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Comparative thermal ecology of copperheads (*Agkistrodon contortrix*) and cottonmouths (*Agkistrodon piscivorus*)

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1. Introduction

Most animals thermoregulate in some fashion, but the ways in which they accomplish thermoregulation differ. Thermoregulation is important because T_b influences nearly all physiological processes (Angilletta et al., 2002b). Endotherms maintain their body temperature (T_b) physiologically over a wide variety of external temperatures (Bennett and Ruben, 1979) whereas ectotherms typically take advantage of variation in environmental temperatures to regulate their T_b through behavioral thermoregulation (Bogert, 1949). The intensity of behavioral thermoregulation is often conceptualized as a continuum of strategies that range from complete thermoconformity, where individuals passively receive the thermal conditions of the environment, to active thermoregulation in which body temperatures are tightly regulated, usually at a level different from the average environmental temperature (Hertz et al., 1993; Angilletta, 2009). The degree to which ectotherms regulate body temperature is further influenced by the costs and benefits of thermoregulation (Huey and Slatkin, 1976), which are determined by the availability of suitable temperatures that allow common activities and function (Huey and Stevenson, 1979). High cost thermal environments may increase mortality risk, energy expended to thermoregulate, and missed opportunities of reproduction or food acquisition (Angilletta, 2009). Some ectotherms have a wide thermal performance breadth (eurythermic), where physiological processes are near optimal over a wide range of body temperatures while others have a narrow thermal breadth (stenothermic) of optimized physiological processes, behaviors, and performance (Huey and Stevenson, 1979; Blouin-Demers et al., 2003). These thermal performance breadths influence the differing strategies used in thermoregulation (Huey and Stevenson, 1979).

Thermoconformity is a viable behavioral strategy when the physiological disadvantages of conformity are low (Ruibal, 1961). Physiological processes such as food assimilation (Du et al., 2007), heart rate (Licht, 1965), and locomotor performance (Kaufmann and Bennett, 1989) can have low or negligible costs when body temperature (T_b) can be maintained near the preferred body temperature range (T_{set}), negating the need for active thermoregulation (Blouin-Demers and Weatherhead, 2001). Conformity may also be the preferred strategy when costs of active thermoregulation are high relative to a resource availability or predation risk (Huey and Slatkin, 1976). Alternatively, some ectotherms invest extensive time and energy into precise thermoregulation. This strategy is common in thermally heterogeneous environments where achieving preferred body temperatures does not have a high cost (due to lower energy expenditures for thermoregulation; Huey and Slatkin, 1976; Blouin-Demers and Nadeau, 2005) or where ecological costs are low from a reduced risk of mortality shuttling over long distances (Angilletta, 2009).

Given the importance of thermoregulation in ectotherms, we examined the thermal ecology of two closely related viperids; the Copperhead (*Agkistrodon contortrix*) and the Cottonmouth (*Agkistrodon piscivorus*) (Gloyd and Conant, 1990). These species share a common evolutionary history (Burbrink and Guiher, 2015), but differ considerably in habitat use and are typically locally parapatric in distribution. Copperheads are typically found in deciduous hardwood forests with high levels of vegetative cover, brush piles, large logs, scattered rocks, and moist leaf litter (Fitch, 1960; Ernst and Ernst, 2003). They are often found near water but rarely observed in it (Fitch, 1960), and tend to be hidden under the cover of leaf litter where they can remain cryptic while waiting to ambush prey (Bechtel, 1978). Conversely, cottonmouths are almost exclusively associated with aquatic habitats and slow-moving bodies of water, from brackish marshes to muddy swamps and sloughs with patchy vegetation and canopy cover (Ernst and Ernst, 2003; Powell et al., 2016). Cottonmouths are frequently observed conspicuously basking on vegetation mats, logs, and hummocks exposed from the water (Burkett, 1966) and may aggregate at remaining waterholes during times of drought (Ernst, 1992).

The objective of this study was to compare the thermal biology of these two closely related, but somewhat ecologically and behaviorally dissimilar species. Since cottonmouths in our study inhabit areas with sparse canopy and large tree gaps, we predict that cottonmouths will thermoregulate more effective. Copperheads for this study were found in closed-canopy hardwood forests which may reduce the thermal heterogeneity of habitats available to the snakes. Because they differ markedly in habitat use, occupying sites with potentially dissimilar thermal environments, we test the hypothesis that habitat use can

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influence the ways in which the species interact with their thermal environments (thermoregulation).

2. Materials and methods

2.1. Locality

Copperheads were located at Land Between the Lakes National Recreation Area (LBL) in Trigg county Kentucky (36.81°N 88.06°W). The landscape is dominated by secondary oak-hickory forest along steep, dry ridges and mesophytic communities in cooler, moist ravines (Close et al., 2002). Cottonmouths were studied at a site adjacent to the Cumberland River and backwater sloughs of Sycamore Creek, Cheatham County Tennessee near Cumberland River Bicentennial Trail (CRBT, 36.28°N 87.08°W). The area is comprised of a large palustrine scrub-shrub wetland (Tiner, 1984) with adjacent agriculture fields and mixed hardwood forest. The two sites are approximately 100 km apart with a difference of approximately 50 m in elevation. Snakes at both sites were collected opportunistically during the active seasons (April-September) and capture locations were recorded using a handheld GPS (Garmin GPSmap 60Sx). Sex was determined in the field via cloacal probing and only male snakes were used in this study because of potential differences in thermal ecology between the sexes (Charland and Gregory, 1990; Graves and Duvall, 1993; Gardner-Santana and Beaupre, 2009). Individuals were assigned to one of two experimental groups; telemetered snakes to carry T_b recording dataloggers in the field, or laboratory gradient snakes to carry dataloggers in a lab experiment used to determine the preferred T_b range.

