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Seasonal changes in thermal environment and metabolic enzyme activity in the diamondback terrapin (*Malaclemys terrapin*)

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ABSTRACT

Diamondback terrapins experience broad fluctuations in temperature on both a daily and seasonal basis in their estuarine environment. We measured metabolic enzyme activity in terrapin muscle tissue to assess thermal dependence and the role of temperature in seasonal metabolic downregulation in this species. Activity of lactate dehydrogenase (LDH), pyruvate kinase (PK), citrate synthase (CS), and cytochrome *c* oxidase (CCO) was assayed at 10, 20, 30, and 40 °C for tissue collected during summer and winter. The Q_{10} for enzyme activity varied between 1.31 and 2.11 within the temperature range at which terrapins were active (20–40 °C). The Q_{10} for LDH, CS, and CCO varied between 1.39 and 1.76 and between 10 and 20 °C, but PK exhibited heightened thermal sensitivity within this lower temperature range, with a Q_{10} of 2.90 for summer-collected tissue and 5.55 for winter-collected tissue. There was no significant effect of season on activity of LDH or PK, but activity of CS and CCO was significantly lower in winter-collected tissue compared with summer-collected tissue. Results indicate that temperature effects contribute to seasonal metabolic downregulation and dormancy in terrapins, but other environmental factors (i.e. oxygen availability), as well as seasonal shifts in blood biochemistry and circulating hormones may also play an important role.

1. Introduction

Temperature has profound effects on the structure and function of metabolic enzymes and biochemical reaction rates (Hochachka and Somero, 2002), which ultimately result in changes in rates of physiological processes and metabolism at the organismal level (Bennett and Dawson, 1976; Huey, 1982). Physiological and behavioral responses to a seasonal decrease in environmental temperature (T_E) vary considerably among reptiles, and may depend both on the magnitude of the temperature change and the duration of cold exposure, as well as ecological factors such as food availability or risk of predation (Huey, 1982; Tsuji, 1988). Reptiles in mildly seasonal climates may maintain activity during the winter months through a combination of behavioral thermoregulation and/or compensation of metabolic capacity at the molecular and cellular levels (Tsuji, 1988; Southwood et al., 2003; Seebacher et al., 2003, 2004). This strategy is advantageous if the energetic benefits of sustaining activity outweigh the costs and result in a selective advantage (Tsuji, 1988; Guderley and St-Pierre, 2002; Wilson and Franklin, 2002; Seebacher, 2005), which is less likely to be the case for terrestrial or semi-aquatic reptiles that experience harsh winters. Marked hypometabolism and entrance into a dormant state characterize the overwintering strategy of several species of turtles that live at mid- to high-latitudes (i.e. *Trachemys scripta*, *Chrysemys picta*, and *Chelydra serpentina*), many of which remain submerged in water or buried in mud for months at a time during cold exposure (Ultsch, 1989, 2006; Ernst and Lovich, 2009). Depression of metabolic rate in dormant turtles is due to the direct effects of temperature on biochemical reaction rates (i.e. Q_{10} effects, Herbert and Jackson, 1985), as well as metabolic downregulation in response to low oxygen conditions. Mechanisms to decrease rates of ATP synthesis and ATP demand during anoxia have been well-studied, and include post-transcriptional regulation of enzyme activity (Storey and Storey, 1990; Storey, 1996), modifications in protein synthesis (Brooks and Storey, 1993; Hochachka et al., 1996), and modifications in selective permeability of membranes and ion transport processes (Bickler and Buck, 2007; Staples and Buck, 2009).

The diamondback terrapin (*Malaclemys terrapin*) inhabits salt marshes, coves, and tidal creeks along the Eastern and Gulf coasts of the United States from Cape Cod, Massachusetts in the north to Corpus Christi, Texas in the south (Hart and Lee, 2006; Ernst and Lovich, 2009). Terrapins forage primarily on small species of crustaceans and mollusks in shallow, flooded marshes at high tide (Tucker et al., 1995; Whitelaw and Zajac, 2002) and retreat to deeper waters or bury in the mud of the intertidal zone during low tide (Spivey, 1998; Harden et al., 2007). Terrapins experience wide daily fluctuations in environmental temperatures, depending on tidal cycles and whether they are utilizing the terrestrial or aquatic

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habitat (Harden et al., 2007). They may also experience wide seasonal fluctuations in temperature, depending on latitudinal location within the species' broad geographic range.

Reports from captive studies and field observations suggest that diamondback terrapins that live in mid- to high-latitudes bury in the mud of the subtidal or intertidal zone and enter a state of dormancy during the winter months. In the northern portion of the range (North Carolina to Massachusetts), winter dormancy extends from October–November until March–April and is initiated at water temperatures (T_W) of 6–13 °C (Coker, 1906; Yearicks et al., 1981; Brennessel, 2006). The period of winter dormancy is much shorter in the southern portion of the range. For example, terrapins in northern Florida are inactive at $T_W \leq 18$C °C, which typically only occurs from December to mid-February (Seigel, 1980; Butler, 2002). Wood (1992) proposed that terrapins in the Florida Keys, where average T_W rarely drops below 20 °C, remain active year-round. The cues that trigger seasonal dormancy in this species have not been studied, but it is likely that temperature plays an important role in seasonal decreases in metabolism and the timing of dormancy behavior (Ultsch, 1989). Several reptile species display heightened thermal sensitivity of metabolic and physiological processes at low temperatures, which contributes to a decrease in activity levels and whole animal metabolism (Bennett and Dawson, 1976; Hochachka and Somero, 2002). Other mechanisms of metabolic downregulation associated with hypoxia and anoxia may also be employed by terrapins (Jackson, 2000), given the prolonged periods of mud burial they are thought to endure during the winter.

