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Water exchange relationships predict overwintering behavior in hatchling turtles

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Abstract

Neonatal ectotherms face a wide range of environmental hazards because of the diverse habitats they inhabit and their small body sizes; this is especially true amongst turtles that live in temperate zones and experience cold winter conditions after hatching. Such hatchlings must balance challenges involving desiccation, freezing, and predation, among other threats. Turtle hatchlings either overwinter in water, terrestrially in relatively shallow nests, terrestrially deep below nests, or terrestrially outside of the nest entirely, and these different microhabitats are associated with different desiccation and freezing risks. We measured desiccation tolerance of individuals of six turtle species, including two (Diamondback Terrapins (*Malaclemys terrapin*, Schoepf 1973) and Eastern Box Turtles (*Terrapene carolina*, Linnaeus 1758)) that utilize a strategy that has not previously been explored, along with Wood Turtles (*Glyptemys insculpta*, Leconte 1830), whose overwintering microhabitat is uncertain. We found additional support for the hypothesis that desiccation resistance is associated with overwintering strategies in hatchling turtles. Further investigation into the overwintering strategies of *M. terrapin* and *T. carolina* would be productive.

Keywords: hatchling, turtle, Diamondback Terrapin, *Malaclemys terrapin*, Eastern Box Turtle, *Terrapene carolina*, Wood Turtle, *Glyptemys insculpta*, desiccation, desiccation tolerance, overwinter, microhabitat, rehydration

Introduction

Neonate ectotherms face a wide range of environmental hazards, in large part because of the diverse habitats they inhabit. This is especially true amongst turtles that live in high latitudes and thus experience cold winter conditions soon after hatching. These hatchlings must overcome important challenges that affect mortality; involving desiccation, freezing, and predation, among other threats (Ultsch 2006; Costanzo et al. 2008; Muldoon and Burke 2012; Costanzo and Lee 2013). Neonate turtles display a surprising diversity of behaviors, including dramatic variation in the time between hatching from the egg and emerging from the nest, and where they go after leaving the nest. Thus four behavioral patterns are known for turtles; hatchlings either overwinter in water (OIW), overwinter terrestrially shallowly in the nest (the cavity initially excavated by the ovipositing female) and are likely exposed to sub-zero temperatures (TIN), overwinter terrestrially deep below the nest and thus perhaps avoid exposure to sub-zero temperatures (TBN), or overwinter terrestrially outside the nest entirely (TON) and likely are exposed to sub-zero temperatures (Costanzo et al. 1995; Ultsch 2006; Burke and Capitano 2011; Muldoon and Burke 2012; Duncan and Burke 2016). Furthermore, when exposed to freezing temperatures, hatchlings can either supercool or freeze (Costanzo and Lee 2013); each strategy has a set of associated challenges, mortality risks, and potential benefits. Members of some species may utilize multiple strategies, for example, Diamondback Terrapins (*Malaclemys terrapin*, Schoepf 1973) employ OIW, TIN, and TON strategies (Ultsch 2006), even within the same population (Muldoon and Burke 2012; Duncan and Burke 2016). Species-specific patterns and possible evolutionary explanations for these patterns are explored by Costanzo et al. (2008).

Baker et al. (2003), Costanzo et al. (2001), and Dinkelacker et al. (2004) (reviewed in Costanzo et al. 2008) investigated adaptations for cold tolerance, desiccation resistance, and rehydration ability in a suite of North American OIW, TIN, and TBN turtle species, and found that species employing TIN and TBN strategies were generally able to supercool and resist freezing at lower temperatures, and were better at resisting desiccation, than were OIW species. They thus hypothesized that adaptations for resisting freezing and desiccation were important challenges to turtle hatchlings overwintering in terrestrial environments. Given that the overwintering microhabitats of many hatchling turtles are poorly known, this relationship offers a method to use laboratory-collected physiological data to test hypotheses about the microhabitats of overwintering hatchling turtles. For example, the mostly aquatic Blanding's turtles (*Emydoidea blandingii*, Gray 1870) often lay nests far from water, hatchlings typically emerge in the fall and apparently wander broadly, eventually overwintering in a variety of forest and marsh habitats (Linck and Gillette 2009, Paterson et al. (2012; 2014)). Details of these microhabitats are unclear, and *E. blandingii* might employ either OIW or TON strategies (or both). Dinkelacker et al.'s (2004) measurements of *E. blandingii* hatchling desiccation resistance abilities were intermediate between typical OIW and TIN values (no TON values were available), indicating that moist overwintering sites were probably required, and TON was unlikely.

