Pérez-Santigosa N, Laza A, Camacho I, Recio F. 2007. Hematologic and biochemical reference intervals of free-living Mediterranean pond turtles (*Mauremys leprosa*). *J Wildl Dis* 43:798–801.

Holtkamp HC, Verhoef NJ, Leijnse B. 1975. The difference between the glucose concentrations in plasma and whole blood. *Clin Chim Acta* 59:41–49.

Huchzermeyer FW. 2003. *Crocodiles: Biology, husbandry and diseases*. CABI Publishing, Wallingford, UK, 337 pp.

Lance VA, Elsey RM. 1999. Hormonal and metabolic responses of juvenile alligators to cold shock. *J Exp Zool* 283:566–572.

Mader DR, editor. 2006. *Reptile medicine and surgery*, 2nd Ed. Elsevier, St. Louis, Missouri, 1264 pp.

Olsson A, Phalen D. 2013. Comparison of biochemical stress indicators in juvenile captive estuarine crocodiles (*Crocodylus porosus*) following physical restraint or chemical restraint by midazolam injection. *J Wildl Dis* 49:560–567.

R Development Core Team. 2015. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org. Accessed December 2015.

Rice AN, Ross JP, Woodward AR, Carbonneau DA, Percival HF. 2007. Alligator diet in relation to alligator mortality on Lake Griffin, FL. *Southeast Nat* 6:97–110.

Saalfeld DT, Conway WC, Calkins GE. 2011. Food habits of American alligators (*Alligator mississippiensis*) in East Texas. *Southeast Nat* 10:659–672.

Thrall MA, Weiser G, Allison RW, Campbell TW, editors. 2012. *Veterinary hematology and clinical chemistry*, 2nd Ed. Wiley-Blackwell, Ames, Iowa, 776 pp.

VetScan I-STAT 1: Operator's Manual. 2009. Abaxis, Inc., Union City, California, 98 pp.

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