The primary goals of our study were to document thermal conditions experienced by terrapins in a mid-latitude, seasonal environment and investigate the effects of temperature on metabolic enzyme activity in terrapin muscle tissue. We used data loggers to record terrapin carapace temperature (T_C) and VHF radiotelemetry to track terrapin movements in a North Carolina estuary from early summer to mid-winter (June 2008–February 2009). Muscle tissue was collected from terrapins captured during the summer and winter, and activity of enzymes associated with anaerobic (lactate dehydrogenase (LDH, EC 1.1.1.27) and pyruvate kinase (PK, EC 2.7.1.40)) and aerobic (citrate synthase (CS, EC 2.3.3.1) and cytochrome *c* oxidase (CCO, 1.9.3.1)) metabolic pathways were assayed at 10, 20, 30, and 40 °C. We calculated Q_{10} values for 10 °C increments over the range of assay temperatures to assess thermal sensitivity of enzymes, and compared enzyme activity of summer and winter-collected tissue to look for patterns indicative of seasonal metabolic downregulation. We predicted that thermal sensitivity of enzymes would be greatest at temperatures associated with entrance into dormancy (10–20 °C), and that enzyme activity in winter-collected tissue would be significantly lower than activity in summer-collected tissue when compared at the same temperature.

2. Materials and methods

2.1. General procedures

Terrapins (N = 10) were initially captured by seining a small cove on the west side of Masonboro Island, NC (34° 10′ 34″ N; 77° 49′ 20″ W, Fig. 1) during June and July of 2008. Water temperature at the time of capture was 26–27 °C. Terrapins were transferred in large, plastic boxes to the University of North Carolina Wilmington (Wilmington, NC, USA), approximately 10 km distance, for processing. Each terrapin was marked with a unique series of notchings on the marginal scutes for future identification. Sex was determined based on tail length and position of cloacal opening, and mass was measured using a top-loading scale (Scout Pro SP6000, Ohaus Corporation, Pine Brook, NJ, USA). All terrapins used for this study were female and weighed between 437 and 706 g at the time of initial capture (Table 1).

A small tissue sample was excised from the flexor tibialis muscle complex of the right rear limb for our investigation of metabolic enzyme activity. Before excising tissue, the incision area was scrubbed with 95% ethanol and Betadine® topical antiseptic and an intramuscular injection of local anaesthetic (2% Lidocaine HCL, Phoenix Pharmaceutical, Inc., St. Joseph, MO, USA) was administered. A 1 cm incision was made in the skin and approximately 20 g of muscle tissue was removed using surgical scissors. Dissolvable sutures (Maxon 3-0, C-14 needle, Henry Schein Inc., Melville, NY, USA) were used to close the incision and the area was treated with topical antibiotic ointment (Furacin, Squire Laboratories, Revere, MA, USA). Muscle tissue samples were immediately frozen in liquid nitrogen and transferred to a −70 °C freezer for storage. Samples were stored for 4 to 6 months between collection and assays (please see Section 2.2 Enzyme assays).

Temperature data loggers (5.9 mm × 17.4 mm, 3.12 g; iButton DS1922L-F51, Dallas Semiconductor, Dallas, TX, USA) were attached to the anterior portion of the terrapin's carapace using quick-setting epoxy putty (Loctite®, Henkel Corporation, Cary, NC, USA). The instrument was positioned to the left of the midline on the second vertebral scute. The data loggers were programmed to record temperature every 30 min for a maximum of 6 months with a resolution of 0.0625 °C and an accuracy of 0.5 °C. Instruments were calibrated in a temperature-controlled water bath (Haake DC10, Thermo Electron Corporation, Newington, NH, USA) against an NIST-certified thermometer prior to deployment, and coated in two layers of protective, waterproof plastic (Plasti Dip International, Blaine, MN, USA). Radiotransmitters (20 mm × 10 mm, 6–9.6 g; model PD-2, Holohil Systems Ltd., Carp, Ontario, Canada) were secured to the right of the midline on the second vertebral scute with quick-setting epoxy putty. Each radiotransmitter emitted a VHF signal at a unique frequency between 150.241 and 150.971 MHz. Tests conducted prior to deploying radiotransmitters demonstrated that the maximum range of signal transmission in the salt marsh environment was approximately 1000 m, but the average range was approximately 500 m.

Terrapins were monitored in the laboratory following surgical procedures and instrument attachment, and released at the site of capture within two days. Terrapin movements were tracked via radiotelemetry from 13 Jun 2008 to 28 Feb 2009 (Table 1). Radiotransmitter signals were detected using a VHF receiver (model TR-4, Telonics, Inc. Mesa, AZ, USA) and H-antennae (model RA-2AK, Telonics, Inc. Mesa, AZ, USA), and efforts were made to visually locate terrapins for which VHF signals were detected. Notes on terrapin activity and habitat (i.e. buried in mud, resting on mud surface, swimming in water) were recorded upon location (results reported in detail elsewhere, Southwood et al., 2009). Radiotracking occurred twice a week from June through September, and once a week from October through February. Terrapins were recaptured in January (N = 4) and February (N = 1) of 2009 to retrieve temperature data loggers and take an additional biopsy sample from the left rear limb using previously described methods. In order to minimize disturbance to the terrapins during their winter dormancy, we conducted muscle tissue sampling at the capture site rather than transporting the terrapins back to the laboratory. Terrapins were released at the site of capture, and we continued to monitor their movements via radiotelemetry for several months following release. We deployed a new temperature data logger on one of the terrapins recaptured in January 2009 (terrapin ACW) prior to release. A subsequent recapture of this terrapin permitted us to retrieve the data logger and obtain temperature data for this terrapin for the period 19 January 2009–28 February 2009.