2.2. Preferred body temperature

A laboratory thermal gradient was used to estimate individual's preferred body temperature range (T_{set}). The gradient provides an environment that is free of ecological costs and constraints that could potentially influence thermoregulation (Hertz et al., 1993). We used three identical thermal gradients, and each was comprised of a large commercial snake cage (Vision cage V632, 183 × 91 cm) cooled at one end to ~16 °C using a copper coil placed underneath the cage circulating water through a Fischer Scientific chiller (model 9010). Adjacent to the cooling coil we used eight 15 cm wide Flexwatt heat strips (12 W/ft) placed side-by side and individually connected to temperature controllers (Omega CSC32) with type-K thermocouples reading the surface temperatures on the floor of each gradient. This design provided a linear thermal gradient of available temperatures between 16 °C and 34 °C on the interior floor of the cage, changing 2.0 °C per 15 cm. Lighting was provided by fluorescent fixtures in the room set to a 12:12 h light:dark photoperiod. Snakes were given ~5 cm of leaf litter (native oaks) as substrate and water was available in small bowls at three locations across the gradient.

To determine the preferred body temperature range (T_{set}), snakes were anesthetized with isoflurane and surgically implanted with a Maxim iButton® DS1922L temperature data logger weighing no more than 5% of the snake's body mass. Loggers were coated in biologically inert rubber (Plasti Dip International, St. Louis Park, MN) and inserted into the coelomic cavity following the protocols of Reinert and Cundall (1982). Postabsorptive snakes were then allowed to recover for a minimum of 24 h before being placed in the thermal gradient and given 24 h to acclimate before initiating measurements. After the acclimation period, snakes remained in the gradient for 72 h with data loggers recording body temperatures every 10 min. Snakes were then removed from the gradient and the data loggers were retrieved using the same surgical procedures as above. The bounds of the central 50% of T_bs during the 72 h test period were considered the preferred temperature range, T_{set}, for each individual (Hertz et al., 1993; Christian and Weavers, 1996).

2.3. Telemetry

Snakes collected for telemetry had a radio transmitter (SI-2, Holohil Systems Ltd., Carp, ON, Canada) implanted in the coelomic cavity approximately one-third of the body length anteriorly to the cloaca following the methods of Reinert and Cundall (1982). These snakes were also implanted with an iButton® DS1922L data logger programmed to record body temperatures (T_b) every 20 min for the length of the active season. The combined mass of the radio transmitter and datalogger did not comprise more than 7% of the total body mass of the snake. After surgery, snakes were given 24–48 h to recover in the lab and were then released in the field at the point of capture. Snakes were relocated with telemetry (Advanced Telemetry Systems R410 receiver and Telonics RA-23 antenna) at least once per week to monitor general location.

2.4. Operative temperatures

To estimate the distribution of body temperatures that snakes could potentially attain at the study sites, hollow copper models were used to estimate environmental operative temperatures (T_e; Bakken, 1992; Peterson et al., 1993; Christian et al., 2016). Models were 30 cm long and 1.9 cm in diameter with caps secured on each end. Temperature data loggers (Maxim iButton® DS1922L) were placed inside each model and recorded operative temperature every 20 min, coinciding with recording of snake T_bs. Data loggers were first coated in rubber (Plasti Dip International, St. Louis Park, MN) for weatherproofing and secured with zip ties along perpendicular axes of the logger. The ties kept the datalogger in the center of the model, reducing conductive heat exchange with the sides of the model (Bakken, 1992). Copper models were designed to be of a similar size, shape, and radiative properties of copperheads or cottonmouths and were painted to approximate the average reflectance of the dorsal surface of the snakes (Peterson et al., 1993).

We deployed 24 operative models at each site, dispersed in a manner to represent as closely as possible the different thermal regimes (thermal microhabitats) available to snakes while they are engaged in above-ground activities (i.e. moving or resting in ambush foraging posture). We placed models in open canopy tree-fall gaps, closed-canopy forests, under cover at the bases of fallen trees, along ecotone edges, and (for cottonmouth sites) in shallow-water areas of the swamp.

