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# Blood Biochemical Reference Intervals for Wild Ornate Box Turtles (*Terrapene ornata*) during the Active Season

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ABSTRACT: Blood biochemical and hematology analyses are helpful indicators of the physiologic health of animals, particularly when making conservation and management decisions for threatened species. In this study, we 1) established blood biochemical reference intervals for two populations of threatened, free-ranging ornate box turtles (*Terrapene ornata*) in northern Illinois during their active season and 2) examined the effects of individual carapace temperature ($T_c$) on blood biochemical variables by using a Bayesian hierarchical framework. Individual blood variables differed throughout the active season (May–September 2015), but there were few distinct patterns in concentrations over time. When controlling for individual variability, blood biochemical variables potassium, sodium, chloride, ionized calcium, hematocrit (percentage of packed cell volume), and osmolality showed no effect of $T_c$ (i.e., slope estimates for these variables were not credibly different from zero) and had little individual variation. Glucose and urea nitrogen were found to have slopes credibly different from zero, with glucose having an estimated positive slope and urea nitrogen having an estimated negative slope, suggesting different relationships in response to $T_c$ when controlling for individual variability. These physiologic blood data will serve as important baseline reference values for the clinical evaluation of wild ornate box turtles presented for veterinary care or for comparison to other studies of wild populations. Further, this study highlights the importance of considering individual-level effects (e.g., $T_c$) on physiologic health variables.

*Key words:* Blood chemistry, free-ranging turtles, physiologic health, radiotelemetry, temperature, *Terrapene ornata*, threatened.

Blood biochemical and hematology studies are important not only for veterinary diagnoses and treatment of captive and wild animals, but they can also be important for scientific research into animal ecology and physiology or for the management and conservation of imperiled species (Cook and O'Connor 2010). Before blood biochemical values can provide useful information on imperiled species, it is necessary to document reference blood values for wild populations by examining their range of variation, as well as their variability with respect to environmental or physiologic factors by analyzing the data on an individual organism level (e.g., LaGrange et al. 2014).

One such imperiled vertebrate species is the prairie-dependent ornate box turtle (*Terrapene ornata*), which is threatened across much of its current range in the central and western US (Dodd 2002), primarily as a consequence of severe habitat loss and alteration. Little is known about the seasonal ecology and physiology of ornate box turtles, and establishing these records is imperative to further our understanding and conservation of this species.

We aimed to 1) determine baseline and temporal blood biochemical values for this species during their active season and 2) examine the effect of individual carapace temperature ($T_c$) on blood biochemical variables. Individual ornate box turtles may vary in their activity level and thus thermal ecology, potentially resulting in individual differences in blood biochemical values driven by temperature.

This study was conducted on 20 free-ranging, adult ornate box turtles (five females and 15 males; mean initial mass=305±33; range=230–360 g) within the Goose Lake Prairie Nature Preserve (41°21′42.4184″N, 88°19′30.734″W) and Wilmington Shrub Prairie Nature Preserve and Kankakee Sands Preserve (41°16′24.312″N, 88°10′3.36″W), located in Grundy and Will counties, Illinois, US (Milanovich et al. 2017). Temperature data loggers (Thermochron iButton data loggers, model DS1921G, Dallas Semiconductor, Dallas Texas, USA), programmed to record $T_c$ every 240 min, and radio transmitters were attached to the anterior carapace of each individual. Once per month from May 2015 to September 2015, we obtained a <0.25-mL blood sample from the dorsal subcarapacial cervical plexus with a 25-gauge needle. To maintain sample quality consistency, we discarded samples obviously contaminated with lymph fluid (Murray 2000), and the same investigators (L.A.H. and J.F.) performed all sampling. Blood samples were immediately transferred to a lithium-heparinized tube and placed on ice, and individuals were immediately released at the point of capture. Within 5 min of blood collection, a 50-μL sample of whole blood was inserted into a CHEM8+ cartridge and analyzed onsite with an i-STAT 1 point-of-care handheld blood analyzer (Abaxis, Inc., Abaxis Veterinary Diagnostics, Union City, California, USA). The CHEM8+ cartridge analyzed the following blood biochemical variables: potassium (mmol/L), sodium (mmol/L), chloride (mmol/L), glucose (mg/dL), ionized calcium (iCalcium; mmol/L), urea nitrogen (mg/dL), creatinine (mg/dL), hematocrit (percentage of packed cell volume), and osmolality (mOsm/kg), calculated by using the equation from Camacho et al. (2015). Body mass (g) data were not recorded consistently for analyses. We report variable concentrations, combined for all months, as measures of central tendency (mean and median) and range, as recommended by Friedrichs et al. (2012; Table 1). Descriptive statistics of variable concentrations by month are given in Supplementary Material Table S1.

We used within-subjects linear models to examine the effect of weighted $T_c$ on eight blood biochemical response variables: potassium, sodium, chloride, glucose, iCalcium, urea nitrogen, hematocrit, and osmolality. Sexes were pooled for all blood biochemical variables except iCalcium ($\text{iCalcium}_{\text{female}}$ and $\text{iCalcium}_{\text{male}}$), which can be elevated in gravid females (Kimble and Williams 2012). We calculated $T_c$ as a weighted mean of daily mean $T_c$ readings recorded 7 d prior to blood sampling (Harden et al. 2015). Not all of the 20 turtles in this study consistently had attached iButtons; thus, 15 (three females and 12 males) were used in the $T_c$ linear model analyses. A Bayesian hierarchical framework was adopted to explicitly consider individual effects (unit heterogeneity) and improve parameter estimation in a case like ours, where small sample sizes can result in biased maximum likelihood estimates (Kéry 2010). The model equations, parameter estimates, and estimation procedures are given in Table S2.