2.2. Enzyme assays

Muscle tissue samples were partially thawed, minced with a razor blade, and diluted 1:20 in ice-cold 75 mmol·L^{-1} Tris–HCl buffer adjusted for pH 7.5 at room temperature. The dilution was kept on ice, homogenized using an UltraTurrax T8 tissue homogenizer (IKA Works Inc., Wilmington, NC, USA), and sonicated using a VirSonic100

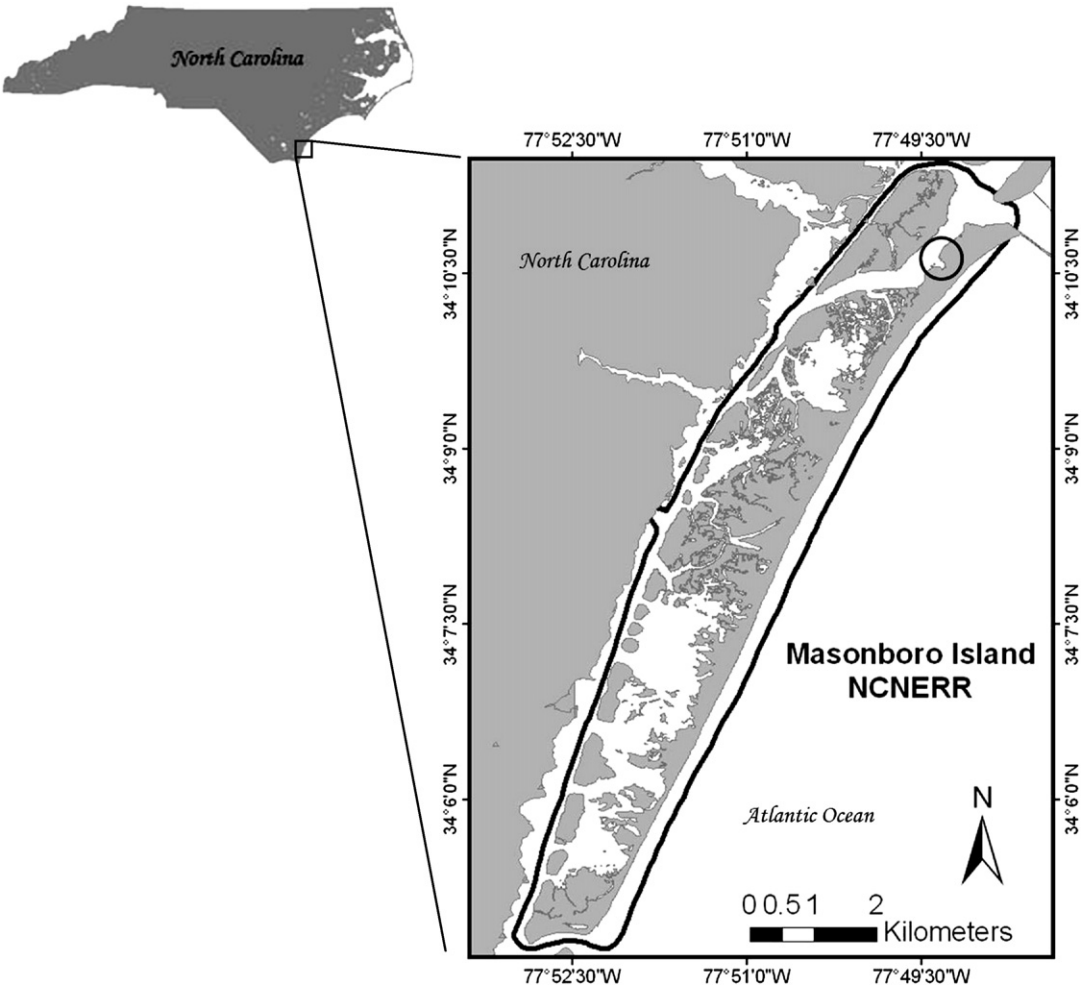

Fig. 1. Map depicting Masonboro Island, part of the North Carolina Estuarine Research Reserve (NCNERR). The reserve boundary is outlined, and the circled area within the reserve boundary designates the site where terrapins were captured in June–July 2008.

ultrasonic cell disruptor (VirTis, Gardiner, NY, USA). Whole homogenate was used for assays, and each assay was run in duplicate. Activities of lactate dehydrogenase (LDH), pyruvate kinase (PK), citrate synthase (CS), and cytochrome *c* oxidase (CCO) were determined at 10 °C, 20 °C, 30 °C, and 40 °C using a Lambda 25 UV/VIS spectrophotometer (Perkin Elmer Instruments, Shelton, CT, USA) with temperature-controlled cells. The millimolar extinction coefficient (ε) and wavelength at which reactions were monitored (λ) are indicated in assay protocols listed below. The order in which activity was measured at different assay temperatures was randomized. Stock solutions for assays were prepared using buffers adjusted for pH at each temperature. All chemicals for enzyme assays were obtained from Sigma-Aldrich™ (St. Louis, MO, USA). Assay protocols are based on those described in Southwood et al. (2003, 2006): LDH: $\lambda = 340$ nm, $\varepsilon = 6.22$, pH 7.5, 50 mmol·L^{-1} imidazole–HCl, 1.5 mmol·L^{-1} β-nicotinamide adenine dinucleotide (reduced NADH), 4 mmol·L^{-1} pyruvate, 1/500 dilution; PK: $\lambda = 340$ nm, $\varepsilon = 6.22$, pH 7.0, 50 mmol·L^{-1} imidazole–HCl, 10 mmol·L^{-1} magnesium chloride, 100 mmol·L^{-1} KCl, 1.5 mmol·L^{-1} β-nicotinamide adenine dinucleotide (reduced NADH), 7 mmol·L^{-1} phospho*enol*pyruvate, 5 mmol·L^{-1} adenine diphosphate, excess LDH, 1/100 dilution; CS: $\lambda = 412$ nm, $\varepsilon = 13.6$, pH 8.0, 100 mmol·L^{-1} Tris–HCl, 0.3 mmol·L^{-1} acetyl-CoA, 0.5 mmol·L^{-1} oxaloacetate, 0.1 mmol·L^{-1} 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), 1/20 dilution; CCO: $\lambda = 550$ nm, $\varepsilon = 29.5$, pH 7.5, 50 mmol·L^{-1} potassium phosphate, 0.05 mmol·L^{-1} cytochrome *c* (reduced), 1/20 dilution.