We repeated Baker et al. (2003), Costanzo et al. (2001), and Dinkelacker et al.'s (2004) (all conducted in the same lab using the same equipment, Costanzo pers. comm.) methodological approach to measure desiccation resistance, testing hatchlings of three additional turtle species, including two that are known to use the TON strategy (Diamondback

Terrapins, *Malaclemys terrapin* (Duncan and Burke 2016), and Eastern Box Turtles, *Terrapene carolina*, Linnaeus 1758 (Burke and Capitano 2011)). As Dinkelacker et al. (2004) did with *E. blandingii*, we tested hatchling Wood Turtles (*Glyptemys insculpta*, Leconte 1830) whose overwintering strategy is somewhat uncertain, with the expectation that our results would indicate which strategy was most likely for this species. We also tested the desiccation resistance of three species (Snapping Turtles (*Chelydra serpentina*, Linnaeus 1758), Northern Map Turtles (*Graptemys geographica*, Lesueur 1817) and *E. blandingii* that had been previously tested by Baker et al. (2003), Costanzo et al. (2001), and Dinkelacker et al.'s (2004), to facilitate comparisons of our results with their results.

Methods

This work was conducted under approval of Hofstra University's Institutional Animal Care and Use Committee (Protocol 15/16-18). We obtained hatchlings of six turtle species from diverse sources in 2016. We collected freshly laid *M. terrapin* eggs from Ruler's Bar Hassock, in Jamaica Bay Wildlife Refuge, on the border of Kings and Queens counties, New York (40.615582, -73.833658) and nearby Laurel Hill County Park, Secaucus, Hudson County, New Jersey (40.760603, -74.088115). We collected freshly laid *C. serpentina* eggs at the headwaters of the Peconic River in Suffolk County, New York (40.874729, -72.847528). We obtained freshly laid *T. carolina* eggs from a private breeding facility on Long Island; they were of local origin but the specific locality is unknown. We obtained *G. insculpta* eggs from Cold Spring Harbor Fish Hatchery and Aquarium; the origin of this stock is unknown, but is mostly likely from the upper Midwest region of the U.S. We incubated the eggs of these four species at 28°C. We also obtained newly hatched *G. geographica* hatchlings from Cold Spring Harbor Fish Hatchery and

Aquarium; the origin of this stock is unknown but was probably New Jersey. We used *E. blandingii* hatchlings that were incubated *in situ* in nests in Massachusetts (exact location withheld for security reasons) and collected immediately after emergence.

We followed the procedures of Costanzo et al. (2001) as closely as possible. After hatching, the turtles were transferred to individual compartments in plastic containers with damp vermiculite and held at 22°C. We gradually reduced the temperature starting on 1 October 2016, down to 10°C by November 1st. They were then held at 10°C for 30 days. We gradually reduced the temperature to 5°C between 1 December and 15 December. Hatchlings were kept at 5°C thereafter.

Evaporative Water Loss Experiment

We followed the procedures of Costanzo et al. (2001) as closely as possible. We removed hatchlings from holding boxes, brushed them clean of substrate material and water, and weighed them to the nearest 0.001g. We placed them individually in 473 ml plastic containers without lids, and placed these in a large desiccation chamber at 75-85% relative humidity. A fan and a vent on the side of the chamber was set to replace air volume ca. 2.5 times/hr. The hatchlings were in darkness at 5°C, except when removed for weighing. We weighed the hatchlings at 24 hour intervals for the next ten days, and tested whether each turtle was responsive by assessing its response to gentle prodding with a probe. We rotated the containers through the desiccator shelves so each hatchling experienced conditions throughout the desiccator.

We calculated evaporative water loss (EWL) as the decrease in mass over this time. We report EWL here two ways, first, as did Costanzo et al (2001) by omitting data from the first and last two days' measurements, and second, using all ten measurements.

