



The blood biochemistry of overwintering diamondback terrapins (*Malaclemys terrapin*)



Leigh Anne Harden^{a,b,*}, Stephen R. Midway^c, Amanda Southwood Williard^a

^a Department of Biology and Marine Biology, University of North Carolina Wilmington, 601S, College Rd., Wilmington, NC 28403, USA

^b Department of Biology, Loyola University Chicago, 442 Life Sciences Building, 1050 West Sheridan Road, Chicago, IL 60660, USA

^c Department of Biology, Coastal Carolina University, Conway, SC 29528, USA

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ABSTRACT

Estuarine ectothermic vertebrates, such as diamondback terrapins (*Malaclemys terrapin*, Schwartz 1955), inhabit a dynamic environment, and many aspects of their biology reflect their ability to withstand and respond to these environmental challenges. The physiological adjustments necessary to maintain water and salt balance and the metabolic adjustments that accompany seasonal changes in activity and behavior have not been well-characterized for overwintering terrapins under field conditions. To investigate terrapin osmotic and metabolic physiology during winter when activity levels are depressed, we obtained repeat blood samples from 10 radio-tagged female terrapins maintained in a semi-natural, open-air salt marsh enclosure in southeastern North Carolina, USA. From November 2011 to April 2012, we measured monthly plasma osmolality, plasma concentrations of inorganic osmolytes (Na^+ , K^+ , Cl^-), and protein catabolic indices (urea and uric acid), as well as monthly plasma concentrations of total Ca^{2+} , lactate, and glucose as metabolic indices. We used linear mixed models to determine the best predictors of blood chemistry, where time (i.e., day) and environmental variables were fixed factors and individual terrapins were random factors. Day was a poor predictor of blood chemistry concentrations, indicating that the progression of winter did not elicit corresponding changes in biochemical indices as documented in other semi-aquatic turtles exposed to more severe winter and/or laboratory conditions. Carapace temperature was the most common predictor of blood chemistry concentrations in all models, underscoring its relative influence on physiology. In contrast to previous laboratory-based studies on the overwinter physiology of terrapins, our study demonstrates that terrapins in their natural environment are able to maintain biochemical homeostasis throughout winter. The use of evasive behavioral strategies may be an important factor for terrapins to reduce the passive exchange of water and salts with the estuarine environment.

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1. Introduction

Seasonal changes in environmental conditions such as temperature, photoperiod, and food and water availability can have profound effects on the physiology of ectotherms. Temperature, in particular, has direct effects on biochemical reaction rates, physiological processes, and whole animal metabolism (Dubois et al., 2009; Hochachka and Somero, 2002). For aquatic ectothermic vertebrates, water and salt concentrations can fluctuate as environmental conditions change, posing osmoregulatory challenges that require morphological, behavioral, and physiological adjustments to minimize dehydration due to excess water loss and salt accumulation (Dunson and Mazzotti, 1989; Dunson and Travis, 1994). Osmoregulation is particularly difficult for estuarine and marine ectotherms that experience periods of curtailed physiologic rates associated with seasonal cold exposure (Gilles-Baillien, 1973). For

euhaline fish, ionoregulatory processes, regulated by the gill membrane enzyme Na^+/K^+ ATPase, can be affected by cold winter temperatures, resulting in functional, ultrastructural and/or morphological changes in Na^+/K^+ ATPase to maintain osmotic balance (Anderson, 2013). Alternatively, some marine turtles undergo a behavioral shift during the cold winter months, in which they make periodic prolonged, aerobic dives to rest on the seafloor (Hochscheid et al., 2005). This intermittent dormancy reduces energetic costs while still permitting physiological functions associated with foraging (Hochscheid et al., 2007), movement, and osmoregulatory processes (i.e., lachrymal salt gland secretion).