Enzyme activity is expressed as U·g^{-1} (μmol substrate converted min^{-1} per gram wet tissue). The thermal sensitivity (Q_{10}) of enzyme activity was calculated for each 10 °C interval using the equation $Q_{10} = (k_2/k_1)^{10/(T2-T1)}$, where k_i is the activity at T_i.

2.3. Statistical analysis

Data were downloaded from recovered data loggers and weekly mean, maximum, and minimum temperatures experienced by terrapins were calculated (13 June–19 January, N = 4; 20 January–28 February, N = 2). Linear regression was used to test for a significant relationship between mass and enzyme activity at different assay temperatures for biopsy samples collected in June and July of 2008 (N = 10). Only data from terrapins for which we had both summer and winter biopsies (N = 5) were used to assess the effects of temperature and season on enzyme activity. A square root transformation was performed on raw enzyme activity data so that assumptions of equal variance and normal distribution could be met, and a balanced split plot model was fitted to the data. Assay temperature and season were treated as fixed effects, and repeated measures from individual terrapins were accounted for as a random effect in the model. The Tukey HSD post-hoc test was used to further analyze significant differences between treatment groups. Statistical analyses were performed using JMP 7.0 software (SAS Institute Inc., Cary, NC, USA). Differences were considered to be statistically significant at

Table 1
Summary information for terrapins captured in June–July 2008 at Masonboro Island, NC. Terrapins were radiotracked for 23–260 days. Four terrapins were recaptured in January 2009 and one terrapin was recaptured in February 2009.

Terrapin ID	Capture and biopsy date	Sex	Initial mass (g)	Temperature monitoring period (days)	No. of visual observations	Recapture and biopsy date
ACK	11 Jun 2008	F	662	213	37	19Jan2009
ACL	11 Jun 2008	F	437	n/a	12	n/a (signal lost 30Jul2008)
ACM	11 Jun 2008	F	556	n/a	20	n/a (signal lost 30Oct2008)
ACN	11 Jun 2008	F	620	n/a	7	n/a (signal lost 29Sep2008)
ACO	11 Jun 2008	F	570	167	32	28Feb2009
ACQ	11 Jun 2008	F	706	207	31	19Jan2009
ACW	11 Jun 2008	F	494	246	38	19Jan2009[a]
HIJ	11 Jun 2008	F	541	n/a	20	n/a (signal lost 13Oct 2008)
AHO	30 Jul 2008	F	596	n/a	8	n/a (signal lost 22Aug2008)
AHP	30 Jul 2008	F	617	n/a[b]	20	19Jan2009

[a] A new data logger was placed on terrapin ACW on 19 January 2009 prior to her release. We subsequently recaptured this terrapin and were able to obtain temperature data for the period of 20 January 2009–28 February 2009.
[b] The temperature data logger was no longer attached to this terrapin upon recapture. Therefore, we have no temperature data for this animal.

$P<0.05$. Values are presented as mean ± standard error of the mean ($X \pm$ S.E.M.).

3. Results

3.1. Thermal habitat

Individual terrapins were monitored via radiotelemetry for 23–260 days. Some terrapins were visually observed reliably throughout the tracking period, whereas others were located sporadically (Table 1). From 13 June 2008 through 1 October 2008, we visually located tagged terrapins 167 times. Terrapins were observed swimming in the water 82.6% of the time, on the mud surface 1.2% of the time, and buried in the mud of the intertidal zone 16.2% of the time during the warm summer months (Fig. 2). Observations of terrapins buried in the mud of the intertidal zone became more frequent as temperatures decreased in the fall. From 2 October 2008 to 28 February 2009, we visually located tagged terrapins 58 times. Terrapins were observed swimming in the water 8.0% of the time, on the mud surface 2.0% of the time, and buried in the mud of the intertidal zone 90.0% of the time during the cold fall and winter months (Fig. 2).

We attempted to recapture terrapins mid-way through the tracking period to replace data loggers and insure continuous temperature recordings. Unfortunately, in most cases the data logger memory capacity exceeded before the terrapin could be recaptured and the instrument replaced, so there are some gaps in temperature coverage. The temperature monitoring period for individual terrapins ranged from 167 to 246 days. Temperature data loggers were successfully retrieved from four terrapins (Table 1).