First rehydration experiment: Sand

Following Costanzo et al. (2001), immediately at the conclusion of the EWL experiment we weighed the hatchlings again, filled each plastic container approximately $\frac{1}{2}$ full with water-saturated sand, and half-buried each hatchling in the container used for the EWL experiment. The water-soaked sand was made by vigorously mixing commercial "play" sand with aged tap water until water ran out of the mix. We put plastic lids with ventilation holes on each container, then held them for ten days at 5°C. The hatchlings were in darkness except for irregular checking to assess the position of each hatchling relative to the surface of the sand, during which hatchlings were otherwise not disturbed. At the end of this experiment we gently brushed each turtle to remove sand, weighed them, cleaned and dried the plastic holding tubs, then returned hatchlings to containers without lids. We held them for 24 hours at 5°C and re-weighed them; these latter measurements were used to determine whether mass (water) had changed.

Second rehydration experiment: Water

Following Costanzo et al. (2001), immediately at the conclusion of the second weighing of the first rehydration experiment we added 70 ml of aged tap water to each container, put plastic lids with ventilation holes on each container, then held them in darkness for ten days at 5°C. At the end of this experiment we gently blotted each turtle to remove surface water, weighed them, cleaned and dried the plastic holding containers, then returned hatchlings to containers

without lids. We held them for 24 hours at 5°C and re-weighed them; these latter measurements were used to determine whether mass (water) had changed.

Measurement of Exposed skin

Following Costanzo et al. (2001), we measured exposed skin as an index of the potential for cutaneous water transfer. Photographs were taken of the cold hatchlings from the ventral aspect, while their heads and limbs were retracted naturally. We used ImageJ (Rasband 2017) to measure the size of plastrons by tracing the margins of the plastrons, including the bridges dorsally to where they met the carapace. We also traced the distal margins of the carapaces, extrapolating where necessary because the view was obscured. We calculated the Critical Exposure Area (= difference between plastron area and carapace area, Costanzo et al. 2001) and the Critical Exposure Index (=ratio of carapace area to plastron area, Costanzo et al. 2001).

Statistical Analysis

We tested multiple-species data sets for normality, and where they were normal, we generally followed the statistical analyses used by Baker et al. (2003), Costanzo et al. (2001), and Dinkelacker et al. (2004). We used a combination of one-way ANOVA and ANCOVA with Post-hoc Tukey HSD comparisons when initial ANOVAs were significant. We compared EWL among the species using ANOVA and ANCOVA, using initial mass as a co-variate. We used paired t-tests with Bonferroni corrections for some species comparisons. We explored the relationship between exposed skin (Critical Exposure Area) and rates of water loss (evaporative water loss (EWL)) via regression analysis.

Results

Four turtle hatchlings died during our experiment; two (one *E. blandingii* and one *M. terrapin*) during the desiccation portion and two (one *E. blandingii* and one *T. carolina*) during rehydration. We omitted data from these individuals from relevant parts of analysis.

We found widespread differences among hatchling body masses, % water loss, water loss rates, and exposure area (Table 1). A one-way ANOVA testing for differences in hatchling body mass revealed significant differences among species ($F_{5,46} = 8.48$, $P < 0.001$). Post-hoc comparisons using a Tukey HSD test indicated that five of 15 species pairwise comparisons were significantly different: *E. blandingii* vs. *G. geographica* ($P = 0.001$), *E. blandingii* vs. *M. terrapin* ($P = 0.017$), *T. carolina* vs. *G. geographica* ($P = 0.035$), *G. geographica* vs. *G. insculpta* ($P < 0.001$), and *M. terrapin* vs. *G. insculpta* ($P < 0.001$).

A one-way ANOVA testing for differences in % water loss over the nine days of the desiccation experiment revealed significant differences among species ($F_{5,46} = 28.84$, $P < 0.001$). Post-hoc comparisons using the Tukey HSD test indicated that ten of 15 species pairwise comparisons were significantly different; the non-significant comparisons were *E. blandingii* vs. *T. carolina* ($P = 0.91$), *E. blandingii* vs. *C. serpentina* ($P = 0.97$), *T. carolina* vs. *C. serpentina* ($P = 0.63$), *T. carolina* vs. *G. geographica* ($P = 0.41$), and *G. insculpta* vs. *C. serpentina* ($P = 0.18$).