2.5. Analyses

To quantify the thermal properties of the habitats available to snakes, we used several commonly applied indices of operative temperatures and of thermoregulation (Hertz et al., 1993; Christian et al., 2016). To assess thermal quality of the habitats we used the d_e index (Hertz et al., 1993; Blouin-Demers and Weatherhead, 2001) calculated as the mean deviations of T_es from the T_{set} range. If T_e is below T_{set}, d_e is measured as the difference between the lower bound of T_{set} and T_e and vice versa for T_es above T_{set}. We also measured the accuracy of thermoregulation, d_b, (Blouin-Demers and Weatherhead, 2002) as the mean absolute value of deviations of T_bs from T_{set} (Hertz et al., 1993). An index of thermoregulatory effectiveness (E) was calculated from the d_e and d_b values, defined as $E = 1 - (\bar{d}_b/\bar{d}_e)$ and generally ranges from 0 (no thermoregulation, strict thermoconformity) to 1 (precise thermoregulation; Hertz et al., 1993). To further compare the effectiveness of thermoregulation between copperheads and cottonmouths we also used Blouin-Demers and Weatherhead's index of effectiveness (d_e-d_b; 2001), hereafter referred to as departure from thermoconformity. Their index (d_e-d_b) removes the ratio that is found in Hertz et al. (1993), permitting species comparisons by looking at the magnitude of the difference between d_e and d_b, and not a ratio that could be potentially skewed by snakes having different thermal environments and strategies of thermoregulation (Blouin-Demers and Weatherhead, 2001). Both effectiveness of thermoregulation and departure from thermoconformity are considered here for comparison with other studies using only the Hertz et al. (1993) index. We calculated exploitation of the thermal environment (*Ex*) for each individual, defined as the time in which T_bs are within T_{set} divided by the time available for T_bs to be within T_{set} (Christian and Weavers, 1996).

Data analysis was conducted using JMP Pro 10 (SAS Institute Inc., Cary, NC). All analyses for thermoregulatory indices were averaged for each individual snake and each operative model over the course of a month. Monthly means were used in linear mixed-model repeated measures analysis of variance (ANOVA) to determine differences between species and sites, among months, and for month*site interactions. Species, site, and month were all fixed effects in the model with either individual snake or individual model being considered a random effect (depending whether considering T_b or T_e). Post-hoc comparisons of species by month were conducted using Tukey HSD tests. Comparisons were considered significant if statistical test results yielded $P < 0.05$

3. Results

We recorded T_bs for 13 cottonmouths for a total of 215,756 T_bs (April 2012-September 2013) and 149,047 T_bs of 15 copperheads (April 2015 to September 2016). We collected 224,029 T_es for cottonmouth habitats and 575,808 T_es for copperhead habitats. Temperatures were combined to generate monthly means for calculation of indices of thermoregulation.

3.1. Thermal preference (Laboratory)

Thirty-five male copperheads and 19 male cottonmouths were used to determine preferred T_b range in laboratory thermal gradient trials. Copperheads had a mean T_b of 26.4 °C and mean upper and mean lower T_{set} of 27.8 °C and 24.9 °C and cottonmouths were nearly identical, having a mean T_b of 26.1 °C with upper and lower T_{set} of 27.9 °C and 24.9 °C, respectively (Table 1). There was not a difference between the two species for any of the metrics describing thermal preference (mean T_b, $F_{1,53} = 0.44$, $P > 0.51$; upper T_{set}, $F_{1,53} = 0.0006$, $P > 0.98$; lower T_{set}, $F_{1,53} = 0.1$, $P > 0.75$).

3.2. Thermal quality of the environment

Grand mean T_es for each month increased from April to July, peaking in July for both sites, and decreased through September (Table 2, Fig. 1). Mean T_e was different among months ($F_{5,191} = 695.71$, $P < 0.0001$) but not between sites or month*site interactions ($F_{1,206} = 1.66$, $P = 0.1994$; $F_{5,191} = 2.09$, $P = 0.0688$; Fig. 1). A Tukey HSD showed that all months were different (pairwise between sites) except June and August. Mean minimum temperatures differed among months and month*site interactions but not between sites ($F_{5,190} = 307.41$, $P < 0.0001$; $F_{5,190} = 4.53$, $P = 0.0006$; $F_{1,212} = 0.06$, $P = 0.80$). Mean maximum temperature was different among months, between sites and month*site interactions (Table 2; $F_{5,190} = 4.28$, $P = 0.001$; $F_{1,207} = 20.81$, $P < 0.0001$; $F_{5,190} = 4.07$, $P = 0.001$). Mean monthly temperatures at both sites reached the T_{set} for each species in only July; however, mean maximum temperatures were above the upper bounds of T_{set} in each month of the active season indicating that microhabitats were available to allow snakes to reach their T_{set}.

Operative temperatures (T_e) indicate that habitats occupied by the species differed significantly in thermal quality (d_e). However, these results may be driven by sample size rather than biological differences, and for every month, mean T_es and mean d_es were less than 1 °C different between the two sites (Table 2). Month, site, and the interaction between month and site were all different in mean d_es ($F_{5,190} = 641.71$, $P < 0.0001$; $F_{1,215} = 7.35$, $P = 0.0073$; $F_{5,190} = 11.27$, $P < 0.0001$) indicating thermal quality is different between habitats among the months of the active season (Table 2). Post-hoc Tukey HSD tests indicates that LBL had a lower d_e than CRBT for June and July. May was the only month where CRBT had a lower d_e than LBL and the sites are not different in August and September. The two sites were also different in max d_e among months, between sites, and month by site interactions ($F_{5,190} = 6.92$, $P < 0.0001$; $F_{1,209} = 11.68$, $P = 0.0008$; $F_{5,190} = 6.44$, $P < 0.0001$). Tukey HSD indicates that the differences in d_e between sites occur in June and July with LBL having a lower mean maximum d_e.