In order to characterize the temperatures at which terrapins are typically active, we divided the temperature records into an active period (June 13–October 1, 16 weeks) and dormant period (Oct 2–Feb 28, 22 weeks) based on visual observations of terrapin behavior (Fig. 2). Mean weekly T_C during the active period was 27.0 ± 0.4 °C (Fig. 3). The mean weekly maximum T_C during the active period was 34.8 ± 0.7 °C, and the mean weekly minimum T_C was 22.8 ± 0.5 °C (Fig. 3). Mean weekly T_C steadily declined through the months of October and November (Fig. 3), and this was accompanied by a pronounced shift in terrapin habitat utilization (Fig. 2). Beginning in early October, the frequency of terrapins sighted swimming in water decreased noticeably as the frequency of terrapins sighted buried in mud increased. The mean weekly T_C during the dormant period was 12.4 ± 1.0 °C. The mean weekly maximum T_C during the dormant period was 17.9 ± 1.0 °C, and the mean weekly minimum T_C was 5.9 ± 1.2 °C (Fig. 3). Temperatures experienced by terrapins were consistently higher than 20 °C throughout the active period and consistently lower than 20 °C for the majority of the dormant period (Figs. 3 and 4). Sub-zero temperatures were experienced by terrapins on multiple occasions during winter dormancy. The lowest T_C was −3.42 °C, recorded from terrapin ACW on 6 Feb 2009.

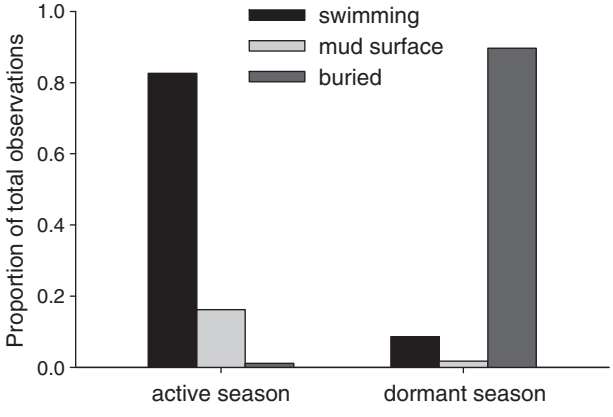

Fig. 2. Visual observations of diamondback terrapins swimming in water, on mud surface, and buried in mud at Masonboro Island, NC from 13 June 2008–1 October 2008 (active season) (N = 10) and 2 October 2008–28 February 2009 (dormant season) (N = 8).

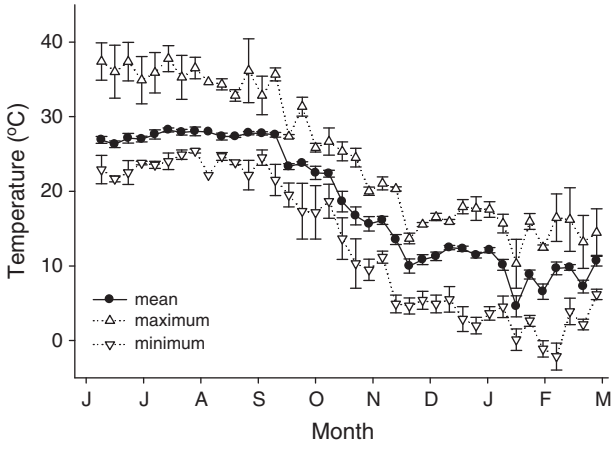

Fig. 3. Weekly mean, maximum, and minimum temperatures recorded by data loggers attached to the carapace of diamondback terrapins at Masonboro Island, NC, USA from June 2008 to February 2009 (13 June–19 January, N = 4; 20 January–28 February, N = 2).

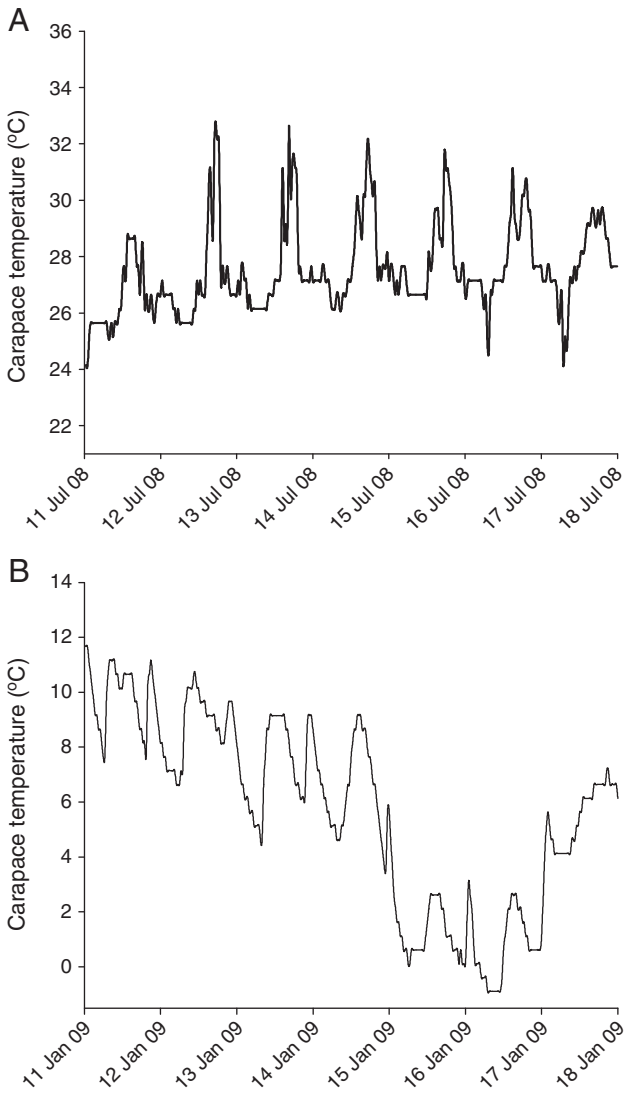

Fig. 4. Carapace temperatures recorded from terrapin ACQ during a one week interval in July 2008 (A) and January 2009 (B). Temperatures varied up to 7 °C within a 24-h period in the summer and up to 9 °C within a 24-h period in the winter. Sub-zero temperatures were experienced sporadically during the winter months.