A one-way ANOVA testing for differences in mg lost/g/day (= EWL), where g was based on weight after first day of desiccation experiment, as in Costanzo et al. 2001) over the nine days of the desiccation experiment revealed significant differences among species ($F_{5,46} = 28.84$, $P < 0.001$). Post-hoc comparisons using the Tukey HSD test indicated that ten of 15 species pairwise comparisons were significantly different; the only non-significant comparisons were *E. blandingii* vs. *T. carolina* ($P = 0.78$), *E. blandingii* vs. *C. serpentina* ($P = 0.97$), *T. carolina* vs. *G.*

geographica ($P=0.56$), *T. carolina* vs. *C. serpentina* ($P=0.46$), and *G. insculpta* vs. *C. serpentina* ($P=0.21$).

We found widespread differences among water gain (both absolute and %) after immersion in wet sand and in water (Table 2). Paired t-tests with Bonferroni corrections to test whether hatchlings of each species gained water during rehydration experiments showed that four species gained significant amounts of water during our sand rehydration experiment: *T. carolina* ($t=3.98$, D.F.=7, $P=0.005$), *G. geographica* ($t=5.24$, D.F.=8, $P<0.001$), *M. terrapin* ($t=4.87$, D.F.=14, $P<0.001$), and *G. insculpta* ($t=4.66$, D.F.=6, $P=0.003$). Similarly, two species gained significant amounts of water during our water rehydration experiment: *T. carolina* ($t=5.86$, D.F.=6, $P=0.001$) and *G. geographica* ($t=5.21$, D.F.=8, $P<0.001$).

A one-way ANOVA testing for differences in weight gain during rehydration in sand revealed significant differences among species ($F_{5,45}=4.45$, $P=0.002$). Post-hoc comparisons using the Tukey HSD test indicated that only two of 15 species pairwise comparisons were significantly different (*T. carolina* vs. *G. geographica* ($P=0.012$), *T. carolina* vs. *M. terrapin* ($P=0.011$)). Body mass was not a significant co-variate in this analysis ($F_{1,44}=0.003$, $P=0.953$).

A one-way ANOVA testing for differences in weight gain during rehydration in water revealed significant differences among species ($F_{5,45}=8.895$, $P<0.001$). Post-hoc comparisons using the Tukey HSD test indicated that six of 15 species pairwise comparisons were significantly different: *E. blandingii* vs. *T. carolina* ($P=0.01$), *E. blandingii* vs. *G. insculpta* ($P=0.001$), *T. carolina* vs. *G. geographica* ($P=0.045$), *T. carolina* vs. *M. terrapin* ($P<0.001$), *G. geographica* vs. *G. insculpta* ($P=0.006$), and *M. terrapin* vs. *G. insculpta* ($P<0.001$). Body mass was not a significant co-variate in this analysis ($F_{1,43}=0.288$, $P=0.594$).

A one-way ANOVA testing for differences in Critical Exposure Area revealed significant differences among species ($F_{5,46}=116.613$, $P<0.0001$). Post-hoc comparisons using the Tukey HSD test indicated that 14 of 15 species pairwise comparisons were significantly different, the only non-significant comparison was *T. carolina* vs. *G. geographica* ($P=1.00$). A one-way ANOVA testing for differences in Critical Exposure Index revealed significant differences among species ($F_{5,46}=418.559$, $P<0.0001$). Post-hoc comparisons using the Tukey HSD test indicated that 11 of 15 species pairwise comparisons were significantly different; the only non-significant comparisons were *E. blandingii* vs. *T. carolina* ($P=0.110$), *E. blandingii* vs. *G. geographica* ($P=1.00$), *E. blandingii* vs. *M. terrapin* ($P=0.073$), and *T. carolina* vs. *M. terrapin* ($P=1.00$).

Regression analysis showed a strong and significant relationship between Critical Exposure Area and EWL ($b= 0.036$, $t(4) = 4.9$, $P = 0.008$, $R^2 = 0.86$). Residuals were evenly distributed and relatively small except for residual associated with *M. terrapin* (range = -1.23 – 2.74, excluding *M. terrapin* residual, -3.28).

Discussion

We found widespread and significant differences in various measures of desiccation resistance (% water loss, water loss rates, rehydration rates, and exposure area) among the six species of hatchling turtles corresponding with differences in overwintering microhabitat. With few exceptions, we found broad agreement among the measures—species which typically overwintered in progressively drier habitats, had progressively lower rates of % water loss, water loss per g/day, and amount of exposed skin, all indicating greater selective pressures to minimize desiccation. Thus, our findings are in concordance with similar studies by Baker et al. (2003), Costanzo et al. (2001), and Dinkelacker et al. (2004).