3.3. Body temperatures

Across the active season body temperatures (T_b) were different among months, between species, and month*species interactions ($F_{5,94} = 232.82$, $P < 0.0001$; $F_{1,22} = 9.48$, $P = 0.0055$; $F_{5,94} = 3.80$, $P = 0.0035$) with a Tukey HSD post hoc test indicating different T_bs in May (19.1 ± 0.3 and 21.4 ± 0.4) and June (23.2 ± 0.4 and 24.6 ± 0.4) with cottonmouths having a higher T_b than copperheads. Both species follow the trend of having the lowest T_bs in April with increasing T_b through July and then decreasing again through September (Fig. 2). Similarly, the two species differ in their deviations from the set-point range (d_b) among months between species and month*site interactions ($F_{5,91} = 267.37$, $P < 0.0001$; $F_{1,17} = 11.11$, $P = 0.0039$; $F_{5,91} = 5.17$, $P = 0.0003$). Post-hoc Tukey HSD test indicates that cottonmouths have a lower d_b in May (4.4 ± 0.1 °C) than copperheads (6.3 ± 0.1 °C), suggesting that cottonmouths are behaviorally thermoregulating more effectively and maintaining T_bs closer to their T_{set}.

3.4. Thermoregulation indices

We used multiple indices to compare copperhead and cottonmouth thermoregulation during the active season. Effectiveness of thermoregulation, (*E*; Hertz et al., 1993), was different between the two species ($F_{1,24} = 29.96$, $P < 0.0001$), among the months of the active season ($F_{5,102} = 16.48$, $P < 0.0001$), and species*month interaction (Fig. 3; $F_{5,102} = 17.47$, $P < 0.0001$). Significant differences between species occurred in May ($E = 0.33 \pm 0.06$ and 0.12 ± 0.02), June ($E = 0.51 \pm 0.03$ and 0.20 ± 0.00.03), and July ($E = 0.59 \pm 0.02$ and 0.15 ± 0.04) with cottonmouths having a higher *E* (thermoregulating more precisely) than copperheads in each month (Fig. 3a; Tukey HSD < 0.05 for each comparison). Significant differences in thermoregulation are apparent when examining departure from thermoconformity (d_e-d_b; Blouin-Demers and Weatherhead, 2001). There was a difference between species ($F_{1,25} = 23.15$, $P < 0.0001$), among months ($F_{5,101} = 2.79$, $P < 0.0212$), and month*species interactions

Table 1

Mean body temperatures (T_b in °C) selected by male *Agkistrodon sp.* in a laboratory thermal gradient with 75% and 25% quartiles of distributions represented. Means ± 1 standard error with ranges in parentheses.

Gradient index	*A. contortrix* (N = 35)	*A. piscivorus* (N = 19)	df	*F*	*P*
25% quartile	24.9 ± 0.5 (24.4–25.7)	24.9 ± 0.8 (24.1–25.7)	1,53	0.100	> 0.75
75% quartile	27.8 ± 0.3 (27.5–28.1)	27.9 ± 0.6 (27.3–28.5)	1,53	< 0.001	> 0.98
Mean	26.4 ± 0.3 (26.1–26.7)	26.1 ± 0.6 (25.5–26.7)	1,53	0.440	> 0.51

Table 2

Mean maximum, grand mean, and mean minimum environmental operative temperatures (T_e) along with mean deviation (d_e), mean maximum deviation and percentage of deviations equal to T_{set} of environmental temperatures from T_{set} for Land Between the Lakes National Recreation Area (LBL) and Cumberland River Bicentennial Trail (CRBT) ± 1 standard error.

Month	Mean Max T_e (°C)		Mean T_e (°C)		Mean Min T_e (°C)		Mean d_e (°C)		Max d_e (°C)		d_e (% = 0)	
	LBL	CRBT	LBL	CRBT	LBL	CRBT	LBL	CRBT	LBL	CRBT	LBL	CRBT
April	37.4 ± 2.6	42.7 ± 1.8	16.2 ± 0.3	15.7 ± 0.2	1.3 ± 0.5	0.2 ± 0.5	9.6 ± 0.1	10.4 ± 0.2	23.9 ± 0.7	24.7 ± 0.5	3.3 ± 0.005	5.3 ± 0.004
May	35.8 ± 2.5	36.3 ± 2.8	18.4 ± 0.3	19.0 ± 0.4	6.1 ± 0.5	7.6 ± 0.8	7.2 ± 0.1	6.4 ± 0.3	19.9 ± 0.9	18.1 ± 1.2	3.8 ± 0.007	7.3 ± 0.01
June	36.5 ± 2.1	45.6 ± 3.1	23.6 ± 0.3	23.7 ± 0.3	13.4 ± 0.3	14.2 ± 0.6	2.9 ± 0.1	3.7 ± 0.2	14.3 ± 1.4	19.1 ± 2.6	18.1 ± 0.016	13.5 ± 0.014
July	36.6 ± 2.1	47.0 ± 3.4	25.1 ± 0.2	25.3 ± 0.3	18.3 ± 0.3	16.5 ± 0.9	1.5 ± 0.1	2.5 ± 0.3	11.2 ± 1.7	19.4 ± 3.3	28.9 ± 0.021	23.2 ± 0.021
August	37.7 ± 2.3	43.5 ± 3.3	24.1 ± 0.3	24.2 ± 0.3	13.8 ± 0.4	14.1 ± 0.8	2.6 ± 0.2	2.8 ± 0.2	14.7 ± 1.6	18.0 ± 2.6	21.1 ± 0.015	19.0 ± 0.018
September	38.6 ± 2.4	39.8 ± 2.8	22.3 ± 0.3	22.4 ± 0.3	10.1 ± 0.4	10.4 ± 0.8	4.3 ± 0.2	4.0 ± 0.2	17.4 ± 1.4	17.5 ± 1.8	11.0 ± 0.008	11.9 ± 0.012