Few estuarine species, and even fewer estuarine reptiles, are obligate to this dynamic estuarine environment (Hart and Lee, 2006). One of the true obligate estuarine reptiles, the diamondback terrapin (*Malaclemys terrapin*) is one such estuarine turtle species endemic to tidally-influenced temperate zone habitats, where salinities range from 11 to full strength seawater (≥ 34 , Dunson, 1970). In this desiccating environment, terrapins use behavioral and physiological adjustments in order to prevent water loss and combat excessive influx of

* Corresponding author at: Loyola University Chicago, 442 Life Sciences Building, 1050 West Sheridan Rd., Chicago, IL 60660, USA. Tel.: +1 610 506 1055.
E-mail address: leighanneharden@gmail.com (L.A. Harden).

salts. Both compensatory and evasive strategies are employed to maintain plasma osmotic pressure 1/3 to 1/2 that of environmental water (Gilles-Baillien, 1970; Kirschner, 1970). Evasive strategies that reduce evaporative water loss and salt and water exchange with environment include impermeable integument, hypophagy, winter mud burial, aestivation, basking, and water retention via urine reduction (Bentley et al., 1967; Brennessel, 2006; Gilles-Baillien, 1973), which all ultimately reduce metabolic costs associated with maintaining osmotic balance with the environment (Bentley et al., 1967). Compensatory strategies that involve active (i.e., energy-requiring) uptake or extrusion of water or salts include the active exchange of organic osmolytes across cell membranes, the secretion of salts from body to environment via lachrymal salt gland in order to adjust osmotic pressure (Cowan, 1981; Dunson, 1970), and the detection, active uptake, and extracellular storage of fresh rain water when readily available (Bels et al., 1995; Davenport and Macedo, 1990; Robinson and Dunson, 1976).

In addition to osmoregulatory adjustments, terrapins may also experience seasonal shifts in metabolic capacity. Overwintering aquatic emydid turtles exhibit various physiological adaptations that allow them to survive the sometimes harsh conditions of winter. The effects of low ambient temperatures, hypoxia, and downregulation of molecular and cellular mechanisms result in a drastic depression of the metabolic rates of overwintering turtles (Jackson, 2000; Southwood Williard and Harden, 2011). Low metabolic rates may be supported by aerobic metabolic pathways for those turtles capable of gas exchange via extrapulmonary respiration in open, flowing water such as: *Pseudemys rubriventris*, *Apalone spinifera*, and *Graptemys geographica* (Crocker et al., 2000; Dunson, 1960; Graham and Guimond, 1995; Jackson, 1979; King and Heatwole, 1999; Ultsch, 1989).

In contrast, other freshwater species such as painted turtles (*Chrysemys picta*) and snapping turtles (*Chelydra serpentina*) often overwinter in anoxic environments (e.g., iced-over water bodies, mud) and thus rely heavily on anaerobic metabolism to meet their low metabolic needs (Jackson, 2000; Ultsch, 1989). This overwintering strategy results in the gradual accumulation of plasma lactate (Reese et al., 2001, 2004) that can exceed 200 mmol/L under stable anoxic conditions (Ultsch and Jackson, 1982; Ultsch et al., 1999). The degree to which turtles accumulate lactate and the means by which they buffer lactate play a major role in determining survival during anoxic submergence (Ultsch and Jackson, 1995). Previous studies have demonstrated that an increased reliance on anaerobic metabolic pathways during winter dormancy is reflected by elevated blood lactate concentrations and subsequent changes in blood Ca^{2+} and Mg^{2+} indicative of lactate buffering with their shell in order to avoid metabolic acidosis (Jackson, 2000, 2002; Jackson and Heisler, 1982; Jackson et al., 1996; Reese et al., 2004).

Terrapins experience shifts in environmental temperatures from summer to winter and correspondingly exhibit dramatic changes in their activity levels in winter, at which point they burrow in the inter- or subtidal mud and can remain inactive for extended time periods (Butler, 2002; Coker, 1906; Haramis et al., 2011; Harden and Williard, 2012; Southwood Williard and Harden, 2011; Yearicks et al., 1981). However, the physiological adjustments underlying the observed behaviors have not been thoroughly investigated. The overwintering strategy of terrapins is interesting from an osmoregulatory and metabolic perspective, given the estuarine habitat in which they live and the potentially high energetic costs of maintaining osmotic and ionic balance. Insight into seasonal adjustments in osmoregulation and metabolism in reptiles may be gained by blood biochemical variables (Costa and Sinervo, 2004) such as ion concentrations, and metabolic products (Dessauer, 1970; Jackson, 2000; Shoemaker and Nagy, 1977; Somero and Hochachka, 1971; Tracy et al., 2006).