Blood biochemical variables individually varied throughout the active season (May to September 2015), but there were no clear patterns over time (Table S1). Moreover, slope estimates for the blood biochemical variables potassium, sodium, chloride, $\text{iCalcium}_{\text{female}}$, $\text{iCalcium}_{\text{male}}$, hematocrit, and osmolality in response to $T_c$ were not credibly different from zero and had very little individual variation (Table S2); thus, these variables were not considered to be measurably different or strongly influenced by temperature. Glucose and urea nitrogen, however, were found to have slopes credibly different from zero, with glucose having an estimated positive slope and urea nitrogen having an estimated negative slope, suggesting different relationships in response to $T_c$ when controlling for individual variability (Table S2). Because biochemistry values can vary among blood analyzers (Stoot et al. 2014), note that these reference intervals are i-STAT specific and should be used as such in future comparisons. Our results suggest that $T_c$ may not strongly affect certain inorganic osmolytes (e.g., ions: potassium, sodium, chloride, and iCalcium) that are important for maintaining cell volume and cellular biochemical reactions (Hochachka and Somero 2002). However, $T_c$ may have an effect on certain organic osmolytes (e.g., glucose and urea nitrogen) that are associated with seasonal variations in diet, metabolism, and water balance. Glucose levels in reptiles can vary greatly and are dependent on temperature and nutrition and environmental stressors (Campbell 2006).

TABLE 1. Blood biochemistry variables for free-ranging ornate box turtles (*Terrapene ornata*) captured over an active season in northern Illinois, USA.[a]

| Variable | $n$ | Mean | Median | Range |
|---|---|---|---|---|
| Carapace temperature (C) | 15 | 21.2 | 21.2 | 16.3–26.7 |
| Potassium (mmol/L) | 19 | 3.6 | 3.6 | 2.0–5.7 |
| Sodium (mmol/L) | 19 | 133.6 | 134.0 | 125.0–143.0 |
| Chloride (mmol/L) | 19 | 102.8 | 103.0 | 89.0–114.0 |
| Glucose (mg/dL)[b] | 18 | 71.8 | 64.5 | 22.0–154.0 |
| Ionized calcium (mmol/L)[b] | 19 | 1.3 | 1.3 | 0.8–1.8 |
| Ionized calcium$_{female}$ (mmol/L)[b] | 4 | 1.3 | 1.3 | 0.9–1.5 |
| Ionized calcium$_{male}$ (mmol/L)[b] | 15 | 1.3 | 1.3 | 0.9–1.8 |
| Urea nitrogen (mg/dL)[c] | 18 | 79.1[c] | 78.0 | 14.0–140+ |
| Hematocrit (%)[b] | 16 | 17.9 | 17.9 | 10–29 |
| Osmolality (mOsm/kg)[c] | 17 | 306.7 | 305.0 | 274.6–345.8+ |

[a] Data were combined for all months (May to September 2015). Creatinine (mg/dL) is absent from this table because concentrations were <0.2 mg/dL for all turtle blood samples.

[b] Denotes data that failed a test of normality.

[c] Denotes urea nitrogen and osmolality values that are likely underestimated due to two urea nitrogen values in May and July that exceeded the analytic range of the analyzer (140 mg/dL).

Urea nitrogen levels can vary substantially in turtles and are associated with protein-based energy sources (during activity or hibernation) and environmental water availability (Yang et al. 2014). Urea nitrogen is a nitrogenous waste product in primarily aquatic turtles (Shoemaker and Nagy 1977), but has also been documented in terrestrial turtles at moderate to high levels during periods of water stress or extreme inactivity, suggesting their use of the urea cycle (Baze and Horne 1970; Christopher et al. 1994; Henen 1997). We observed that standing water was readily available to active ornate box turtles, and that they were often found within water during hotter months, which may help to explain the decreases in urea nitrogen as $T_c$ increases.

There is little previous data regarding ornate box turtles blood biochemical variables; however, published values for osmolality, sodium, potassium, calcium, chloride, and urea nitrogen fall within our reference intervals (Baze and Horne 1970; Dessauer 1970). Further, we can compare our results to studies on the closely related eastern box turtle (*Terrapene carolina*). Our results fall within normal ranges of potassium, sodium, chloride, hematocrit, and glucose measured in healthy, free-living eastern box turtles at the Maryland Zoo (Adamovicz et al. 2015) and within the normal range of sodium in free-living eastern box turtles in Tennessee and Illinois (Lloyd et al. 2016). Additionally, potassium was lower, and glucose was higher when compared with wild, active eastern box turtles in Indiana (Kimble and Williams 2012). There have been studies investigating temperature effects on ornate box turtle body heat dissipation (Sturbaum and Riedesel 1977) and heart rate (Actams and DeCarvalho 1984); however, these studies have been conducted in controlled environments. Our study, conversely, investigates the relationship of blood biochemistry to temperatures of free-ranging ornate box turtles in their natural environment. Environmental cues, such as temperature and rainfall, largely drive activity levels and behaviors of terrestrial turtles (Converse and Savidge 2003), and thus their physiology, but there is often considerable variation among individuals (e.g., eastern box turtles; DeGregorio et al. 2015). We accounted for individual differences in ornate box turtle $T_c$ in an attempt to elucidate true patterns of blood chemistry. Temporal analysis of baseline values of blood chemistry for this threatened species may be increasingly important as suitable prairie habitat decreases, novel diseases emerge, and environmental stresses increases.

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## SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2017-09-222.

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