3.2. Metabolic enzyme activity

Linear regression analyses indicated that there was no significant relationship between LDH, PK, CS, or CCO activity and terrapin mass at any of the assay temperatures tested ($r^2 = 0.001 - 0.283$, $P = 0.113–0.784$) . Assay temperature had a significant effect on activity of LDH ($F = 159.3432$, df = 3, $P < 0.0001$), PK ($F = 176.0264$, df = 3, $P < 0.0001$), CS ($F = 18.0317$, df = 3, $P < 0.0001$), and CCO ($F = 27.5830$, df = 3, $P < 0.0001$) (Fig. 5). The Tukey HSD post-hoc analyses showed that enzyme activity at each assay temperature was significantly different from all other temperatures tested for LDH and PK. There was no significant difference in CS activity between 20 and 30 °C, however, CS activity at 10 °C was significantly lower than at CS activity at 30 °C and 40 °C and CS activity at 40 °C was significantly higher than CS activity at 10 °C and 20 °C. Activity of CCO was significantly lower at 10 °C and 20 °C compared with CCO activity at 30 °C and 40 °C. There was no significant difference in either LDH activity ($F = 0.0057$, df = 1, $P = 0.9406$) or PK activity ($F = 1.0743$, df = 1, $P = 0.3154$) for tissue collected during summer and winter, but winter-collected tissue had significantly lower CS activity ($F = 25.1518$, df = 1, $P < 0.0001$) and CCO activity ($F = 39.0722$, df = 1, $P < 0.0001$) compared with summer-collected tissue. There was no significant interaction between season and assay temperature for LDH ($F = 0.0751$, df = 3, $P = 0.9725$), PK, ($F = 1.4125$, df = 3, $P = 0.2757$), CS ($F = 0.2732$, df = 3, $P = 0.8439$), or CCO ($F = 1.9830$, df = 3, $P = 0.1572$), indicating that the effect of temperature on enzyme activity does not vary between seasons.

The Q_{10} value for enzyme activity within the temperature range at which terrapins are active (20–40 °C) varied between 1.31 and 2.11 (Fig. 6). The Q_{10} for LDH, CS, and CCO varied between 1.39 and 1.76 and between 10 and 20 °C, but PK exhibited heightened thermal sensitivity within this lower temperature range, with a Q_{10} of 2.90 for summer-collected tissue and 5.55 for winter-collected tissue (Fig. 6).

4. Discussion

The estuarine diamondback terrapin encounters highly variable thermal conditions on both a daily and seasonal basis (Figs. 3 and 4). Given this dynamic thermal environment, it is perhaps not surprising that this species displayed low to moderate thermal sensitivity of metabolic enzyme activity (Q_{10} 1.31–2.11 at 20–40 °C, Fig. 6) over the wide range of temperatures in which activity is maintained. We predicted a heightened thermal sensitivity of metabolic enzyme activity at temperatures associated with entrance into dormancy (Herbert and Jackson, 1985), but a statistically significant shift in thermal sensitivity (as assessed by the interaction between season and assay temperature) was not detected with the split plot model. The mean Q_{10} values for LDH, CS, and CCO between 10 and 20 °C (Q_{10} 1.39–1.76) were similar to values observed between 20 and 40 °C (Fig. 6). Interestingly, pyruvate kinase displayed an increase in thermal sensitivity at low temperature, with an average Q_{10} of 2.90 at 10–20 °C in summer-collected tissue and 5.55 at 10–20 °C in winter-collected tissue (Fig. 6). Pyruvate kinase is the final enzyme in the glycolytic pathway of carbohydrate metabolism, and catalyzes a reaction resulting in substrate-level phosphorylation of ADP to form ATP and production of pyruvate. Pyruvate may either be further modified to produce substrates that enter oxygen-dependent metabolic pathways or used as substrate for lactic acid fermentation in the absence of oxygen. Pyruvate kinase is, therefore, important in terms of both aerobic and anaerobic means of ATP production from carbohydrate metabolism. The trend towards increased thermal sensitivity of PK in the range of 10–20 °C may contribute to a decreased rate of flux through glycolysis at the onset of winter dormancy.

Carbohydrates are the primary fuel utilized during winter dormancy in the majority of turtle species studied, particularly those that experience anoxic conditions while overwintering (Jackson, 2000), and a decrease in glycolytic capacity is typically associated with a decrease in overall metabolism. The degree to which terrapins experience anoxic conditions during winter burial is not known, but there is some evidence that they have regular access to air and maintain aerobic metabolism during dormancy (please see discussion below). A shift from carbohydrate to lipid metabolism could offset the lower rates of flux through glycolysis under aerobic conditions, but the role of fat oxidation in ATP production during winter dormancy in terrapins has not been explored. An assessment of β-oxidation enzymes (i.e. HOAD) may shed some light on this topic.