We investigated changes in osmotic and ionic status of terrapins throughout the winter by measuring plasma osmolality, inorganic osmolytes (Na^+ , K^+ , Cl^-), the organic osmolyte urea, and uric acid. We also explored the metabolic status of terrapins by measuring plasma Ca^{2+} , lactate, and glucose levels. Although we did not measure bound

forms of the lactate buffer, calcium (i.e., CaLactate +), we did measure free Ca^{2+} and free lactate, which are indicative of the lactate buffering mechanism described for aquatic turtles (Jackson, 2000, 2002; Jackson and Heisler, 1982; Jackson et al., 1996; Reese et al., 2004). These data were interpreted in light of terrapin habitat use and environmental data. We then predicted that throughout winter months, blood chemistry would 1) reflect physiological adjustments to minimize water loss and salt gain (e.g., increase in osmolality, increase in urea), 2) reflect increased reliance on anaerobic metabolism throughout winter burial (i.e., increase in lactate and potential buffer Ca^{2+}), and 3) vary predictably with alterations in environmental factors that affect water availability and rate processes.

2. Materials and methods

2.1. Study site and field methods

To test these predictions regarding osmotic and metabolic physiology, we maintained terrapins in an open-air enclosure on the landward side of Masonboro Island National Estuarine Research Reserve (NERR) in Byron's Creek, North Carolina, USA (34° 08' 08" N, 77° 50' 57" W, Fig. 1) that encompassed typical terrapin habitat and allowed terrapins to experience natural environmental shifts (see Harden et al., 2014 for details on materials, dimensions, and construction of enclosure).

Environmental data were obtained from a National Oceanic and Atmospheric Administration, Office of Ocean and Coastal Resource Management, National Estuarine Research Reserve System-wide Monitoring Program station located 2 km from our Byron's Creek terrapin enclosure. Salinity and tidal creek temperature data from this monitoring station were collected at 30 minute intervals by a YSI 6600EDS data sonde (YSI Inc., Yellow Springs, OH, USA) and total rainfall (mm) was also recorded at 30 minute intervals collected by a tipping bucket rain gauge (Campbell Scientific, Inc., Logan, UT, USA, Model# TE525, rainfall per tip: 0.01 in.) mounted on the monitoring station. We also measured shallow mud temperature (2 cm) salinity with HOBO data loggers (HOBO® UTBI-001 and U24-002, respectively Onset Computer Corporation, Bourne, MA) located within the terrapin enclosure, but due to equipment malfunction, salinity measurements were not recorded consistently throughout the duration of the study thus the NOAA monitoring station data were used for analyses. We were confident with this substitution in data because long-term salinity measurements from NOAA monitoring station and from the enclosure conductivity logger were significantly correlated ($r = 0.527$, $p < 0.001$) and these tidal creeks are well-mixed estuarine systems. Mud salinity was also measured by centrifuging core samples taken from the enclosure periodically throughout the winter and using a refractometer on the suspended liquid.

In nearby tidal creeks and coves, we used large >100 m gillnets to collect 10 female terrapins (300–700 g, Table 1), which were relocated to the enclosure in Byron's Creek. Terrapin collection sites were within 5 km of the enclosure site. Terrapins were sexed, aged, measured, and given a unique 3-letter code (e.g., APV) notched into the marginal scutes following processing protocols outlined by Dorcas et al. (2007). Temperature data loggers (iButtons, programmed to record carapace temperature, T_c , every 30 min) and radio transmitters (frequencies: 150.162–150.838 MHz) were attached to the anterior carapace using quick-setting marine grade epoxy putty (see Harden et al., 2014 for more details). This datalogger measured the temperature of the carapace surface, thus reflected the immediate environment of the turtle. Furthermore, previous studies have found carapace temperatures T_c to be strong indicators of body temperature T_b in small to medium-sized turtles (*C. picta*: Grayson and Dorcas, 2004; *Cuora flavomarginata*: Chen and Lue, 2008; *Platysternon megacephalum*: Shen et al., 2013), such as the terrapins used in this study.

Terrapins were released into the enclosure on 22 September 2011, and were allowed a period of 45 days to acclimate behaviorally and

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