Our finding of a strong positive relationship between Critical Exposure Area and EWL supports the concept that the primary region of water loss is the exposed skin between the plastron and carapace. We note that *M. terrapin* is unusual, in that this species has even lower rates of water loss than would be expected based on its amount of exposed skin and the general relationship between exposed skin and EWL alone. Costanzo et al. (2001) (also reviewed in Costanzo et al. 2008) further described a general relationship between turtle species with hatchlings that had relatively high Critical Exposure Areas and their overwintering strategies, in that these species were likely to be especially vulnerable to ice and ice nucleating agents, thus precluding supercooling as an option for surviving freezing conditions. For example, our results suggest that supercooling is not likely to be a viable option for *G. insculpta*.

We note that the specimens of two species we studied (*G. geographica* and *E. blandingii*) were collected after hatching, following unknown incubation conditions. This may have affected the results of our experiments because incubation conditions are known to affect a wide range of hatchling characteristics, such as survivorship, body size, locomotor performance, and growth (e.g., Janzen 1993; Roosenburg and Kelley 1996; Demuth 2001).

The three previous studies to measure desiccation resistance in hatchling turtles (Baker et al. 2003; Costanzo et al. 2001; and Dinkelacker et al. 2004) were all performed in the same laboratory using the same equipment (Costanzo, pers. comm.), so they are directly comparable to each other. Despite our attempts to replicate the methods of previous papers, we note that our desiccation rate values, especially Evaporative Water Loss (=EWL) are generally higher than the values reported in those papers. In fact, four of our six EWL values were greater than the highest EWL value reported in any of those papers. We tested three species also previously

tested; our values ranged from 256%-477% greater than previously reported values. We note that we found the same EWL pattern among the three species (*C. serpentina* > *E. blandingii* > *G. geographica*) as did Baker et al. (2003), Costanzo et al. (2001), and Dinkelacker et al.'s (2004); we attribute these differences in values to differences in initial hydration and the efficacy of the desiccation chambers. However, these differences suggest we cannot directly compare our quantitative results with those of previous works.

We report here the first measures of desiccation resistance and Critical Exposure Area in hatchlings of TON (terrestrial overwintering outside the nest) turtle species, and find that one (*M. terrapin*) is exceptionally desiccation tolerant (very low % water loss, low water loss/day, low Critical Exposure Area, low Critical Exposure Index). These are lower values than previously reported for hatchlings of any turtle species. We suggest that *M. terrapin* may have such exceptional desiccation resistance because some remain buried in overwintering refugia for a very long period (up to eight months, Muldoon and Burke 2012; Duncan and Burke 2016) and the sand substrate of their refugia is subject to salt spray from nearby marine sources (Burke, pers. obs.). The low Critical Exposure Area of this species suggests that it may be able to resist the initiation of freezing due to ice and ice nucleating agents, and therefore supercooling may be a potential option for surviving freezing conditions. This suggestion is concordant with laboratory experiments by Baker et al. (2006), who found that *M. terrapin* hatchlings were able to supercool to very low temperatures while avoiding spontaneous freezing. However, in clear contrast to our findings of relatively low Critical Exposure Area for this species, Baker et al. also showed that *M. terrapin* hatchlings in contact with nucleating agents readily succumbed to

inoculative freezing, which is likely to happen under natural nesting conditions. These conflicting findings should be further investigated.

Our results showed that the EWL rates and Critical Exposure Areas of another TON species (*T. carolina*) are intermediate between TIN (overwintering terrestrially shallowly in the nest) and OIW (overwintering in water) species, much like that of *E. blandingii* (Dinkelacker et al. 2004; 2005). The overwintering microhabitats of *T. carolina* are known from three reports (Madden 1975; Burke and Capitano 2011; Melvin 2017), which all suggest that this species uses both TIN and TON strategies. This should be explored in greater detail and more locations, especially exploring soil moisture conditions.

G. insculpta hatchling overwintering locations are also poorly known. Tuttle and Carroll (2005) tracked hatchling *G. insculpta* from nests to streams, but did not observe overwintering sites. *G. insculpta* hatchlings have been reported to overwinter “on the shores of a creek” (Paterson et al. 2012). However, Dragon (2014) reported that *G. insculpta* hatchlings overwinter in water, as do adult *G. insculpta*. Our findings that *G. insculpta* hatchlings have very low desiccation resistance and high Critical Exposure Areas strongly support the hypothesis that *G. insculpta* avoid freezing and are typical OIW strategists.