Activity of CS and CCO was significantly lower in winter-collected tissue compared with summer-collected tissue (Fig. 5). The seasonal trend in activity observed for these enzymes indicates that factors other than temperature contribute to metabolic downregulation during the winter (Olson, 1987; Seebacher, 2005). Metabolic depression in response to anoxia has been well-documented in freshwater turtles that overwinter in environments with limited access to oxygen, such as buried in mud or submerged in ice-covered ponds (Ultsch, 2006). These remarkable animals lower rates of ATP utilization by reducing protein turnover (Land et al., 1993; Land and Hochachka, 1994) and decreasing energy expenditure associated with maintenance of ion gradients across membranes (Perez-Pinzon et al.,

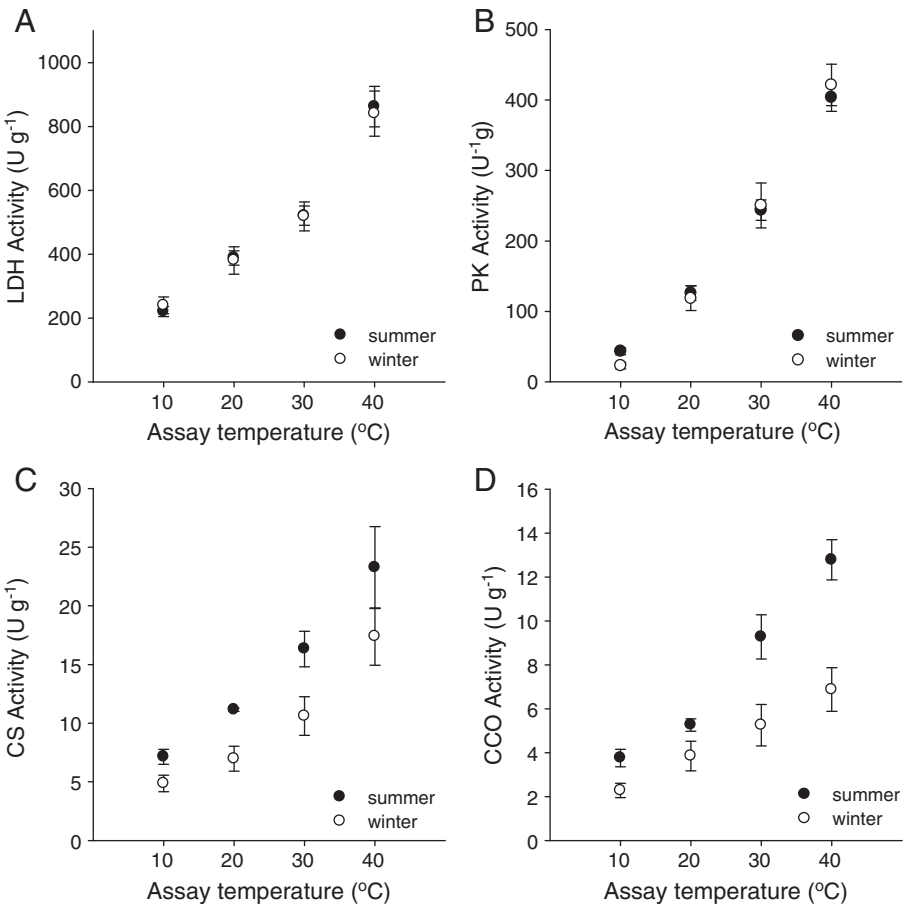

Fig. 5. Activity in $U \cdot g^{-1}$ for (A) lactate dehydrogenase (LDH), (B) pyruvate kinase (PK), (C) citrate synthase (CS), and (D) cytochrome *c* oxidase (CCO) measured in tissue collected from diamondback terrapins in summer (●) and winter (○) (N = 5). Assay temperature had a significant effect on activity of all enzymes ($P<0.0001$). There was no significant difference in activity of summer- and winter-collected tissue for LDH or PK, but the activity of CS and CCO was significantly lower in winter-collected tissue compared with summer-collected tissue. There was no statistically significant interaction between assay temperature and season for any of the enzymes, indicating that the overall pattern of thermal sensitivity was similar between seasons.

1992; Buck and Hochachka, 1993), and simultaneously lower rates of ATP production by modifying the activity of regulatory enzymes in ATP-producing pathways via phosphorylation and binding to subcellular components (Brooks and Storey, 1989; Storey and Storey, 1990; Storey, 1996). The molecular and cellular responses to anoxia permit these turtles to drastically lower their metabolic demands, such that ATP requirements may be met primarily through inefficient anaerobic pathways. Reliance on anaerobic glycolysis in overwintering freshwater turtles is supported by exceptionally large glycogen stores and enhanced buffering capacity to maintain acid-base balance in the face of high levels of accumulated lactate (Jackson, 2000).

The terrapins we located during the winter portion of this study were usually buried to a depth of approximately 5–10 cm in the mud of the intertidal zone. Oxygen availability would obviously be low during burial, but the shallow depths at which terrapins bury and the fact that they would have access to air during low tide does not preclude continued reliance on aerobic metabolic pathways. It is possible that terrapins move vertically within the mud to periodically access the surface and breathe. Visual observations made during the course of our radiotracking study confirmed that terrapins occasionally emerged from the mud and moved short distances during the winter (Fig. 2). Furthermore, blood samples obtained from terrapins at our field site showed low levels of plasma lactate during the winter, which implies that terrapins do not rely heavily on anaerobic metabolism during dormancy (Harden and Southwood Williard unpublished data, http://student.uncw.edu/lah4492/Harden_SICB_poster_2010.pdf). The common map turtle (*Graptemys geographica*), a close relative and potential sister taxa to the terrapin (Lamb and Osentoski, 1997), is anoxia intolerant and relies on aquatic respiration to maintain aerobic metabolism during winter dormancy (Graham and Graham, 1992; Crocker et al., 2000; Reese et al., 2001). If terrapins have regular access to air and maintain aerobic metabolism during the winter, it is unlikely that the seasonal decrease in enzyme activity observed in our study is due to anoxia-induced mechanisms of metabolic downregulation. A more detailed investigation of burial behavior and blood biochemistry would be useful in assessing the degree to which terrapins experience hypoxic or anoxic conditions in the winter, and the potential shifts in reliance on aerobic and anaerobic metabolic pathways.