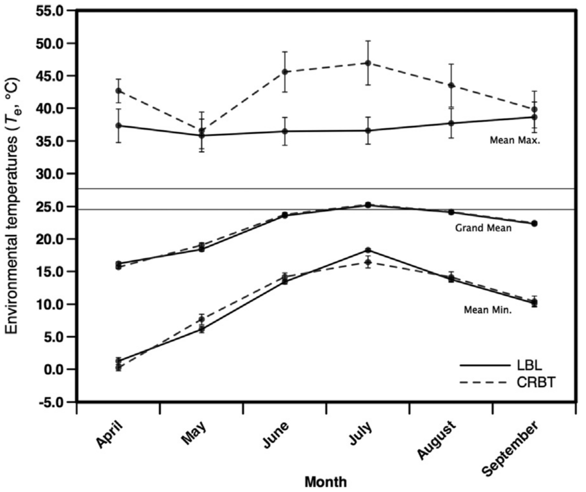

Fig. 1. Mean minimum, grand mean, and mean maximum operative environmental temperatures (T_e) for each month of the active season at Land Between the Lakes National Recreation Area (LBL) and Cumberland River Bicentennial Trail (CRBT; +/- standard error bars). Horizontal lines denote the preferred body temperature ranges for copperheads and cottonmouths.

($F_{5,101}$ = 11.2, $P < 0.0001$) across the active season. Cottonmouths thermoregulated more precisely than copperheads in May (Fig. 3b; 2.14 ± 0.40 vs. 0.90 ± 0.14), June (2.00 ± 0.12 vs. 0.58 ± 0.08), and July (1.74 ± 0.16 vs. 0.22 ± 0.06; Tukey HSD < 0.05 for all).

The index of thermal exploitation (*E*x) was significantly different between species overall ($F_{1,25}$ = 5.49, $P = 0.03$) and among months ($F_{5,96}$ = 23.0, $P < 0.0001$), but not for the interaction of month*-species (Fig. 3c; $F_{5,96}$ = 1.7, $P = 0.14$). Cottonmouths consistently maintained a higher *E*x throughout the active season (4 of 6 months) although differences were small in July (52.3 ± 3.36 and 51.39 ± 3.22) and August (54.95 ± 3.46 and 54.77 ± 3.33).

4. Discussion

Overall, copperheads and cottonmouths exhibited remarkably similar patterns in monthly body temperatures and thermoregulation across much of the active season. Despite being different structurally, thermal environments for the two species were also remarkably similar. This suggests that the two species face similar thermal challenges and interact with their thermal environments in a similar manner, overall. However, cottonmouths generally tend to thermoregulate more effectively, especially in late summer when their habitats offer higher maximal temperatures. In May, June, and July cottonmouths maintained higher thermoregulatory effectiveness than copperheads, which tend to be closer to thermoconformity, passively enduring operative temperatures. The two species appear to exploit their thermal environments similarly, maintaining their T_bs within T_{set}, when T_{set} is available, just over 50% of the time. Most of the active season, neither species seem to utilize the highest temperature microhabitats available.

Thermoregulation can be costly for ectotherms, especially when habitats are not spatially or temporally thermally heterogeneous (Huey and Slatkin, 1976). It may mean that the organism has to expend considerable effort to find suitable temperatures, which can be energetically expensive (Huey and Slatkin, 1976). While seeking suitable environmental temperatures for thermoregulation, individuals may also have increased exposure to predators. Adult cottonmouths (730.3 g ± 87.6 g) are considerably larger than adult copperheads (285 g ± 22.9 g), and larger body size may allow cottonmouths the opportunity to spend more time exposed while searching for suitable temperatures because they have fewer predators relative to smaller sized copperheads of the same age (Mushinsky and Miller, 1993; Ernst and Ernst, 2003). This is supported by observations of adult