Costanzo et al. (2001) investigated rehydration rates of hatchlings of eight species of turtles and found that only one (Ornate Box turtles, *Terrapene ornata*) gained a significant amount of mass (water) while in wet sand. In contrast, we found that four species (*T. carolina*, *G. geographica*, *M. terrapin*, and *G. insculpta*) gained mass while in wet sand. We are confident these increases were actually water and not sand adhering to hatchlings because we carefully removed visible sand. Our results indicate that at least for some turtle species, it may be

possible to gain or lose water while they remain in contact with soil in terrestrial refugia. This has important implications for the risk of desiccation and ability to rehydrate if they overwinter on land, as do *T. carolina* and *M. terrapin* (see above).

Our results replicate the pattern of results reported by previous work and extend them to three new turtle species and one new overwintering strategy. This supports the hypothesis that desiccation resistance, rehydration ability, and measures of exposed skin are characteristics of turtle hatchlings subject to adaptive pressures associated with overwintering microhabitats. Further explorations of these patterns will lead to a better understanding of the evolutionary and ecological characteristics of this poorly understood life history stage.

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Table 1. Names, (sample size), typical hatchling overwinter strategy, and mean values (and standard deviations) for four measures of desiccation resistance for six species of turtles.

Species	Overwinter Mode	Body mass (g)	% Body water lost overall	EWL ^a (mg/g/d)	Critical Exposure Area ^b (mm ²)	Critical Exposure Index ^b
<i>Malaclemys terrapin</i> (15)	TIN/TON ^c	6.9 (0.46)	2.1 (0.65)	4.2 (1.69)	191.5 (23.89)	1.40 (0.04)
<i>Graptemys geographica</i> (9)	TIN ^d	6.4 (0.93)	7.1 (1.21)	9.5 (1.74)	266.7 (18.34)	1.51 (0.07)
<i>Terrapene carolina</i> (8)	TON/TON ^c	7.6 (1.32)	10.0 (6.4)	12.3 (7.23)	249.2 (74.41)	1.39 (0.07)
<i>Emydoidea blandingii</i> (9)	OIW ^c	8.0 (0.45)	11.6 (2.22)	14.5 (2.47)	335.9 (36.59)	1.50 (0.03)
<i>Chelydra serpentina</i> (4)	OIW ^e	7.1 (0.39)	13.0 (3.07)	16.1 (3.13)	414.2 (44.16)	3.16 (0.20)
<i>Glyptemys insculpta</i> (7)	OIW/TON ^c	8.5 (0.80)	17.7 (1.62)	21.1 (2.10)	603.8 (31.07)	1.87 (0.06)

a: EWL = Evaporative water loss, see Methods

b: see Methods

c: see text above

d: Nagle et al. 2004

e: Finkler and Kolbe 2008

Table 2. Names, (sample sizes), mean values (and standard deviations) for two measures^c of rehydration ability for six species of turtles.

Species	Mass at	After sand	% increase	After water	% increase
	start (g)	rehydration (g)		rehydration (g)	
<i>Malaclemys terrapin</i> (15)	6.73 (0.46)	6.8 (0.43)	1.6 (1.37)	6.8 (0.45)	-1.1 (2.15)
<i>Graptemys geographica</i> (9)	5.9 (0.89)	6.0 (0.87)	1.4 (0.92)	6.1 (0.85)	0.9 (0.59)
<i>Terrapene carolina</i> (8)	6.2 (1.47)	7.2 (1.52)	5.0 (4.25)	7.8 (1.40)	5.8 (4.12)
<i>Emydoidea blandingii</i> (8)	7.1 (0.44)	7.3 (0.36)	3.2 (3.11)	7.3 (0.42)	-0.3 (2.24)
<i>Chelydra serpentina</i> (4)	6.2 (0.54)	6.24 (0.55)	1.3 (0.64)	6.32 (0.52)	1.4 (0.97)
<i>Glyptemys insculpta</i> (7)	7.0 (0.73)	7.3 (0.75)	4.0 (2.23)	7.8 (0.91)	6.5 (7.00)

c: see Methods