Another factor that could contribute to seasonal metabolic downregulation of enzyme activity is alterations in circulating levels of thyroid hormones, such as triiodothyronine (T3) and thyroxine (T4). Several turtle species (*C. picta, Gopherus agassizii,* and *Chelonia mydas*) exhibit seasonal shifts in circulating levels of plasma T4 in particular, with highest levels of T4 occurring during summer and lower levels occurring during winter (Licht et al., 1985; Kohel et al., 2001; Southwood et al., 2003). Although a link between T4 and metabolic enzyme activity has not been demonstrated in turtles, studies conducted with lizards show that increases in plasma T4 are associated with increases in aerobic enzyme activity (CS) and a general increase in aerobic capacity and metabolic rate (John-Alder, 1983, 1984, 1990a,b). Levels of circulating thyroid hormones may be influenced by a number of factors that change seasonally, such as

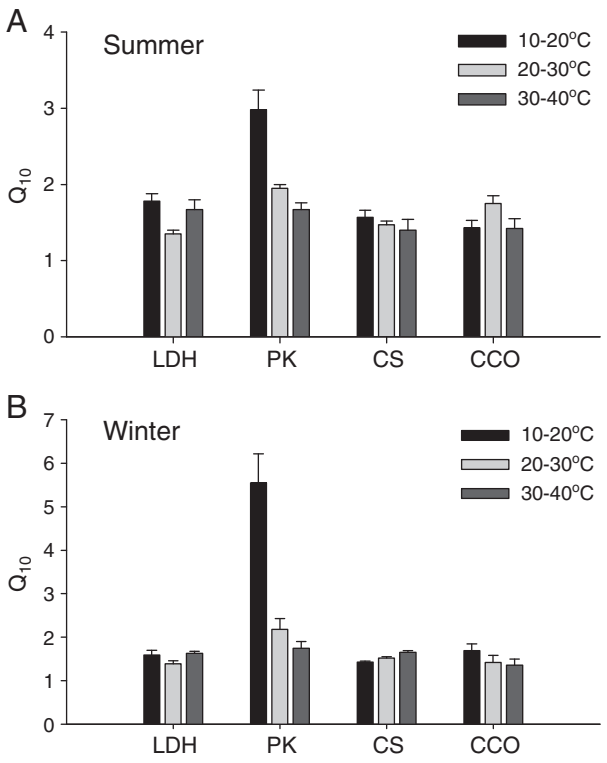

Fig. 6. Q_{10} of LDH, PK, CS, and CCO activity in muscle tissue collected during the summer (A) and winter (B). PK was the only enzyme that showed heightened thermal sensitivity at the range of temperatures associated with entrance into dormancy (10–20 °C).

nutritional status, reproductive status, temperature, and photoperiod (Lynn, 1970). An investigation of seasonal changes in thyroid hormones of terrapins has not been conducted, but could provide valuable information regarding the role of endocrine factors in seasonal shifts in biochemical reactions and overall metabolism.

Accumulation of nitrogenous waste products due to anuria is another factor that could contribute to metabolic suppression during winter dormancy in terrapins. Terrapins exhibit significantly higher plasma levels of urea during winter dormancy (80–85 mM) compared with levels in mid-summer (15 mM, Gilles-Baillien, 1973). Numerous studies have demonstrated the disruptive effects of urea on protein structure and enzyme function, (Hand and Somero, 1982; Yancey, 1994; Fuery et al., 1997; Cowan and Storey, 2002), and urea accumulation (80–220 mM) in hibernating and aestivating lungfish and amphibians is thought to reversibly inhibit enzyme activity and trigger metabolic downregulation during periods of decreased activity or dehydration (Funkhouser et al., 1972; Griffith, 1991; Withers and Guppy, 1996; Muir et al., 2007, 2008, 2010). Muir et al. (2010) demonstrated that mass-specific oxygen consumption of terrapin muscle tissue at 10 °C was significantly lower when treated with physiologically relevant levels of urea (80 mM) compared with controls, thus providing support for the urea-induced hypometabolism hypothesis. In addition to contributing to metabolic suppression, urea may also play a role in survival during exposure to sub-zero temperatures during winter dormancy (Costanzo and Lee, 2005; Costanzo et al., 2006). Hatchling terrapins in the northern portion of the geographical range are repeatedly exposed to sub-zero temperatures when they overwinter in terrestrial nests, and high rates of survival are attributed to an ability to tolerate osmotic stress caused by ice formation in the tissues (Baker et al., 2006). Freeze tolerance in hatchling terrapins is promoted by accumulation of organic osmolytes such as urea, glucose, and lactate (Costanzo et al., 2006). The adult terrapins in our study periodically experienced sub-zero temperatures, and terrapins that overwinter at higher latitudes are expected to experience more frequent exposure to freezing conditions. The role of urea in seasonal metabolic downregulation and freeze tolerance in adult terrapins, particularly in the northern portion of the range, is worthy of further investigation.

In conclusion, we found that terrapins experience broad fluctuations in temperature on both a daily and seasonal basis in their estuarine habitat. Low to moderate thermal sensitivity of metabolic enzymes permits continued functioning over the wide range of temperatures in which terrapins maintain activity, and a trend towards heightened sensitivity of the glycolytic enzyme PK at low temperatures may contribute to a decrease in carbohydrate metabolism at the onset of winter dormancy. Our observations of terrapin behavior are in agreement with previous observations of the timing of winter dormancy in terrapins, and results from metabolic enzyme activity analyses lend support to the idea that dormancy is induced at temperatures between 10 and 20 °C. Nevertheless, lower levels of enzyme activity in winter-collected tissue compared with summer-collected tissue when tested at the same assay temperature (Fig. 5) indicate that temperature is not the sole factor contributing to metabolic downregulation during the winter. The exact mechanisms by which enzyme activity is modulated on a seasonal basis have yet to be elucidated.

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