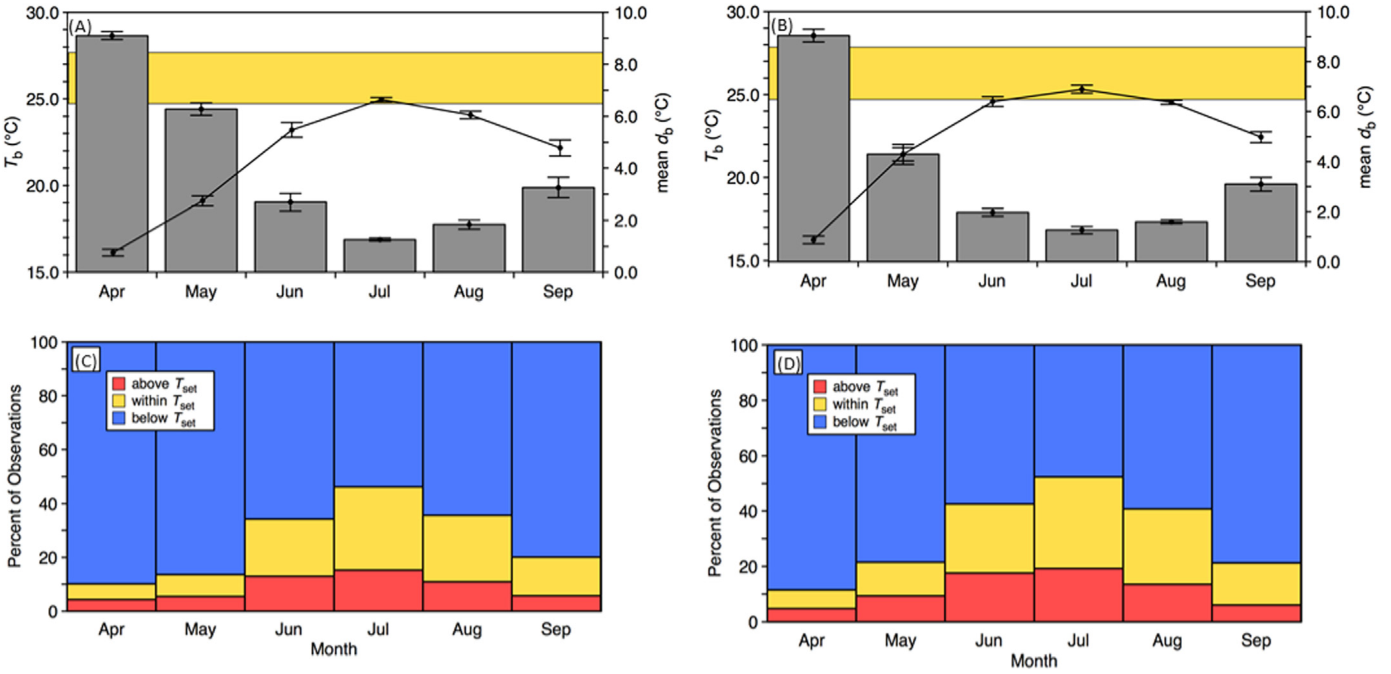

Fig. 2. A & 2B. The yellow bar was the preferred body temperature range of copperheads and cottonmouths respectively (T_{set}). The solid line represents the mean monthly body temperature (T_b). Columns represent the mean deviations in body temperature from the set-point range (d_b). Error bars are ± 1 standard error. Fig. 2C & D. Percentage of observations in which copperheads and cottonmouths body temperatures were above, below, or within their preferred body temperature range (T_{set}).

cottonmouths occurring farther from aquatic foraging areas than juvenile cottonmouths, which is potentially driven by a higher predation risk for juveniles (Eskew et al., 2009). High predation risk may outweigh the thermal benefits that copperheads receive when leaving refugia to seek favorable thermal environments. Remaining in cooler environments may also reduce the energy costs, subsequently decreasing foraging demands (Brown and Weatherhead, 2000) and risk of predation.

Behavioral differences may account for some of the variation in thermoregulation between species. At our sites, cottonmouths emerge from hibernation in limestone bluffs and disperse through a narrow hardwood corridor to an adjacent buttonbush swamp where they spend the majority of their active season. Copperheads in our study, however, typically hibernate in small mammal burrows in closed-canopy forests until spring emergence. They do not appear to show long-distance dispersal behavior away from hibernacula that is typical of cottonmouths. Cottonmouths may be dispersing to structurally heterogeneous sites (swamps with patchy canopy cover and emergent vegetation) that contain a diversity suitable thermal microhabitats, allowing them to thermoregulate more efficiently than copperheads which typically occupy homogeneous closed-canopy forest. Similar movement patterns have been found in black ratsnakes and northern watersnakes with overlapping ranges and a similar preferred temperature range (T_{set}). Black ratsnakes have lower T_bs in the field than northern watersnakes, and this is likely a result of a wider thermal performance breadth in black ratsnakes, allowing them to perform adequately at these lower temperatures (Brown and Weatherhead, 2000; Blouin-Demers and Weatherhead, 2001; Blouin-Demers et al., 2003).

On the ambush predator foraging continuum, copperheads are thought to be a more active forager than cottonmouths, although both shift ontogenetically from sit-and-wait predators as neonates to more active ambush predator as adults (Beaupre and Montgomery, 2007; Eskew et al., 2009). The higher movement rates copperheads exhibit may produce a behavioral tradeoff between foraging and precise thermoregulation (Belliure et al., 1996). Christian and Weavers (1996) observed a similar pattern when comparing four species of varanid lizards in Australia, with *Varanus panoptes* being the most active species but thermoregulating with the least precision. In Asian monitor lizards, *Varanus bengalensis* and *V. salvator*, are sympatric with contrasting terrestrial (*V. bengalensis*) and semi-aquatic (*V. salvator*) habitat use. *Varanus salvator* maintained lower T_bs with less variability than *V. bengalensis* (Wikramanayake and Dryden, 1993) which is opposite of what we found with copperheads and cottonmouths. Our semiaquatic species (cottonmouth) had a higher T_b than our terrestrial species although only significantly different in May. However, our results do support the anecdotal trend that Wikramanayake and Dryden (1993) found indicating that *V. salvator* likely had a lower T_b due to heavy habitat canopy cover (and presumably lower T_es) near the riparian areas that *V. salvator* inhabited compared to inland areas that *V. bengalensis* prefer. This information may explain the slightly lower T_b found with copperheads since operative models indicate that the heavy canopy cover of LBL has a lower mean maximum temperature for periods of the active season. Cottonmouths may also be using riparian vegetation to maintain a more consistent T_b than copperheads when air temperatures are not ideal, a similar behavior demonstrated by *V. salvator* (Wikramanayake and Dryden, 1993). A better understanding of microhabitat use by both copperheads and cottonmouths may help us understand the interaction between habitat structure and behavioral thermoregulation by both species.

Cottonmouths are appreciably larger in adult body size than copperheads, which may aid them in maintaining preferred body temperatures, even when conditions are not favorable (Shine and Madsen, 1996). The average adult copperhead in our study was 746.9 mm SVL with an average mass of 285 g, whereas cottonmouths averaged more than twice that with a mean mass of 730 g and a mean SVL of 836.6 mm. Thermal inertia associated with additional mass may allow cottonmouths to maintain higher T_bs while traveling through thermally unfavorable habitats or while foraging in water (Bartholomew, 1966). Australian tiger snakes maintain a higher overall T_b when limited to semiaquatic vs terrestrial activity and have increased performance in foraging and predator escape (Aubret and Michniewicz, 2010). Entering the water with a higher T_b may delay the reduction in locomotor

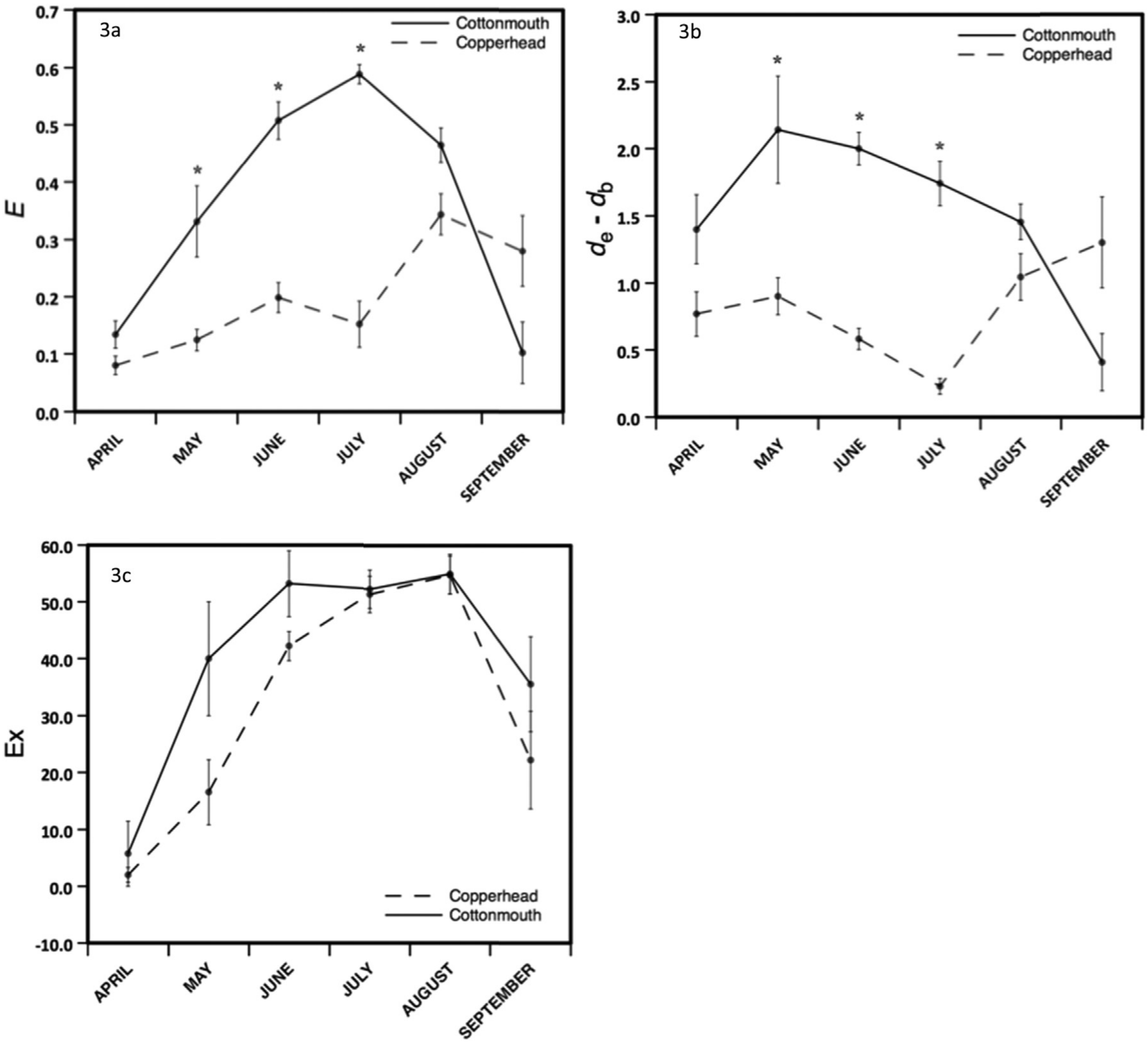

Fig. 3. a. Mean effectiveness of thermoregulation (*E*) over the active season 3b. Mean departure from thermoconformity (d_e- d_b) for each month of the active season 3c. Mean thermal exploitation (*E*x) for each month of the active season. Asterisks indicate significance with a post hoc Tukey HSD.

performance associated with a cooling body temperature and extend the time that an ectotherm can forage or evade a predator in the water (MacKinnon et al., 2006). Locomotor performance has been shown to decline for other species after entering cool water, causing them to be slightly hypothermic. Both the Australian water rat (*Hydromys chrysogaster*) and the *Triturus* newt had reduced locomotor performance after entering cooler water (Dawson and Fanning, 1981; Gvozdik and Van Damme, 2008). Since locomotor performance can be linked to fitness (Angilletta et al., 2002a), it is beneficial for ectotherms to maintain a T_b that increases performance (Huey and Kingsolver, 1989; Marvin, 2003), which may help explain higher precision of thermoregulation in cottonmouths.

We estimated the T_{set} for copperheads to be 24.9–27.8 °C and the T_{set} of cottonmouths to be 24.9–27.9 °C. Other studies of these same species have shown wider preferred T_b ranges than the range that we estimated. Copperheads have been previously estimated to have a preferred T_b range of 23–30.5 °C (n = 20; Sanders and Jacob, 1981) encompassing our estimated range. Differences in calculated T_{set} range may also be partially driven population differences. Cottonmouths were previously estimated to have a T_{set} of 22.6–29.2 °C in Texas (n = 8; Keck, 1998) and 23.5–29.5 °C in Southwest Missouri (n = 4; Menzel, 2008), which also encompasses our calculated set-point range. Copperheads and cottonmouths appear to have a lower T_{set} than other North American snakes. They have a lower T_{set} than massasauga rattlesnakes (*Sistrurus catenatus*) at 30.0–33.6 °C (Harvey and Weatherhead, 2010, 2011), milksnakes (*Lampropeltis triangulum*) at 29.0–31.0 °C (Row, 2005), and black ratsnakes (*Pantherophis obsoletus*) at 26.5–29.8 °C (Blouin-Demers and Weatherhead, 2001). This suggests that copperheads and cottonmouths routinely prefer lower temperatures than other snakes, even those occurring at higher latitudes, which typically have lower or broader T_{set} ranges than the same species at lower latitudes (Stevens, 1989; Weatherhead et al., 2012).

Departure from thermoconformity (d_e-d_b) was also lower for copperheads (0.76 ± 0.1) and cottonmouths (1.6 ± 0.1) relative to massasauga rattlesnakes (3.3 ± 0.1; Harvey and Weatherhead, 2011) and black ratsnakes of Texas (3.24 ± 0.25), Illinois (3.08 ± 0.38), and Ontario (3.66 ± 0.29; Weatherhead et al., 2012). However, cottonmouths in this study had a higher d_e-d_b index (1.6 ± 0.1) than those in Missouri (0.69; Menzel, 2008) and a higher exploitation index (*E*x) for both copperheads (33.6% ± 2.9) and cottonmouths (44.9% ± 3.0) than previously reported for cottonmouths (35.9%; Menzel, 2008) and black ratsnakes of Texas (23.9% ± 1.5), Illinois (26.7% ± 2.3), and Ontario (21.6% ± 1.5; Weatherhead et al., 2012). This indicates that copperheads and cottonmouths trend more toward thermal conformity than some other snake species but do take advantage of temperatures above their minimum T_{set} more often than black ratsnakes.

The grand mean for effectiveness of thermoregulation (*E*) was higher for our copperheads (0.20 ± 0.02) and cottonmouths

(0.35 ± 0.02) than previously estimated by Menzel (2008) for cottonmouths of Southwest Missouri (0.14). This suggests that copperheads and cottonmouths routinely thermoregulate more effectively than other species. When the lower bounds of T_{set} are attainable, cottonmouths exploit their environment to a higher degree than previously reported with both species having a higher *E*x than black ratsnakes from other studies. Overall this suggests that copperheads and cottonmouths perform better at lower temperatures than some other North American snakes by tolerating cooler temperatures but maintaining T_{b}s in their T_{set} range more frequently when the environment allows.

Although both species have nearly identical T_{set} range, cottonmouths tend to thermoregulate more precisely than copperheads during the months of May, June, and July, and both species seem to regulate their T_{b} less efficiently than other snakes (Harvey and Weatherhead, 2011; Weatherhead et al., 2012). This study gives us an overall view of how two closely related yet ecologically different species thermoregulate over the course of the active season, but future studies may need to focus on thermoregulation at a finer scale. Finer resolution may yield information about the importance of particular thermal microhabitats snakes use, giving insight into the most important microhabitat structures. Investigation of differences in diel cycles could potentially explain the temporal differences in thermoregulation. It would also be advantageous to monitor the behavior and microhabitat use concurrently with recording T_{b}. This information would allow for more nuanced inferences on the thermal ecology of both species and give more insight into the driving forces behind the differences in thermoregulation.

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Declarations of interest

None.

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